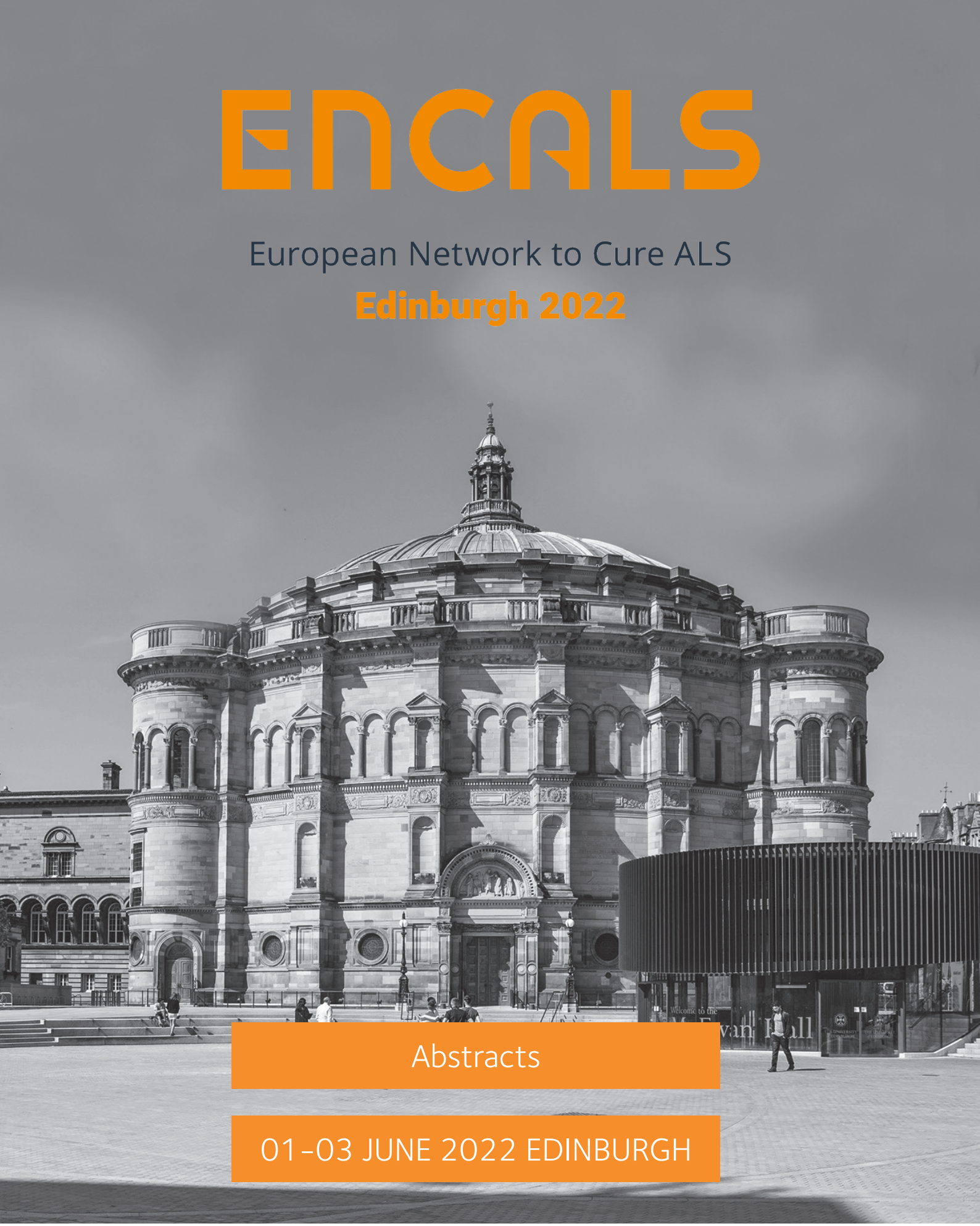
ENCALS

European Network to Cure ALS

Edinburgh 2022



Abstracts

01-03 JUNE 2022 EDINBURGH

THE UNIVERSITY of EDINBURGH

EUAN MACDONALD CENTRE

Vital research into motor neuron disease

Abstracts for Oral Presentations

Session 1: 10.30 to 12.30 – Presymptomatic Disease

1. Pre-symptomatic mild cognitive and behavioural impairment in ALS-frontotemporal spectrum disorder (ALS-FTSD): A conceptual framework.

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Introduction: Neurodegenerative diseases pass through a pre-symptomatic or prodromal stage that may include mild clinical manifestations that do not yet reach the threshold for a clinical diagnosis. Criteria exist for defining cognitive and behavioural impairment in ALS (ALSci/ALSbi), capturing the spectrum of frontotemporal disorders (ALS-FTSD), but are lacking for pre-symptomatic disease.

Methods: We have developed a framework for identifying and characterising mild cognitive (MCI) and behavioural (MBI) impairment as prodromal features of ALS. This framework builds on the existing ALSci/ALSbi criteria but is more inclusive of other cognitive and behavioural features and places an emphasis on decline from premorbid or baseline functioning. It also recognizes that MCI/MBI may be *uncertain* when objective evidence of decline is absent; only a single cognitive test (excluding letter fluency) is impaired or shows decline; confounding factors are present; or subjective report is the only evidence of impairment/decline. We recommend comprehensive neuropsychological assessment of all major cognitive domains and the use of standardised tests appropriate for repeated assessment with good normative data. Inclusion of a brief standardised multi-domain assessment that is sensitive to impairment in ALS will allow for assessment throughout the course of the disease. This framework is currently being applied in the *Pre-symptomatic Familial ALS (Pre-fALS)* study.

Results: Application of the framework during multi-disciplinary consensus meetings has shown it to be practical and intuitive. Illustrative cases from *Pre-fALS* will be discussed. Efforts are currently underway to characterise the entire *Pre-fALS* cohort (n=221).

Discussion: Our approach differs somewhat from that currently used in the FTD community. We place greater emphasis on formal neuropsychological assessment conducted or supervised by a neuropsychologist. Our approach also recognises the pragmatic consideration that pre-symptomatic individuals may be evaluated by ALS neurologists who may feel less comfortable using clinical judgement to designate MCI and MBI. Implementation of this framework will help to define the timing of neuropsychological symptoms relative to motor manifestations of disease and is essential for advancing the goal of early therapeutic intervention.

2. EEG changes in cognitive networks in asymptomatic C9orf72 repeat expansion carriers.

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Background: While previous studies of asymptomatic carriers of C9orf72 hexanucleotide repeat expansions have reported a wide range of alterations in cognition, behaviour, brain metabolism [1] and structure [2], early electrophysiological changes remain largely unexplored. Timely detection and characterisation of disease manifestations can lead to new therapeutic strategies that are based on targeted treatments. Here, we aim to evaluate changes in the cognitive cortical networks in C9orf72 asymptomatic gene carriers (AGC) using electroencephalography (EEG).

Methods: High-density EEG was recorded during the randomised sustained attention to response task (SART) in 16 AGC and 43 age-matched controls. The task requires participants to respond every time they see a number (1-9, Go) except for the number 3 (NoGo). We have assessed five behavioural measures of performance: NoGo accuracy (percentage of three-digit stimuli followed by response omission), Go accuracy (percentage of non-three digit stimuli followed by a response in the permitted time window), anticipation error (clicking less than 200 ms after a Go stimulus) and response time. Using source-localisation, we evaluated the event-related potentials associated with the task: N2 (200-350ms post-stimulus) and P3 (350-500ms); both known to be impaired in ALS [2]. Statistical comparisons were done using Mann-Whitney test.

Results: Controls and AGC did not differ significantly in accuracy (NoGo: $p = 0.64$, Go: $p = 0.21$). However, AGC had slower response time ($p < 0.001$) and committed more anticipation errors ($p = 0.07$). The P3 activation was found significantly increased in AGC in the right superior and medial frontal gyri ($p < 0.05$; with false discovery rate (FDR) correction), whereas the N2 activation was significantly increased in the right inferior parietal lobule and superior-medial frontal cortices ($p < 0.05$; FDR).

Discussion: These results show the potential of EEG to capture functional changes associated with the asymptomatic carriers of C9orf72. The identification and characterisation of biomarkers that can be linked to the early development of ALS, can help in the early diagnosis and early treatment strategy development, as well as enhance our understanding of causal (patho)physiological processes.

Reference: De Vocht J, et al. JAMA Neurology. 20202. Walhout R, et al. Neurology. 20153. McMackin et al. Cerebral Cortex. 2020

3. Distinct neural signatures of pulvinar in C9orf72 ALS mutation carriers.

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Background: Thalamic alterations have been reported in presymptomatic and symptomatic Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) patients with the C9orf72 mutation as a main feature (Querin et al., 2022). Presymptomatic and FTD C9orf72 mutation carriers showed a specific reduction in pulvinar volume, a higher-order nucleus of the thalamus, when compared to healthy participants (Bocchetta et al., 2020, 2021). For the first time, ALS mutation carriers and wild-type patients will be compared to determine if pulvinar changes occur, and how the underlying functional connectivity changes affect this region.

Methods: Structural (T1w) and resting-state functional MRI data were collected with a 1.5T scanner from 19 participants diagnosed with ALS and carrying the C9orf72 hexanucleotide repeat expansion (ALSC9+), 19 patients diagnosed with ALS but not affected by C9orf72 mutation (ALSC9-), and 19 ALS mimics patients (ALSm). All participants were accurately matched for age and sex, while ALS patients were also paired for total ALSFRS-R score and disease duration. Pulvinar volume, corrected for total intracranial volume, was obtained using automatic segmentation (Freesurfer). Seed-to-voxel functional connectivity analyses, after standard image processing on resting state functional magnetic resonance imaging (CONN), were performed using five bilateral anatomical seeds of pulvinar functional parcellation (i.e. dorsomedial, ventromedial, lateral, anterior, and inferior) (Guedj et al., 2020). Significant differences between groups were tested using a factorial design.

Results: ALSC9+ had a significantly reduced pulvinar volume compared to ALSC9- and ALSm ($p<0.003$). Compared to ALSm, ALSC9- showed increased inferior and lateral pulvinar connections with bilateral occipito-temporal-parietal regions, while ALSC9+ showed no differences. Between-groups differences showed a reduced pulvinar-occipital connectivity for anterior and inferior pulvinar seeds in ALSC9+ patients when compared to ALSC9-.

Conclusion: When compared with wild-type ALS patients with similar disease burden, ALSC9+ patients show substantial structural and functional alterations in the pulvinar. Pulvinar subregions may play a crucial role in disease progression and development in patients with C9orf72 mutation because they function as a timekeeper for large-scale cortical networks (Fiebelkorn et al., 2019).

4. Neuropsychological endophenotypes in first- and second-degree relatives of people with ALS.

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Background: People with ALS often experience cognitive changes, such as executive dysfunction and language deficits. Family aggregation studies have found that first and second-degree relatives of people with ALS report higher rates of neuropsychiatric illness, indicating that ALS risk genes, such as C9orf72, may have pleiotropic (i.e., multiple) effects. This also suggests that endophenotypes (i.e., biological traits intermediate to the genetic cause and the disease phenotype) of ALS are present and identifiable in ALS kindred.

Aims: To investigate cognitive endophenotypes in non-affected relatives of ALS patients.

Methods: First- and second-degree relatives of ALS patients (n=148) and controls (n=60) completed a neuropsychological assessment, including measures of intellectual functioning (Wechsler Abbreviated Scale of Intelligence- 2nd Edition; WASI-2), language (Boston Naming Test; BNT), executive functioning (Colour Word Interference Test; CWIT, Digit Span; DS, Verbal Fluency), social cognition (Reading the Mind in the Eyes Test; RMET) and memory (Rey Auditory Verbal Learning Test; RAVLT, Logical Memory from the Wechsler Memory Scale – 3rd Edition; LM). ALS relatives and controls were compared using Welch's t-tests or Wilcoxon rank tests. Analyses of variance (ANOVAs) were carried out to examine the effect of family history (FALS vs SALS). Lastly, C9orf72 positive (n=39) and negative ALS relatives (n=158) were compared.

Results: ALS relatives scored significantly worse than controls on WASI-2 IQ (d = 0.54, 95% CI: 0.13, 0.95), phonemic verbal fluency (d = 0.81, 95% CI: 0.37, 1.24), CWIT inhibition errors (d = 0.49, 95% CI: 0.17, 0.80), DS backwards span (d = 0.8, 95% CI: 0.41, 1.19), BNT spontaneous correct (d = 0.91, 95% CI: 0.61, 1.20), RAVLT 5 trial total recall (d = 0.67, 95% CI: 0.26, 1.08) and LM delayed recall (d = 0.7, 95% CI: 0.29, 1.11). Post-hoc Bonferroni comparisons indicated that relatives of FALS patients showed particularly strong deficits in phonemic verbal fluency compared to controls ($p<.001$) and relatives of SALS patients ($p<.05$). Analysis of C9orf72 subgroups revealed that semantic verbal fluency was significantly lower in C9orf72 negative than C9orf72 positive relatives (d = -1.25, 95% CI: -2.16, -0.31).

Conclusions: These findings suggest a cognitive endophenotype exists in non-affected members of ALS kindreds. Deficits on these tasks may signify disruption to underlying fronto-striatal networks.

Session 2: 13.30 to 15.00 – Imaging and Biomarkers

5. Neuroimaging correlates of domain-specific cognitive deficits in amyotrophic lateral sclerosis.

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Background: It has proved difficult to characterize the longitudinal course of cognitive and behavioural symptoms in Amyotrophic lateral sclerosis (ALS). In patients with ALS widespread grey and white matter degeneration can be identified using brain MRI. Therefore, the objective of this study was to evaluate whether specific cognitive and behavioural deficits correlate with distinct neurodegeneration patterns on brain MRI.

Methods: We performed T1 weighted brain MRI and DTI in 293 patients and 237 controls. 337 additional follow-up visits were conducted in 171 patients at a 3-6-month interval. Cortical thickness and white matter fractional anisotropy were calculated. The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was administered within seven days from each MRI scan. For 190 patients, the ECAS behavioural screen was administered from proxies. Negative binomial models and logistic regression were used to analyse domain level cognitive performance and behavioural changes, respectively. An interaction analysis was performed on patients and controls, to explore whether cognitive findings are disease specific. Longitudinal changes of domain-associated brain structures were analysed using linear mixed models.

Results: Deficits in the five cognitive domains were associated with specific neurodegeneration patterns: Language impairment was significantly associated with (left predominant) frontal, temporal, parietal and subcortical grey matter neurodegeneration; Fluency with widespread cortical and subcortical grey matter involvement; Memory dysfunction with hippocampal and medial-temporal atrophy; Executive dysfunction impairment was exclusively correlated with widespread white matter impairment; Visuospatial scores did not correlate with MRI parameters. Correlates of behavioural symptoms were limited to white matter impairment. Interaction analyses showed that most ECAS-MRI correlations (75.7% in grey matter, 52.7% in white matter) were stronger in ALS than in controls. Longitudinal analyses show that all cognitive domain-associated grey matter involvement worsened over time.

Interpretation: ALS-related cognitive and behavioural changes are associated with distinct domain-specific cerebral neurodegeneration patterns. MRI is able to capture the heterogeneity of cognitive involvement in ALS and proves to be a useful longitudinal biomarker for progression of extra-motor neurodegeneration.

6. ALS subgroups based on EEG measures recorded during sustained attention to response task performance.

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Background: Previous findings from our group, based on resting-state EEG alone, revealed the presence of network-based subphenotypes [1]. The sustained attention to response task (SART) engages both cognitive and motor functions [2]. The heterogenous nature of ALS, which may involve motor and cognitive impairment, may be clarified by subphenotyping with measurements of networks engaged by the SART. Here, we present evidence that SART evoked potential based clustering can detect stable subphenotypes of ALS patients which differ in cognitive prognosis.

Objective: To stratify ALS patients with cluster analysis, based on the N2 peak recorded EEG measures recorded during SART performance.

Method: EEG was recorded in 23 ALS patients during SART performance [2]. The event related potential was found for each channel, and the mean of the N2 peak (which is related to motor control) was calculated, giving 128 measures per subject. The SPARCL algorithm was used for feature selection, which removed irrelevant measures. Hierarchical and spectral clustering were implemented. Cluster Validity Indices (CVIs) were calculated to assess the goodness of each cluster assignment. P-values for each solution were calculated by generating random samples from a uniform box aligned with the principal components of the data. Stability under small perturbation was quantified using Adjusted Rand Index (ARI; [0,1], 1 the most stable).

Results: Feature selection chose 41 key measures. Spectral clustering found one significant (p=0.016) and reasonably stable (ARI=0.722) solution with 3 clusters (sizes = 5, 8, 10). Hierarchical clustering found an almost identical significant and stable solution, with only one patient classified differently. There were no analogous solutions in controls. A significant difference was found between clusters in terms of non-ALS-specific ECAS (p=0.007), and memory (p=0.020).

Conclusion: Evidence for cluster structure was found in SART EEG data, with two different algorithms independently yielding almost identical solutions. Cluster profiling revealed that the smallest of these clusters may be linked to abnormal non-ALS-specific ECAS and memory. While our sample is not large enough yet to draw more general and strong conclusions from this specific solution, the findings demonstrate that SART EEG may provide a valuable tool for ALS stratification, warranting further investigation.1. Dukic et al, 2021, Brain2. McMackin et al, 2020, Cereb. Cortex

7. Brain sensorimotor integration after focal muscle-tendon vibration in amyotrophic lateral sclerosis.

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Background: Sensitive alterations have been found at an early stage of the disease in SOD1 murine model of amyotrophic lateral sclerosis (ALS) (1) and in patient spinal cord (2). Furthermore, somatosensory evoked potentials decrease in patients with weak motor dysfunctions (3).

Objective: To evaluate the integration of proprioceptive sensory afferents at cortical and sub-cortical levels in early stage of ALS

Methods: 21 ALS patients underwent fMRI during muscle-tendon vibrations applied on the less affected or no affected hand (MRC score ≥ 4). Their results were compared to those of 23 age and gender-matched healthy controls. Vibrations were applied on the first finger dorsal interosseous muscle (FDI) and on extensor digitorum muscle (ED; fingers III & IV). fMRI acquisitions were performed while the participants had their eyes closed (to enhance kinesthesia). Kinesthesia was assessed after fMRI session. The brain activation maps were analyzed from fMRI data and cerebellum, putamen, thalamus, motor areas and somatosensory areas were selected as region of interest for the second step of analyze.

Results: Preliminary analysis of the activation maps indicates that patients and healthy controls have similar activation in motor areas (4 and 6 Brodmann areas) on contralateral side of vibration site. However, patients exhibited hyper-activation compare to controls in motor and premotor areas (6 and 8 Brodmann areas) on the ipsilateral side of the vibrations. Furthermore, we found significant hyper-activation on ipsilateral side in sensorimotor areas and in cerebellum. In the end, kinesthesia decreases significantly in patients.

Conclusions: These preliminary results suggest that activity in cortical and sub-cortical structures involved in sensorimotor integration are altered in patients, particularly on ipsilateral hemispheric to stimulation side which might be related to disturbed interhemispheric relationships in ALS.

Bibliography:(1) Fischer and al, 'The WldS Gene Modestly Prolongs Survival in the SOD1G93A FALS Mouse'. Neurobiology of Disease. https://doi.org/10.1016/j.nbd.2005.01.008.(2) Iglesias and al, 'Electrophysiological and Spinal Imaging Evidences for Sensory Dysfunction in Amyotrophic Lateral Sclerosis'. BMJ Open. https://doi.org/10.1136/bmjopen-2015-007659.(3) Sangari and al, 'Abnormal Cortical Brain Integration of Somatosensory Afferents in ALS'. Clinical Neurophysiology. https://doi.org/10.1016/j.clinph.2017.12.008.

8. Diagnostic utility of nerve excitability tests in ALS.

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Background: Hyperexcitable motor neurons are a ubiquitous feature of ALS. Nerve excitability tests have become increasingly available to study these neurons' aberrant behavior. Previous studies found distinctive abnormalities in patients with ALS, even preceding axon loss. Despite being reliable and non-invasive, the diagnostic value of peripheral nerve excitability tests remains to be defined.

Objectives: To determine the diagnostic accuracy of excitability measures in patients with suspected motor neuron disorders (MND).

Methods: We included patients referred to our neuromuscular outpatient clinic with suspected MND. Routine diagnostic strategies were performed in all subjects. Final diagnoses were based on relevant consensus criteria. We performed nerve excitability tests on the abductor pollicis brevis (APB) and selected strength-duration-time-constant, superexcitability and S2 as predictors. Axon loss of the APB was quantified with motor unit number estimates (MUNE). Patients reported their experienced burden during measurements with visual analog scores (VAS). Lastly, we examined the addition of altered nerve excitability to the El-Escorial and Awaji criteria. Altered excitability was considered evidence of lower motor neuron dysfunction, thereby replacing needle-EMG findings.

Results: We included 165 patients, of which 114 (69%) were classified as ALS. Patients were age-gender matched, with shorter median disease durations from onset in ALS (10 vs. 12 months; $p<0.001$). Median VAS scores were low in both groups (ALS=2, non-ALS=2; $p=0.3$). Excitability measures had high overall diagnostic accuracy (AUC [CI]=0.89 [0.83-0.94]). Subgroup analyses in patients with or without axon loss in the APB produced AUCs of 0.85 [0.77-0.93] and 0.92 [0.82-0.98], respectively. Addition of nerve excitability to the El-Escorial and Awaji criteria increased their sensitivities by 14% and 15%, respectively, while replacing needle-EMG findings of 92 (55%) patients with excitability measures. Specificities were unaffected by the addition of excitability measures.

Discussion and conclusion: Nerve excitability measures can accurately classify ALS patients, even before axon loss can be detected. This non-invasive and tolerable technique complements utilized diagnostic criteria and improves their sensitivities, even when omitting regular invasive and painful electrodiagnostic techniques. Testing multiple muscle-nerve combinations may further improve classification.

Session 3: 15.30 to 17.15: Neuropsychology/ Neuropathology

9. Examining the nature of phonemic verbal fluency in the Familial ALS cognitive endophenotype.

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Background: Verbal fluency (VF) deficits are characteristic of ALS-related cognitive impairment. Initiation apathy has previously been associated with VF deficits in ALS patients[1]. There is increasing evidence of phonemic VF deficits in asymptomatic relatives of ALS patients, including unaffected C9orf72-carriers[2], & relatives of Familial-ALS (FALS) patients, irrespective of gene-status[3]. This suggests the presence of a cognitive endophenotype in ALS relatives, though the nature of which is not yet understood. This study will examine if apathy influences VF performance in asymptomatic relatives of ALS patients.

Methods: 46 FALS relatives & 36 apparently Sporadic ALS (SALS) relatives were recruited through the Irish MND Clinic as part of a larger study on gene-pleiotropy in ALS. 26 healthy controls were recruited through the group control participant bank. Phonemic VF was assessed using the FAS test. Each participant also completed the self-report Dimensional Apathy Scale (DAS).

Results: FALS relatives performed significantly worse on the FAS task relative to controls & SALS relatives (both $p<0.001$). SALS & control groups did not differ ($p=0.57$). There were no group differences on any of the DAS domains, including initiation apathy ($p=0.804$). Neither initiation apathy, emotional apathy nor DAS total score accounted for VF scores in any of the groups. However, VF performance was negatively correlated with the DAS executive domain (rho = -0.195, $p=0.04$), accounting for 4.4% of the variance in FAS total z score ($p=0.027$). This trend was not evident in the FALS group (rho=0.12, $p=0.43$), but showed a medium negative correlation for the SALS & control groups (rho= -0.39, $p =0.028$; rho= -0.37, $p=0.047$), accounting for 20.4% ($p=0.005$) & 14.6% ($p=0.04$) of the variance in FAS scores respectively.

Conclusion: Verbal fluency is a higher order executive task requiring input from multiple cognitive processes. Although VF deficits are present in FALS relatives, apathy is unlikely to be the primary driver. Executive apathy may mediate non-FALS VF performance & initiation apathy may later compound distinct executive impairment in ALS patients, but it is not a feature in the endophenotype. Future work will examine the nature of VF deficits in relatives & assess their stability over time, in which, this candidate endophenotype may prove significant in the elucidation of the complex genetic mechanisms in ALS.1Radakovic et al.,20172Lule et al.,20203Costello et al.,2018

10. Self-perceived quality of life and cognitive and behavioural impairment in ALS, UEA/EMC.

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Background: Cognitive and behavioural impairment can occur in up to 50% of people with amyotrophic lateral sclerosis (pwALS), with 15% developing frontotemporal dementia (FTD). With increased utility of the ALS-Frontotemporal Spectrum Disorder (ALS-FTSD) diagnostic criteria, the impact of these impairments on caregivers are well-documented. However, quality of life (QoL) and cognitive and behavioural impairment in pwALS has been seldom researched.

Objective: The aim was to determine if there was a relationship between QoL as perceived by the pwALS and cognitive and behavioural impairment.

Method: This was a prospective cohort study of 122 pwALS and their caregivers from a multidisciplinary ALS clinic. Patient demographics, clinical variables, ALS Functional Rating Scale-Revised (ALSFRS-R), Edinburgh Cognitive & Behavioural ALS Screen (ECAS) and the ALS Specific QoL Measure (ALSSQoL) were available. ALS-FTSD diagnostic categories were derived from ECAS scores. QoL group comparisons were performed between ALS-FTSD categories.

Results: The cohort was 68.9% (N=84) male, mean age 61.7 (9.9) years, mean disease duration 12.1 (17.7) months and mean ALSFRS-R score 34.3 (6.5). 27.9% (N=34) had ALS cognitive impairment (ALSci), 13.9% (N=17) had ALS behavioural impairment (ALSbi), 18.9% (N=23) had both impairments (ALScbi), 38.5% (N=47) had no impairment (ALSCN) and one had ALS-FTD. ALS-FTSD category comparison showed a significant difference in the ALSSQoL score ($p<0.01$), ALSbi had significantly lower overall QoL than those with ALSci ($p<.01$), with no other differences between ALS-FTSD categories. A breakdown of QoL domains showed a significant difference in QoL for negative emotion ($p<.01$), with ALSbi having more negative emotions compared those with ALSci ($p<.01$) and ALSCN ($p<.05$). There was a significant difference in QoL in interaction with people & environment ($p<.01$), with ALSbi having lower QoL in that domain than ALSci ($p<.01$) and ALSCN ($p<.01$). Finally, a significant difference was found for the QoL intimacy domain ($p<.01$), with ALScbi showing lower intimacy QoL compared to ALSCN ($p<.01$).

Conclusions: Behavioural but not cognitive impairment may negatively impact QoL as perceived by pwALS. Those with behavioural impairment reported increased negative emotions, fewer/poorer interaction with people and environment and poorer intimacy. Behavioural impairment not only impacts the caregivers but also the QoL of pwALS.

11. Patients with amyotrophic lateral sclerosis and cognitive deficits are impaired in recognizing negative facial emotions.

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Background and Objectives: Evidence for the presence of emotion recognition disturbance in amyotrophic lateral sclerosis (ALS) is ambiguous. Likely explanations for this variability are heterogeneity of clinical symptomatology with regards to cognition, behaviour and affect, as well as heterogeneity of instruments used to assess emotion recognition. Here, we focused on ALS patients without and with cognitive disorders. These patients were tested with the Facial Emotion Intensity Rating Task - Congruent and Incongruent (FEIRT-CIC), a recently developed sensitive and short test of facial emotion recognition. We aimed to determine whether ALS patients present difficulties in discriminating among the four basic negative facial emotions (i.e., anger, disgust, fear and sadness).

Materials and Methods: We compared performance on negative facial emotion discrimination between 37 ALS patients [25 without cognitive deficits (pure ALS) and 12 with cognitive deficits (ALSci) according to the Strong et al. (2017) diagnostic criteria] and 37 healthy controls (HC), matched for age, sex and education. Scores of a control task (gender discrimination task) and the FEIRT-CIC were compared across groups using the Wilcoxon-Mann-Whitney-test and Receiver Operating Characteristic analyses.

Results: Overall, ALS patients were not impaired in discriminating between gender presented as facial features. They were, however, impaired in discriminating negative emotions overall compared with HC ($p=.05$). ALS subgroup analyses revealed the ALSci group to be impaired on negative emotion discrimination ($p=.002$) with an AUC of .83 (95% CI .67–.99). This deficit was of similar severity for the four negative emotions. In contrast, pure ALS patients showed no impairment in emotion discrimination.

Conclusions: We showed that the capacity in negative emotion discrimination in ALS patients varies depending on whether a cognitive impairment is present or not. As a next step, we will analyse whether ALS subgroups with behavioural disorders are more impaired in negative emotion discrimination than the ALSci subgroup.

12. Motor, cognitive and behavioral profiles of C9orf72-related amyotrophic lateral sclerosis.

Eleonora Colombo (1), Barbara Poletti (1), Alessio Maranzano (1,2), Silvia Peverelli (1), Federica Solca (1), Claudia Colombrita (1), Silvia Torre (1), Cinzia Tiloca (1), Federico Verde (1,2), Ruggero Bonetti (1,2), Laura Carelli (1), Claudia Morelli (1), Antonia Ratti (1,3), Vincenzo Silani (1,2), Nicola Ticozzi* (1,2).

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Background and aims: Amyotrophic lateral sclerosis (ALS) individuals carrying the hexanucleotide repeat expansion (HRE) in the C9orf72 gene (C9Pos) have been described as presenting distinct features compared to the general ALS population (C9Neg). Here we aim to identify the phenotypic traits more closely associated with the HRE and analyze the role of the repeat length as a modifier factor in C9Pos patients.

Methods: We studied a cohort of 960 ALS patients, including 101 familial (FALS) and 859 sporadic cases (SALS). Motor phenotype was determined using the MRC scale, the lower motor neuron score (LMNS) and the Penn upper motor neuron score (PUMNS). Neuropsychological profile was studied using the Italian version of the Edinburgh Cognitive and Behavioral ALS Screen (ECAS), the Frontal Behavioral Inventory (FBI), the Beck Depression Inventory-II (BDI-II) and the State-Trait Anxiety Inventory (STAI). A two-step PCR protocol and Southern blotting were performed to determine the presence and the size of C9orf72 HRE, respectively.

Results: C9orf72 HRE was detected in 55/960 (5.7%) ALS patients, 19 of which were FALS and 36 SALS. C9pos patients showed a younger onset, higher odds of bulbar onset, increased burden of UMN signs, reduced survival, and higher frequency of concurrent dementia. Concerning cognitive phenotype, we found an inverse correlation between the HRE length and the performance at ECAS ALS-specific tasks ($p=0.031$). C9Pos patients also showed higher burden of behavioral disinhibition ($p=1.6x10-4$) and lower degree of depression ($p=0.015$) and anxiety ($p=0.008$) when compared to C9Neg cases.

Conclusion: Our study provides an extensive characterization of motor, cognitive and behavioral features of C9orf72-related ALS and, importantly, indicates that the C9orf72 HRE size may represent a modifier of the cognitive phenotype.

13. Synaptic proteomics reveal distinct molecular signatures of cognitive change and C9orf72 repeat expansion in the human ALS cortex.

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The two major hypotheses of ALS pathogenesis (dying-forward and dying-back) place synapses at their core. Furthermore, increasing evidence is bringing the focus ever closer on synaptic dysfunction as a central and possibly triggering factor in ALS. Despite this, we still know very little about the molecular profile of an ALS synapse. To address this gap we designed a synaptic proteomics experiment to assess a more complete and unbiased change in the synaptic proteome in the ALS brain. We isolated synaptoneurosomes from fresh-frozen post-mortem human cortex using a standard serial-filtration protocol. To minimise inter-individual differences we pooled samples from 18 ALS cases and 11 age/gender matched controls. We analysed samples from the primary motor cortex (BA4) and the dorsolateral prefrontal cortex (BA9) and stratified the ALS group into further experimental groups based on cognitive profile (ECAS score) and C9ORF72-RE status. This resulted in 10 experimental groups, allowing us to assess regional differences and the impact of cognitive decline and C9ORF72-RE on the synaptic proteome, using a 10-plex TMT-based proteomics approach. We identified almost 6000 proteins in our synaptoneurosomes and using a robust bioinformatics approach, validated the strong enrichment of synapses. Interestingly, we found more than 30 ALS-associated proteins at the synapse, including TDP-43, FUS, SOD1 and c9orf72. We identified almost 500 proteins with altered expression levels in ALS synapses (+/- 20% vs control), with region-specific changes highlighting proteins and pathways with intriguing links to neurophysiology and pathology. Stratifying the ALS cohort by their cognitive status revealed almost 150 ALSci-specific alterations in BA9, highlighting novel synaptic proteins that may go some way to explain the synaptic vulnerability in these patients. Stratifying by C9ORF72-RE status revealed 330 protein alterations in the C9ORF72-RE+ve group, with KEGG pathway analysis revealing strong enrichment for glutamatergic receptor signalling. We have validated some of these changes by western blot and at a single synapse level using array tomography imaging. In summary, we have generated the first unbiased proteomic screen of the human ALS synaptic proteome, revealing novel insight into this key compartment in ALS pathophysiology.

14. NanoString molecular barcoding of patient tissue identifies molecular signatures of clinical heterogeneity in C9orf72-ALS.

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Amyotrophic lateral sclerosis (ALS) exists on a pathogenetic disease spectrum with frontotemporal dementia (FTD), with patients sometimes experiencing elements of both conditions (ALS-FTSD). For mutations associated with ALS-FTSD, such as the C9orf72 hexanucleotide repeat expansion, the factors influencing where an individual may lie on this spectrum require further characterisation. Using digital pathology analysis and random forest modelling we have previously shown that microglial staining is predictive of C9orf72-ALS disease status, and that microglial activation is elevated in the language region (BA39) for language-impaired cases. Here we used NanoString molecular barcoding with a panel of 770 neuroinflammatory genes to interrogate this dysregulation at the level of gene expression. We identified 21 top hits for dysregulated neuroinflammatory genes in the motor cortex, with enrichment of microglial and inflammatory response gene sets in disease. Our analyses also revealed two distinct C9orf72-ALS subtypes, with inflammatory signatures that segregate with glial TDP-43 burden and language impairment. Validation was performed using BaseScope(TM) in situ hybridisation and immunohistochemistry. These data imply that distinct molecular signatures can be detected within well curated and deeply clinically phenotyped cohorts that could hold promise for future targeted therapies.

Thursday 2nd June

Session 4: 8.30 to 10.30: Genomics

15. Whole-genome sequencing reveals that variants in the Interleukin 18 Receptor Accessory Protein 3'UTR protect against ALS.

Chen Eitan(1†*), Aviad Siany(1†), Elad Barkan(2), Tsviya Olender(1), Kristel R. van Eijk(3), Matthieu Moisse(4,5), Sali M. K. Farhan(6,7), Yehuda M. Danino(1), Eran Yanowski(1), Hagai Marmor-Kollet(1), Natalia Rivkin(1), Nancy-Sarah Yacovzada(1,2), Shu-Ting Hung(8-10), Johnathan Cooper-Knock(11), Chien-Hsiung Yu(12,13), Cynthia Louis(12,13), Seth L. Masters(12,13), Kevin P. Kenna(3), Rick A. A. van der Spek(3), William Sproviero(14), Ahmad Al Khleifat(14), Alfredo Iacoangeli(14), Aleksey Shatunov(14), Ashley R. Jones(14), Yael Elbaz-Alon(1), Yahel Cohen(1), Elik Chapnik(1), Daphna Rothschild(2,15,16), Omer Weissbrod(2), Gilad Beck(17), Elena Ainbinder(17), Shifra Ben-Dor(17), Sebastian Werneburg(18), Dorothy P. Schafer(18), Robert H. Brown Jr(19), Pamela J. Shaw(11), Philip Van Damme(4,5,20), Leonard H. van den Berg(3), Hemali P. Phatnani(21), Eran Segal(2), Justin K. Ichida(8-10), Ammar Al-Chalabi(14,22), Jan H. Veldink(3), Project MinE ALS Sequencing Consortium, NYGC ALS Consortium and Eran Hornstein(1)

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The non-coding genome is substantially larger than the protein-coding genome but has been largely unexplored by genetic association studies. Here, we performed region-based rare-variant association analysis of >25,000 variants in untranslated regions of 6,139 amyotrophic lateral sclerosis (ALS) whole-genomes and those of 70,403 non-ALS controls. We identified Interleukin-18 Receptor Accessory Protein (IL18RAP) 3′UTR variants as significantly enriched in non-ALS genomes and associated with a five-fold reduced risk of developing ALS, and this was replicated in an independent cohort. These variants in the IL18RAP 3′UTR reduce mRNA stability and the binding of double-stranded RNA-binding proteins. Finally, the variants of IL18RAP 3′UTR confer a survival advantage for motor neurons because they dampen neurotoxicity of human iPSC-derived microglia bearing an ALS-associated expansion in C9orf72, and this depends on NF-κB signaling. This study reveals genetic variants that protect against ALS by reducing neuroinflammation and emphasizes the importance of non-coding genetic association studies.

16. Identifying genetic subtypes of amyotrophic lateral sclerosis using latent class analysis

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Background: Amyotrophic lateral sclerosis has a highly heterogenous clinical presentation and a multifaceted genetic basis in which increasing numbers of genes are reliably implicated. Current classifications of ALS do not readily translate to meaningful subgroups for treatment, nor do they reliably predict clinically important factors like survival. The problem of correct classification potentially affects every person with ALS, and is critical for the development of precision medicine and personalized treatments. Using large datasets of genetic and clinical data of patients from international initiatives (Project MinE and STRENGTH), we aimed to identify and characterise homogenous subgroups of patients using machine learning clustering methods.

Methods: Latent class analysis was applied to identify homogenous (latent) subpopulations within the Project MinE and STRENGTH datasets including genetic and clinical data. We consider key clinical variables such as sex, age of onset, diagnostic delay, site of onset, survival time. Genetic trends are examined in terms of common variation (via polygenic risk scores) and rare variants occurring in 36 genes linked to ALS. Initial analyses are conducted on independent sub-samples within Project MinE, drawn from the United Kingdom and the Netherlands, and subsequent analyses examine trends across the international Project MinE and Strength samples.

Results: In total, data of 17,215 patients from 13 European and North American countries were collected. The ALS subgroups we identify are discriminated primarily by diagnostic delay and we observe differences in the prevalence of key genetic factors across these subgroups. Subgroups present distinct progression trends and association with different clinical outcomes. The structure of the subgroups identified appear to be preserved across people with ALS sampled across the different countries represented in the dataset.

Conclusion: Homogenous subgroups of ALS identified using latent class analysis are preserved across countries. Distinct genetic and progression trends characterise these groups which may be indicative of relationships between the genetic architecture of ALS and clinical disease presentations.

17. Phenotype analysis of FUS mutations in ALS.

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Background. Mutations in Fused in Sarcoma (FUS) are among the most common genetic causes of ALS worldwide. They are supposedly characterized by a homogeneous pure motor phenotype with early-onset and short disease duration. However, a few FUS-mutated cases with a very late disease onset and slow progression have been reported.

Objective: To analyze genotype-phenotype correlations and identify the prognostic factors in FUS-ALS cases.

Methods. We identified and cross-sectionally analyzed 22 FUS-ALS patient histories from a single-center cohort of 2615 genetically tested patients and reviewed 289 previously published FUS-ALS cases. Survival

analysis was performed by Kaplan–Meier survival curves followed by log-rank test and multivariate Cox-analysis.

Results. Survival of FUS-ALS is age-dependent: in our cohort, early-onset cases had a rapid disease progression and short survival (p=0.000003), while the outcome of FUS-mutated patients with mid-to-late onset did not differ from non-FUS ALS patients (p=0.437). Meta-analysis of literature data confirmed this trend (p=0.00003). This survival pattern is not observed in other ALS-related genes in our series. We clustered FUS-ALS patients in three phenotypes: (a) axial ALS, with upper cervical and dropped-head onset in mid-to-late adulthood; (b) benign ALS, usually with a late-onset and slow disease progression, (c) juvenile ALS, often with the bulbar onset and preceded by learning disability or mild mental retardation. Those phenotypes arise from different mutations.

Discussion. We observed specific genotype-phenotype correlations of FUS-ALS and identified age at onset as the most critical prognostic factor. Our results demonstrated that FUS mutations underlie a specific subtype of ALS and enable a careful stratification of newly diagnosed FUS-ALS cases in terms of clinical course and potential therapeutic windows. This will be crucial in the light of incoming gene-specific therapy.

18. Genetic architecture of primary lateral sclerosis.

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Primary lateral sclerosis (PLS) is the rarest form of motor neuron disease (MND), a condition in which the nerve cells controlling movement degenerate and die. The symptoms include progressive weakness and stiffness of the legs, gradually affecting the arms and face. Although PLS does not typically shorten life dramatically, it greatly decreases the quality of life. So far, there is no known genetic cause of PLS. While PLS mostly develops in midlife, there is a juvenile form (JPLS), which occurs in childhood and young adults. JPLS is caused by mutations in a gene called ALS2. One of the biggest challenges faced by people with PLS is delayed diagnosis and misdiagnosis, since the initial symptoms can be similar to amyotrophic lateral sclerosis (ALS), the most common form of MND. In the absence of a concrete genetic test that differentiates PLS from other MNDs, this delay in diagnosis is inevitable. The aim of our study is to investigate the genetic causes of PLS by generating the first large scale genetic dataset of PLS patients. Through the international Project MinE initiative, we got access to the whole genome sequencing and clinical data of 82 PLS patients with “definite” diagnosis, 6550 ALS patients and 2444 controls. To our knowledge this is the largest cohort of PLS patient genomic data. The cohort consisted of equal ratio of male to female patients (41 each). Due to the rare nature of PLS we employed the candidate gene-based approach Variant calling. Our candidate genes included 154 genes that were previously associated with MND, obtained from AlSoD database and ERLIN2, PARK2, MMACHC, SYNE2 and TTBK2 genes which were previously associated with PLS. We identified known pathogenic variants in FIG4, FUS, SPG7 and SPG11 genes in four different PLS patients. In addition, we identified predicted pathogenic variants in ERLIN1 and UNC13A genes in two different PLS patients. We also investigated the role of pathogenic expansions in PLS and no C9orf72 pathogenic expansions were found in our PLS cohort. Finally, we observed that the mean age of onset was 59.05 years and 60.05 years for our PLS and ALS cohort, respectively. This contrasts with the previously reported mean age of onset of 50 years and 60 years between the PLS and ALS patients, respectively.

19. Genetic data of 10,996 ALS patients and 7,403 controls shows that missense variants in the tail domain of NEFH increase the risk of ALS.

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Introduction: The neurofilament heavy chain protein is implicated as a biomarker for ALS, however the role of NEFH gene variants is unclear. Several studies suggest that small deletions in the lysine-serine-proline (KSP) repeat segment of the tail domain might affect ALS risk, but this has not been robustly replicated. Understanding of the contribution of other types of NEFH gene variants to ALS susceptibility is also limited. Despite this, NEFH is part of most ALS sequencing panels. Objectives: To determine if genetic variants in the NEFH gene modify ALS risk by performing 1) a meta-analysis of genetic association studies of NEFH in ALS, and 2) a comprehensive investigation of NEFH variation in ALS using the Project MinE whole-genome sequencing data set (8,902 samples; 6,469 cases and 2,434 controls). Methods: Peer reviewed studies indexed in PubMed, Medline or Embase, published between January 1993 and October 2021 were included if they reported NEFH variant frequencies in people with ALS and controls using next-generation sequencing, microarray or PCR-based approaches. Random and fixed-effect meta-analysis was performed by the inverse variance weighting method. Burden analysis was performed using CMC, Madsen-Browning and SKAT-O methods and grouping variants by functional domain (tail, rod and head), and effect (e.g. missense, synonymous, frameshift, etc) and frequency in control databases (gnomAD non-neuro European (non-Finnish) and Project MinE controls). Results: Forty variants were identified from 12 case-control studies in a total of 9,496 samples (4,527 cases and 4,969 controls). Fixed and random effects meta-analysis found that rare (MAF<1%) tail missense variants increased ALS risk (Fixed: OR 3.94 (1.76-8.78) p=0.0008; Random: OR 4.93 (1.50-16.19), p=0.0085). A total of 591 rare variants, mostly novel (79.4%), were found in the Project MinE cohort. Burden analysis showed ultra-rare and rare tail domain variants as being associated with ALS (Ultra-rare: Madsen-Browning p=0.0240, OR 1.68; Rare: SKAT-O p=0.0366, OR 1.13), replicating the meta-analysis findings. Rod ultra-rare intronic variants (CMC p=0.0064, Madsen-Browning p=0.0040, SKAT-O p=0.0102, OR 1.37) were also enriched in cases. Conclusions: This study confirms that NEFH tail domain variants are associated with ALS risk. The results support the inclusion of NEFH in ALS genetic screening panels and provide important information for the genetic counselling of patients and their families.

20. Dissecting the pathogenic role of Ataxin-2 repeat expansions in ALS.

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Ataxin-2 (ATXN2) is a RNA-binding protein involved in RNA processing and metabolism that recent studies have identified as a risk factor for ALS. The ATXN2 gene contains repeated CAG sequences that generally consist of 22 repeats in healthy individuals. Intermediate repeat expansions (27-33) have been identified as an ALS-modifying factor. In order to study how ATXN2 intermediate repeat expansions affect the development and disease severity of ALS, we have generated transgenic mice carrying the human ATXN2 gene with either healthy (Q22) or ALS-associated (Q33) repeat expansions. In addition, we have obtained double mutant mice using a previously described ALS mouse model harbouring human mutant TDP-43 in combination with the ATXN2 gene. Double mutant mice carrying intermediate ATXN2 repeat expansions (Q33TDP) show a progressive reduction of motor function accompanied by Purkinje cell degeneration in the cerebellum. Whereas spinal cord motor neuron loss or TDP43 mislocalization are not observed in Q33TDP mice, the assembly of stress granules is impaired in Q33TDP motor neurons. RNA sequencing of whole spinal cords has revealed that Q33TDP mice have a distinct transcriptomic profile, including a downregulation of the inflammatory response and upregulation of oxidative phosphorylation. By using our Q33TDP mouse model and iPSC derived from ALS patients carrying ATXN2 intermediate repeat expansions, we have found that mitochondrial function is altered in Q33TDP motor neurons and ATXN2 iPSC-derived motor neurons. Interestingly, we also observe a change in Q33TDP microglia morphology. Furthermore, RNA sequencing of spinal cord isolated microglia shows DEGs related to phagocytosis. Finally, ATXN2 iPSC-derived organoid microglia have a higher expression of disease-associated microglial genes, some of which are also found in Q33TDP microglia. Altogether, our study shows for the first time that ATXN2 intermediate repeat expansions cause several ALS-relevant phenotypes, where mitochondria and microglial function are significantly impaired.

10.10- 10.30: Rapid Fire Posters (1of 2)

21. Understanding Disease Trajectory in Amyotrophic Lateral Sclerosis.

Ahmad Al Khleifat

King's College London

Background and methods: My research attempts to explain ALS phenotype through genotype, focussed on structural variation and disease progression, using bioinformatics as a means of bringing these elements together.

Results and contribution: I have shown that structural variation is a frequent event in ALS, and that an insertion in ERBB4 greatly predisposes to respiratory onset ALS, an otherwise rare phenotype (Al Khleifat, A. et al. npj Genom. Med. 2022). My findings suggest that respiratory onset ALS may be a specific genetic subtype, and potentially explains why progression is slower than expected in those with respiratory onset. I showed that longer telomeres are associated with ALS (Al Khleifat, et al. ALS & FTD 2019), replicating this across international cohorts, and that the same pattern of telomere elongation is seen in ALS brain tissue, despite the lack of mitosis in neurons. I investigated plasma NfL in 2311 individuals across 15 neurodegenerative diseases. Using the single-molecule array (SIMOA), I showed that NfL was significantly increased in all cortical neurodegenerative disorders, and particularly ALS and FTD. I further demonstrated that plasma NfL is clinically useful in detecting FTD in people with psychiatric disorders such as moderate and severe depression, and in identifying frontotemporal dementia in people with cognitive impairment (Ashton, N.J., Janelidze, S., Al Khleifat, A. et al.Nat Commun. I organised NEUROHACK; a 4-day competitive international hackathon in January 2022 at hubs in London and Los Angeles. Over 160 participants from six continents were selected to attend the event. As part of the hackathon, three pilot grants of £10,000 were awarded to NEUROHACK teams to continue to develop the most promising ideas, two of which were awarded to MND teams. I created the Trans-Ancestral Genetics working group that is part of the Project MinE consortium, with a focus on diversifying genetic research in ALS to include multi-ethnic populations and accurately represent ALS genetics-related disease risks in all populations.

Conclusions: I have explored the relationship between ALS phenotypes, disease progression, survival and genetics; developing and using various tools for measurement and assay of these parameters. My work has led to the discovery of a number of genetic factors that contribute to the risk of ALS with some being investigated further given their potential as drug targets for the development of new therapies.

22. Developing a systematic framework to identify, evaluate and report evidence for drug selection in motor neuron disease clinical trials.

Charis Wong (1-3)*, Alessandra Cardinali (1, 3, 4), Bhuvaneish T. Selvaraj (1-4), Rachel S Dakin (1-3), Neil Carragher (5), Suvankar Pal (1-3), Siddharthan Chandran (1-4), Malcolm Macleod (1) on behalf of the ReLiSyR-MND group.

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Despite decades of clinical trials, effective disease modifying treatment options for motor neuron disease (MND) remain limited. There is a pressing need to innovate how we identify and evaluate candidate drugs in clinical trials. Motor Neuron Disease - Systematic Multi-Arm Adaptive Randomised Trial (MND-SMART; ClinicalTrials.gov number: NCT04302870) is an adaptive platform trial aimed at testing a pipeline of candidate drugs in a timely and efficient manner. We aim to develop a systematic and structured framework to identify, evaluate and prioritise candidate drugs for evaluation in MND-SMART, taking into consideration emerging data in different domains and expert opinion. Currently, the domains incorporated in our workflow include (i) published literature through Repurposing Living Systematic Review-Motor Neuron Disease (ReLiSyR-MND), a machine-learning assisted systematic review evaluating clinical studies of MND and other neurodegenerative diseases which may share similar pathological pathways, animal in vivo MND studies and in vitro MND studies; (ii) unbiased in vitro high throughput drug screening; (iii) pathway and network analysis, and (iv) pharmacological, feasibility and clinical trial data by mining drug, chemical and clinical trial registry databases. We compile an integrated list of candidate drugs including drugs which have been described in at least one clinical publication, positive hits in any of our in-house MND in vitro assays, and drugs which target pathways and networks of interest. For each drug, we obtain predictions on blood brain barrier permeability from admetSAR (http://lmmd.ecust.edu.cn/admetsar2/). Next, we generate a list of prioritised drugs deemed suitable for imminent repurposing in MND-SMART, taking into consideration availability in oral formulation, prescription-only medicine status in the British National Formulary, and evaluation of safety profile by clinical triallists in the context of common comorbidities. We further identify, evaluate, and synthesise evidence across the different domains for prioritised drugs and report these using automated workflows as interactive, curated, living evidence summaries. These summaries can be used to inform expert panel discussions on drug selection for future arms of MND-SMART at trial adaptation epochs.

23. Genome-wide assessment of genetic modifiers in ALS progression.

Ramona Zwamborn*, Michelle de Groot , Project MinE sequencing consortium, Wouter van Rheenen , Jan Veldink

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by dysfunction and subsequent loss of cortical and spinal motor neurons (MN). Previous studies demonstrated that alterations in synapses and neuronal activity are part of the underlying pathomechanisms in both in vitro and in vivo models, but their contribution to neuronal death is still under debate. Specifically, the influence of hyper- or hypoactivity on cellular disease progression is highly controversial since both phenotypes have been described and found to be harmful in ALS MN. In this study, we employed high definition multielectrode array (HD-MEA) techniques to longitudinally monitor the electrophysiological properties of hiPSC-derived C9orf72-mutant MN. We found an early hyperactivity of ALSC9orf72 MN, which drastically decreased upon aging while neurodegeneration started to occur. In accordance with previous findings of synaptic alterations in ALS MN, we could also observe a generally reduced network synchroneity in ALSC9orf72 MN cultures. Next, we performed a longitudinal transcriptomic analysis to elucidate the molecular causes triggering the loss of activity properties in mutant neurons. Consistent with our HD-MEA findings, we observed an up-regulation of synaptic transcripts in ALSC9orf72 MN at the earlier time point, which was followed by a significant reduction over time. By administration of the SK channel inhibitor Apamin, which has previously been shown to be neuroprotective in ALS MN, we were able to achieve beneficial effects on an electrophysiological as well as transcriptional level. Altogether, this study suggests phenomena of synaptic maturation as possible explanation for contradicting evidence on electrophysiological alterations in ALSC9orf72 MN, provides an insight into the longitudinal development of their neuronal activity and links these functional changes to aging-dependent transcriptional programs.

24. The Sustained Attention to Response Task evokes sensorimotor beta ERD/ERS and enables quantification of motor and cognitive pathophysiology.

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Objective: To identify sources generating cortical oscillations during performance of the sustained attention to response task (SART) and identify sources of their abnormalities in amyotrophic lateral sclerosis (ALS).

Methods: A randomised SART was undertaken by 30 ALS patients and 40 controls during 128-channel electroencephalography. Linearly constrained minimum variance (LCMV) beamforming was applied to localise sources of event-related (de)synchronisation (ERD/ERS) associated with performing the SART. We investigated relationships between these oscillations at specific cortical sources and measures of task performance and motor, cognitive and behavioural change in ALS.

Results: During correct Go responses, beta ERD and subsequent beta ERS were deemed to primarily originate from the sensorimotor (SM1) and posterior parietal cortices (PPC), more so in the left hemisphere (contralateral to the hand used to perform the task). During correct response withholding (NoGo), beta ERD demonstrated similar origins, but beta ERS was less potent in the left SM1 and PPC. Both beta ERD and beta ERS in the left SM1 and PPC showed significant correlations to SART response time and accuracy measures. ALS patients showed greater anticipation and poorer Go trial response accuracy compared to controls. ALS patients also demonstrated lesser beta ERD in the left PPC during Go trials and the left SM1, PPC and fusiform cortex during NoGo trials. Lower beta ERS in ALS originated from these and additional sources, including the left temporal pole and medial and lateral frontoparietal regions. Theta ERS and alpha ERD were predominantly localised to sensation and cognition-associated regions and also showed significant correlations to SART performance measures, with theta ERS uniquely relating to task performance in ALS patients.

Conclusions: The SART evokes beta ERD/ERS characteristic of established sensorimotor activation and inhibition measures, alongside theta and alpha ERD/ERS in non-motor domains, enabling dissection and quantification of normal, compensatory and pathological motor and cognitive cortical physiology in ALS.

25. Profiling brain morphologic features of motor neuron disease caused by TARDBP mutations: an MRI-based study.

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Objective: Mutations in the TARDBP gene are a rare cause of genetic motor neuron disease (MND). Morphologic MRI features of MND patients carrying this mutation have been poorly described in literature. Our objective was to investigate distinctive clinical and MRI features of a relatively sized sample of MND patients carrying TARDBP mutations.

Methods:. 11 MND patients carrying a TARDBP mutation were enrolled. 11 patients with sporadic MND (sMND) matched by age, sex, clinical presentation and disease severity were also selected, along with 22 healthy controls. Patients underwent clinical and cognitive evaluations, as well as 3D T1-weighted and diffusion tensor (DT) MRI sequences on a 3 Tesla scanner. GM atrophy was first investigated at a whole-brain level using voxel-based morphometry (VBM). GM volumes of 90 Automated Anatomical Labeling (AAL) regions of interest were also obtained. Lastly, tractography was performed to obtain mean DT MRI metrics of the corticospinal tracts (CST). Clinical, cognitive and MRI features were compared between groups using age- and sex-adjusted ANOVA models, Bonferroni-corrected for multiple comparisons.

Results: Compared with sMND, TARDBP patients showed a trend toward faster disease progression rate (p=0.056). Compared with controls, GM volume loss on VBM was greater in TARDBP patients at the level of the right lateral parietal cortex. A significant reduction of GM volumes was found in the left precuneus and right angular gyrus of TARDBP patients, compared to controls (p=0.002), whereas sMND showed GM volumes comparable with those of controls. At tractography, the left CST showed increased radD in both sMND (p=0.043) and in TARDBP compared to controls, even if the latter did not reach statistical significance (p=0.059). The right CST instead showed decreased FA in only in TARDBP patients, compared to controls (p=0.044).

Conclusions: TARDBP patients showed a distinctive parietal pattern of cortical atrophy and a greater damage of the CST compared with controls, that sMND patients matched for disease severity and clinical presentation were lacking. This would suggest that TDP-43 pathology may spread more rapidly in TARDBP mutation carriers, causing greater morphologic alterations in both grey and white matter. Study funding. Italian Ministry of Health (GR-2011-02351217; GR-2013-02357415; RF-2011-02351193), AriSLA (ConnectALS), and European Research Council (StG-2016_714388_NeuroTRACK).

26. Dysregulation of extracellular vesicle formation and release in astrocytes from ALS patients.

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Introduction: In ALS, astrocytes exhibit a toxic phenotype that mediates motor neuron degeneration. In vitro, astrocytes exhibit toxicity through both cell-to-cell contact and secreted factors, thus indicating that inter-cellular communication is dysregulated. Extracellular vesicles (EVs) represent one of many modes of communication between astrocytes and neurons. The 4G2C hexanucleotide repeat expansion in the gene C9orf72 represents the most common genetic cause of ALS. Work published by our group shows that astrocytes generated from C9orf72-ALS patient fibroblasts have impaired secretion of EVs, that mediate neuronal toxicity compared to healthy control astrocytes. More recently C9orf72 has been reported to interact with proteins associated with endosomal trafficking and EV secretion.

Aim: We aim to determine which part of the EV formation and secretion pathway is impaired in C9orf72-ALS patient derived astrocytes.

Methods: Human induced astrocytes were generated from three unaffected individuals and three individuals carrying the hexanucleotide repeat expansion in the C9orf72 gene. The protein levels of early and late endosomal markers were measured via western blotting. The morphological characterisation of early endosomes was conducted using confocal microscopy, whilst multivesicular bodies and lysosomes were manually quantified blinded based on the morphological characteristics using electron microscopy.

Results: We found that C9orf72-ALS astrocytes display an increase (fold-change 1.5) in the early endosomal regulator protein EEA1 compared to astrocytes derived from healthy controls. This increase is accompanied by early endosome morphological changes that we are currently quantifying whilst blind using confocal microscopy. Consistent with the potential accumulation of early endosomes and the inability to transition to late endosomes, C9orf72-ALS astrocytes display a reduction in the number of multivesicular bodies. Interestingly, this phenotype is also accompanied by clustering and accumulation of lysosomes, indicating that multiple blockage points might be present in vesicle formation and degradation.

Conclusion: Our findings provide evidence that the phenotype of reduced EV secretion that we have previously observed may be the result of increased trafficking towards the lysosomal compartment and reduced maturation of early endosomes into multivesicular bodies.

Session 5: 11.00 to 12.30: Cell & Molecular Biology (1of 2)

27. ALS-causing KIF5A mutant proteins form aggregates.

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Heterozygous mutations in KIF5A was found to lead to ALS, extending the list of genes linked with this disorder (Brenner et al., 2018; Nicolas et al., 2018). The ALS-causing KIF5A mutations are clustered in a narrow hot spot, in or close to exon 27. These mutations either affect the splicing of exon 27 or are deletions within exon 26 or exon 27. Therefore, the normal stop codon is lost and a downstream sequence in the 3'-UTR is recognized as a novel stop codon, resulting in aberrant transcripts with a frameshift that is predicted to give rise to slightly elongated proteins with always the same new 40-amino acid peptide at the C-terminus. To evaluate how these KIF5A mutations differ from the wild type on molecular level to cause pathology, we cloned wild type and two KIF5A mutants (c.2993-1G>A and c.3019A>G). Overexpression of the constructs in HEK293 cells resulted in cytoplasmic distribution of wild type KIF5A, whereas aggregate-like structures appeared in both mutants a) upon the direct observation of EGFP-tagged KIF5A constructs and b) when using a commercial KIF5A antibody recognizing both wild-type and mutant proteins. These aggregate-like structures did not colocalize with a certain cellular component such as ER, Golgi, mitochondria, or early endosomes. While TDP-43 localization was not affected by mutant KIF5A, p62 and ubiquitin were partially and occasionally colocalized with the aggregate-like structures. Moreover, we confirmed the altered distribution of mutant proteins by subcellular fractionation and subsequent immunoblotting. Finally, by co-expression of wild type and mutant proteins, we found that mutant KIF5A proteins lead to aggregation of wild type protein as well, suggesting a dominant negative role of the mutations on the disease.

28. HNRNPK counteracts DNA damage as part of RNA toxicity in C9orf72 ALS/FTD.

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The 'GGGGCC' repeat expansion in C9orf72 is the most common cause of ALS and FTD. 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/FTD has not been elucidated yet. In particular, RNA toxicity is not fully understood. In this study, a C9orf72 zebrafish model was used to identify and investigate HNRNPK as a modifier of RNA toxicity. Using zebrafish, iPSC-derived MNs and postmortem material, we found that a loss-of-function of HNRNPK is associated with C9 ALS/FTD. In C9 ALS/FTD patient tissue, we discovered an increased nuclear translocation, but reduced expression of a downstream target of HNRNPK involved in the DNA damage response. Our findings confirm that both HNRNPK and its target are involved in DNA damage and its repair. Finally, we show that increasing the expression of HNRNPK was sufficient to mitigate DNA damage in our C9 RNA toxicity zebrafish model. Overall, our study strengthens the relevance of RNA toxicity as a pathogenic mechanism in C9 ALS and demonstrates its link with an aberrant DNA damage response, opening novel therapeutic strategies for C9 ALS/FTD.

29 Updates on seeding studies: SOD1 prions transmit aggregation and fatal ALS-like disease – Introducing Strain C.

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Evolving evidence suggest the presence of propagating prion-like species in several types of neurodegenerative diseases associated with misfolding of host-encoded non-mutated proteins. Among these are AD, PD, the tauopathies, HD and ALS. A hallmark of prions is the presence of different conformational aggregate strains with different biological activities. Another characteristic of prions is that subsequent prion passage within the same host can lead to a shorter incubation period. In ALS, using binary epitope mapping (BEM) we identified two structurally different strains of mutant hSOD1 aggregates (named A and B) in the CNS of Tg mice models expressing full-length hSOD1 variants. When seeded into spinal cords of adult hSOD1G85R Tg mice, these strains transmit exponentially propagating templated hSOD1 aggregation selectively in the motor system with concomitant development of muscle wasting and paresis. To further investigate prion-like properties of hSOD1 strains in ALS, and explore the spreading characteristic of the disease in vivo, stereotaxic inoculation of hSOD1 aggregate strains into spinal cords of pre-symptomatic Tg-hSOD1D90A mice were performed. This mutation is essentially wt-SOD1 like. Normally, Tg-hSODD90A only develop MND phenotype when the transgene is homozygous. Mice hemizygous for the hSOD1D90A transgene insertion do not spontaneously develop ALS pathology and have a normal murine lifespan (>700d). First & second passage studies were performed to investigate further prion-like properties of hSOD1. Inoculations of strain A or B seeds into the lumbar spinal cord of 100-day-old hemizygous hSOD1D90A mice induced progressive hSOD1 aggregations and premature fatal ALS-like disease after ≈250 and ≈350 days, respectively. BEM analysis of aggregates in the terminal stage lead to a surprise discovery: Inoculation of strain A into hemizygous hSOD1D90A mice gave rise to a new strain named C, which has the C-terminal end apparently recruited to the aggregate core. Second passage inoculations were then performed in hemizygous hSOD1D90A mice, using spinal cord homogenates with strain C aggregates as seeds. The novel prion strain was much more efficient, and transmitted progressive hSOD1 aggregation and ALS-like disease which was fatal 100 days after inoculation. We provide further evidence of the similarities between hSOD1 and the prion protein. Our data suggest that mutations in SOD1 are inducing aggregation and ALS pathology via a prion mechanism.

30. Defective cyclophilin A induces TDP-43 proteinopathy: implications for amyotrophic lateral sclerosis and frontotemporal dementia.

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Background: Aggregation and cytoplasmic mislocalization of TDP-43 are pathological hallmarks of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) spectrum. However, the molecular mechanism by which TDP-43 aggregates form and cause neurodegeneration remains poorly understood. Cyclophilin A, also known as peptidyl-prolyl cis-trans isomerase A (PPIA), is a foldase and molecular chaperone. We previously found that PPIA interacts with TDP-43 and governs some of its functions. PPIA deficiency accelerates disease in a mouse model of ALS and its reduction correlates with a worse disease phenotype in ALS patients. Here we investigated PPIA function as a player of TDP-43 pathology.

Methods: We characterized PPIA knock-out mice (PPIAko) throughout their entire lifespan and performed MRI analysis, histology, electrophysiology, cognitive and motor tests, evaluation of neuroinflammation and TDP-43 pathology. We screened ALS patients for coding, non-synonymous and loss-of-function SNPs in the PPIA gene. We performed molecular dynamics (MD) simulations of PPIA structures. We studied function of PPIA mutants in vitro.

Results: PPIAko mice develop a neurodegenerative disease with key behavioural features of FTD, marked TDP-43 pathology and late-onset motor dysfunction. In the mouse brain, deficient PPIA induces mislocalization and aggregation of the GTP-binding nuclear protein Ran, a PPIA interactor and a master regulator of nucleocytoplasmic transport, also for TDP-43. Moreover, in absence of PPIA, TDP-43 autoregulation is perturbed and TDP-43 and proteins involved in synaptic function are downregulated, leading to impairment of synaptic plasticity. Finally, we found that PPIA was downregulated in several ALS and ALS-FTD patients and identified a PPIA loss-of-function mutation in a sporadic ALS patient. The mutant PPIA has low stability, altered structure and impaired interaction with TDP-43.

Conclusions: Our findings indicate that PPIA is involved in multiple pathways that protect CNS from TDP-43-mediated toxicity. In fact, if deficient and/or dysfunctional PPIA can trigger TDP-43 proteinopathy leading to neurodegeneration. PPIAko mice recapitulate key features of ALS-FTD and are useful experimental model to investigate the mechanisms of TDP-43 proteinopathy, with the aim of developing novel therapeutic approaches.

31. iPSC-derived motor neurons from C9orf72 ALS/FTD-patients display defects in lysosomal function and homeostasis.

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Amyotrophic lateral sclerosis or ALS is an adult-onset progressive neurodegenerative disease caused by the selective death of both upper and lower motor neurons (MNs) (1). An abnormal hexanucleotide repeat expansion (HRE) in chromosome 9 open reading frame 72 (C9orf72) is the most common cause of ALS and frontotemporal dementia (FTD) (2). Though clinically distinct, ALS, FTD, and many other neurodegenerative diseases (NDs) are all characterized by the accumulation and aggregation of (misfolded) proteins. This might not be surprising since aging, which is the major risk factor in all NDs, is directly associated with a decline in cellular proteostasis. In addition, neurons are extremely long and highly polarized cells that are vulnerable to this age-associated decline of the cellular protein quality control system (3). Since many ALS/FTD-causing mutations induce protein stress, we aim to better characterize possible defects in the autophagy-lysosome pathway (4). Our lab recently identified defects in axonal transport, a cellular process pivotal for the correct function of the autophagy-lysosome pathway (5). In this study, we generated MNs derived from iPSCs bearing a C9orf72 HRE mutation as well as from their CRISPR-Cas9 isogenically controlled counterparts. In addition to the mitochondrial transport defects, we identified dysfunctions in lysosomal motility in the C9orf72 mutant motor neurons as an early factor in disease pathogenesis. Moreover, in both iPSC-derived motor neurons and post mortem patient samples, several signs for dysregulation of the autophagy-lysosome pathway are present. Finally, we used antisense oligonucleotides (ASOs) that silence the C9orf72 HRE or the C9orf72 promotor region and evaluated their therapeutic effect on the identified phenotypes.

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Session 6: 13.30 to 14.55 Cell and Molecular Biology (2 of 2)

32. Different cellular environments shape TDP-43 function with implications in neuronal and muscle diseases.

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TDP-43 aggregation and redistribution are recognised as a hallmark of amyotrophic lateral sclerosis and frontotemporal dementia. However, TDP-43 inclusions have recently been described in the muscle of inclusion body myositis patients and TDP-43 alterations have been reported in the neurons of patients affected by Niemann Pick C (NPC), an autosomal recessive disorder due to NCP1 gene mutations that is characterized by cholesterol abnormal accumulation with visceral and neurological symptoms, especially cerebellar ataxia. All these observations, highlight the need to understand the role of TDP-43 beyond the central nervous system and the ALS/FTD spectrum. Taking advantage of RNA-seq, we have recently directly compared TDP-43-mediated RNA processing in various model systems that include muscle and neuronal cell lines. Using this approach, we have been able to identify a potentially new important modifier of ALS/FTD disease, Nitric Oxide Synthase 1 Adaptor Protein (NOS1AP), whose mRNA is a direct TDP-43 target and whose modulation alone can rescue TDP-43 pathology in flies. In primary mouse cortical neurons, we have shown that TDP-43 mediated downregulation of NOS1AP expression strongly affects NMDA-receptor signaling pathway. In human patients, its downregulation strongly correlates with TDP-43 proteinopathy as measured by cryptic STMN2 and UNC13A exon inclusion. Conversely, using a human NPC cellular model we have found that the most promising target which could explain TDP-43 alterations consisted of ITPR1 (inositol 1,4,5-trisphosphate), a gene able to mediate the calcium release from the endoplasmic reticulum (ER). Interestingly, mutations in this gene have already been reported in patients affected by Spinocerebellar Ataxia 15/29 and in our validation experiments, silencing of ITPR1 in SH-SY5Y cell lines confirmed that its knockdown could promote TDP-43 cytoplasmic localization and increased phosphorylation. Taken together, our investigations show that TDP-43 displays a disease-characteristic behaviour targeting unique transcripts in each cell-type. This is presumably due to characteristic expression of RNA-binding proteins, especially hnRNPs, which influence TDP-43's performance and define cell-type specific RNA processing.

33. Why should we care about astrocytes in a motor neuron disease?

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ALS is defined as a motor neuron disorder. During disease progression, motor neurons within the brain and spinal cord die, which causes progressive muscle wasting and eventually death of patients. This we know. But what about the non-neuronal cell population? Increasing attention to the glial effects in ALS is revealing a larger and larger involvement of these 'supporter cells' and this sparks the interest for novel therapeutic entry points. In this study, we investigated the astrocyte contributions to ALS in the context of FUS mutations. Using two FUS-ALS patient-derived induced pluripotent stem cell (iPSC) lines carrying the mutations R521H and P525L, respectively, we generated FUS-ALS astrocytes and compared these cells to their individual CRISPR-Cas9 gene-edited isogenic control counterparts. We found a FUS-ALS-mediated dysregulation of astrocyte homeostasis characterized by calcium hyperactivity, mutation-dependent transcriptional changes, increased reactivity, and emission of pro-inflammatory cytokines. Next, we integrated our astrocytes in our previously established human motor unit microfluidics model comprised of iPSC-derived motor neurons and human primary myoblast-derived myotubes, and evaluated the astroglial effects on human neuromuscular junctions (NMJ). Interestingly, FUS-ALS astrocytes appeared toxic to the motor neuron network, compromised the motor neuron-neurite outgrowth as well as NMJ formation and functionality. Isogenic control astrocytes were able to alleviate or rescue all of these toxic impairments. Diving further into the mechanism of toxicity, we uncovered a striking functional heterogeneity through a dynamic interplay between 1) loss-of-support mechanism through downregulation of multiple genetic pathways involved in maintenance of optimal neuronal homeostasis, and 2) gain-of-toxicity mechanism via increased astrocyte reactivity and cytokine secretion. Finally, we found that FUS-ALS astrocytes unsuccessfully attempt to counteract this toxicity through upregulation of the WNT/β-catenin pathway in motor neurons. In line with the growing interest in the effects of non-neuronal cells in ALS, our data demonstrate a central role of astrocytes in this neurodegenerative disorder.

34. Cell-autonomous immune dysfunction driven by disrupted autophagy in C9orf72-ALS microglia contributes to neurodegeneration.

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The most common genetic mutation found in familial and sporadic amyotrophic lateral sclerosis (ALS), as well as fronto-temporal dementia (FTD), is a repeat expansion in the C9orf72 gene. C9orf72 is highly expressed in human myeloid cells, and although neuroinflammation and microglial pathology are widely found in ALS/FTD, the underlying mechanisms are poorly understood. Here, using human induced pluripotent stem cell-derived microglia-like cells (hiPSC-MG) harbouring C9orf72 mutation (mC9-MG) together with gene-corrected isogenic controls (isoC9-MG) and C9ORF72 knock-out hiPSC-MG (C9KO-MG), we show that reduced C9ORF72 protein is associated with impaired phagocytosis and an exaggerated inflammatory response upon stimulation with lipopolysaccharide, driven by sustained activation of NLRP3 inflammasome and NF-□B signalling. Analysis of the hiPSC-MG C9ORF72 interactome revealed an association of C9ORF72 with key regulators of autophagy, a process involved in the homeostatic regulation of the innate immune response. We found impaired initiation of autophagy in C9KO-MG and mC9-MG. Furthermore, through motor neuron-microglial (MN-MG) co-culture studies, we identified that autophagy deficit in mC9-MG led to increased vulnerability of C9 MNs to excitotoxic stimulus. Pharmacological activation of autophagy ameliorated the sustained activation of NLRP3 inflammasome and NF- B signalling, reversed the phagocytic deficit found in mC9-MG and also reduced MN death in MN-MG co-cultures. We validated these findings in blood-derived macrophages from people with C9orf72 mutation. Our results reveal an important role for C9ORF72 in regulating microglial immune homeostasis and identify dysregulation in human myeloid cells as a contributor to neurodegeneration in ALS/FTD.

35. Astrocyte-induced DNA damage as a mechanism of motor neuron death in ALS.

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Astrocytes derived from sporadic and familial ALS patients and models have been consistently shown to be toxic to motor neurons. ALS astrocyte toxicity is transmitted to motor neurons through conditioned medium, but the mechanisms and molecules involved remain elusive. Several studies have shown increased DNA damage as a consistent feature of sporadic and familial ALS. Our data shows that astrocytes derived from familial ALS patients carrying a repeat expansion in the C9ORF72 gene can induce DNA damage in healthy mouse motor neurons. It was hypothesised that astrocyte-induced DNA damage could contribute to motor neuron death in ALS.

To test this, induced astrocytes (iAstrocytes), which retain hallmarks of ageing, were obtained from sporadic ALS (n=3), C9ORF72-ALS (n=3) and SOD1-ALS (n=2) patient and healthy control (n=3) fibroblasts, and the conditioned media was used to treat healthy human iPSC-derived motor neurons.

It was found that conditioned media from C9ORF72-ALS and sALS iAstrocytes, but not SOD1-ALS iAstrocytes, could induce a significant increase in DNA damage response activation, measured as γH2AX nuclear activation (sALS vs control p=0.03; C9 vs control p=0.007), in treated motor neurons within 24 hours. DNA damage response activation preceded cell death, as a significant increase in activated caspase-3 was only observed in motor neurons 72 hours after treatment. Importantly, we demonstrate through DNA damage repair (DDR) kinetic studies that motor neurons treated with ALS astrocyte conditioned medium do not seem to activate the cascade of DDR events downstream of γH2AX. ALS astrocyte conditioned medium treatment does lead to a significant increase in p62 accumulation in motor neurons (p=0.0015), which may contribute to the observed absence of DNA repair factor activation.

In conclusion, our work identifies DNA damage as a possible mechanism by which ALS astrocytes induce motor neuron death. C9ORF72-ALS dipeptide repeat proteins have previously been shown to induce DNA damage when transfected into cells and present a compelling candidate for the cause of C9ORF72-ALS iAstrocyte-induced DNA damage. Furthermore, p62 was identified as potentially involved in astrocyte-induced DDR impairment and we will investigate whether blocking astrocyte-induced increase in p62 modifies the motor neuron response and has therapeutic potential. We would like to thank the individuals who donated skin biopsies and the funders, the MRC and Astrazeneca.

36. Involvement of inhibitory neurons in amyotrophic lateral sclerosis and frontotemporal dementia linked to Fused in Sarcoma protein.

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Mutations in the Fused in Sarcoma (FUS) gene, encoding for a ubiquitous and multifunctional DNA/RNA-binding protein, cause the most severe forms of amyotrophic lateral sclerosis (ALS), particularly when the nuclear localisation signal (NLS) of FUS is truncated. This truncation leads to the cytoplasmic mislocalisation of the protein, which is also observed in ALS and frontotemporal dementia (FTD) patients devoid of FUS mutations. Knowing that pathogenic mechanisms linked to FUS mislocalisation and the potential role of FUS in either ALS or FTD are yet to be understood, our laboratory generated a mouse model displaying a constitutive and ubiquitous NLS deletion. This effectively induced a cytoplasmic delocalisation of FUS and mildly progressive motor impairments (Scekic-Zahirovic et al., 2016 and 2017). More recently a cortical hyperactivity was highlighted in these mice. This was associated with molecular and ultrastructural alterations of GABAergic synapses, together with FTD-like behavioural dysfunctions (Sahadevan et al., 2021; Scekic-Zahirovic et al., 2021). The aim of my PhD is then to understand the contribution of inhibitory neurons in FUS-ALS and FUS-FTD. In order to conduct my project, we developed two new mouse models using a Cre-Lox recombination technology and mice in which the Cre recombinase expression is restricted to vesicular GABA transporter (VGAT)-positive neurons. (1) By crossing these mice with mice in which Fus can be truncated upon recombination, we obtained mice in which Fus is mutated solely in inhibitory neurons. In parallel, (2) by crossing these mice with mice displaying a constitutive NLS deletion which can be rescued to the wild type situation upon recombination, we truncated Fus in every cell type except inhibitory neurons. With histological studies we validated the recombination efficiency and are investigating the potential effects on neurons degeneration and pathological hallmarks appearance. These models are now being characterised for behavioural outcomes to determine if Fus truncation in inhibitory neurons is (1) sufficient and/or (2) necessary to induce ALS and FTD-like symptoms.

Session 7: 15.20 to 17.00: Drug Discovery

37. Modulation of TDP-43 by TTBK1 inhibitors: A new therapeutic approach for Amyotrophic Lateral Sclerosis and others TDP-43-pathies.

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The discovery of an effective treatment for neurodegenerative diseases, in particular ALS is probably among the most important challenges that the scientific community needs to achieve. Innovative drug design and development may fulfill this goal in well oriented target-based programs.TDP-43 is the main proteinopathy presents in ALS, being the modulation of this protein one of the main therapeutic most pursued therapeutic approaches of our research [1]. Being our main goal the discovery of small molecules able to avoid posttranslational modifications of this nuclear protein, we have investigated efforts on different protein kinases inhibitors [2]. That is the case of TTBK1, a CNS-specific kinase recently involved in TDP-43 phosphorylation [3]. Using a chemical genetic approach, we have discovered some new leads that have been optimized by medicinal chemistry programs to obtain good candidates [4]. These compounds are able not only to decrease TDP-43 phosphorylation in vitro, including immortalized lymphocytes from ALS patients, but also to recover the nuclear localization of this protein. Our most advanced compound has also shown an excellent pharmacokinetic profile with high CNS penetration in vivo. Moreover, after its chronic administration to a Tg-TDP-43 murine model, a significant reduction of TDP-43 phosphorylation in spinal cord is observed which may be associated to the motorneuron preservation and immunomodulation produced by our TTBK1 inhibitor [5].Based on these recent data, we may postulate TTBK1 inhibitors as a new and potential pharmacotherapy for ALS that merits to be developed in clinical trials.

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16.00- 16.20: Rapid Fire Posters (2 of 2)

38. In ALS dysfunction of nucleoporin 107 impairs autophagy contributing to TDP-43 aggregation.

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Nucleocytoplasmic communication is altered in ALS. Nucleoporins (NUPs)are essential components of this transport. Previous results indicate that NUPs homeostasis may be changed in ALS. This work has studied the potential relationship between protein aggregation (using TDP-43 as a paradigm), NUPs subcellular distribution, and cellular stress response. To do this, we analyzed the levels of several NUPs by immunodetection techniques in isolated tissues and nuclei extracted post mortem from ALS patients and a transgenic murine model of ALS in several stages of the disease and both genders. In addition, we performed cell culture studies to elucidate the possible mechanisms that influence NUPs-mediated TDP-43 dysregulation. The results demonstrate changes in the levels of NUPs involved in recognizing transporter proteins in both post-mortem tissues from ALS patients and in mice showing MN demise. On the other hand, the silencing of one of the NUPs, NUP107, caused an increase in the levels of TDP-43 and its phosphorylation and an increase in the formation of its cytoplasmic aggregates. In addition, this was associated with autophagic response alterations, evidenced by the rise of LC3II, p62, and the levels of general protein ubiquitination. Similarly, oxidative stress and osmotic stress in vitro caused an increase in the pathological characteristics of TDP-43 mentioned above, an increase associated with changes in the expression of NUPs and their cellular distribution. These findings demonstrate that the deterioration of NUPs in the ALS framework may contribute to the alteration of intracellular traffic resulting in the aggregation of the protein involved in motoneuronal neurodegeneration, such as TDP-43.

39. Senescent astrocytes drive neurodegeneration via extracellular vesicles in ALS-FTD.

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ALS is a non-cell autonomous neurodegenerative disease characterized, among other factors, by altered intercellular communication and nucleocytoplasmic transport defects. Glial cells contribute to ALS pathology and play a key role in the progression of the disease, but we still lack a thorough understanding of the underlying molecular mechanisms. Cell cycle dysregulation and senescence are gaining increasing attention in the field of neurodegenerative diseases. In this context, a recent publication in Cell has identified p53, a transcription factor involved in cell cycle regulation, as a driver of neurodegeneration in several C9orf72 ALS models. In the last years, our lab has been investigating the molecular mechanisms of glia-to-neuron miscommunication in a model of ALS. One of the possible ways in which cells communicate with each other is through extracellular vesicles (EVs), nanoparticles that transport proteins, lipids, and nucleotides from one cell to the other. Using transgenic mice expressing mutant TDP-43 Q331K and RNA sequencing, we observed that EVs derived from mutant astrocytes are sufficient to induce DNA damage and death in wild-type (WT) neurons. We analyzed the proteome of EVs derived from WT and mutant astrocytes by mass spectrometry and found that the protein cargos differ significantly between control and disease condition. Interestingly, many differentially enriched proteins are targets of c-myc, a transcription factor that stimulates cell cycle progression. Importantly, c-myc protein nuclear localization and transcriptional activity are downregulated in TDP-43 Q331K astrocytes compared to WT astrocytes. Accordingly, TDP-43 Q331K astrocytes show reduced proliferation in vitro and increased senescence-associated beta-galactosidase (SA-beta-gal) compared to WT astrocytes. Finally, transgenic flies with specific c-myc silencing in the glia have markedly reduced locomotor ability and survival compared to normal flies, suggesting that prematurely senescent glial cells might participate in neurodegeneration.

40. Dynamic Expression Profiles of Stressed iPSC-MNs by Translating Ribosome Affinity Purification (TRAP) from C9orf72-ALS Patients.

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Introduction: The G4C2 Hexanucleotide Repeat Expansion (HRE) in the gene C9orf72 is the commonest genetic cause of Amyotrophic Lateral Sclerosis (ALS). The intronic repeat RNA accumulates intracellularly as RNA foci and is translated non-canonically into dipeptide repeat proteins, both of which have been shown to affect RNA metabolism. Furthermore, impaired global translation and perturbed stress granule dynamics have been associated with C9orf72-ALS. We hypothesize that the C9orf72 HRE leads to differences in translating mRNAs (translatome) in motor neurons at baseline, after transient oxidative stress or recovery.

Methods: In this study, we captured the translatome by Translating Ribosome Affinity Purification (TRAP), which immunoprecipitates ribosome-bound mRNAs by a tagged exogenous ribosomal protein (RPL-22) introduced by lentiviral transduction. We optimized TRAP with a ubiquitous promoter in iPSC-MNs from three C9orf72-ALS patients and three age- and gender-matched healthy controls. Oxidative stress was induced by 0.5 mM sodium arsenite treatment for one hour, and then removed for recovery for up to 24 h prior to harvesting.

Results: We validated expression of exogenous proteins in iPSC-MNs and showed little impact from lentiviral transduction on the transcriptome. Enriched RNA pull-down was observed in the TRAP samples (IP) of the transduced group versus the non-transduced group. RNA-Seq on whole RNA samples also confirmed that the IP samples were depleted of certain types of non-coding RNAs as well as mitochondrial RNAs, which are translated by mitochondrion-specific ribosomes. Compared with the transcriptomic input fraction (IF) samples, the IP samples reflected earlier changes after stress and recovery for 2 h. Dysregulated global translational activity after 4 h and 24 h of recovery was observed in one C9orf72-ALS line versus control measured by modified SUnSET assay ($p = 0.025$ and $p= 0.003$ respectively, independent t test).

Conclusion and Plans: This study successfully applied TRAP in iPSC-MNs. This method provides a tool to investigate the dynamic changes in translatome of C9orf72-ALS iPSC-MNs at baseline, after stress and recovery.

41.Aging-dependent activity impairments of human C9orf72-mutant motor neurons are accompanied by aberrant transcriptional programs.

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by dysfunction and subsequent loss of cortical and spinal motor neurons (MN). Previous studies demonstrated that alterations in synapses and neuronal activity are part of the underlying pathomechanisms in both in vitro and in vivo models, but their contribution to neuronal death is still under debate. Specifically, the influence of hyper- or hypoactivity on cellular disease progression is highly controversial since both phenotypes have been described and found to be harmful in ALS MN. In this study, we employed high definition multielectrode array (HD-MEA) techniques to longitudinally monitor the electrophysiological properties of hiPSC-derived C9orf72-mutant MN. We found an early hyperactivity of ALSC9orf72 MN, which drastically decreased upon aging while neurodegeneration started to occur. In accordance with previous findings of synaptic alterations in ALS MN, we could also observe a generally reduced network synchroneity in ALSC9orf72 MN cultures. Next, we performed a longitudinal transcriptomic analysis to elucidate the molecular causes triggering the loss of activity properties in mutant neurons. Consistent with our HD-MEA findings, we observed an up-regulation of synaptic transcripts in ALSC9orf72 MN at the earlier time point, which was followed by a significant reduction over time. By administration of the SK channel inhibitor Apamin, which has previously been shown to be neuroprotective in ALS MN, we were able to achieve beneficial effects on an electrophysiological as well as transcriptional level. Altogether, this study suggests phenomena of synaptic maturation as possible explanation for contradicting evidence on electrophysiological alterations in ALSC9orf72 MN, provides an insight into the longitudinal development of their neuronal activity and links these functional changes to aging-dependent transcriptional programs.

42. Using optogenetics to model activity-dependent neurodegeneration in amyotrophic lateral sclerosis.

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Background: The hallmark of amyotrophic lateral sclerosis (ALS) is degeneration of motor neurons (MNs). There is increasing evidence that hyperexcitability or excitotoxicity mechanisms may underlie the selective vulnerability of MNs in ALS. To determine the relationship between activity and MN degeneration requires a system in which activity can be controlled. Here we present a novel in vitro model with optogenetic control of induced pluripotent stem cell (IPSC)-derived MN firing. We use this to explore the impact of activity on maturity of IPSC-MNs and activity-related disease mechanisms in C9orf72 ALS (C9-ALS). Further we generate 2D and 3D "disease in a dish" co-culture systems using optogenetic MNs and muscle to model the neuromuscular junction (NMJ) in health and disease.

Aims: To establish optogenetic control of IPSC-MNs alone and in co-culture with muscle and use this model to study effects of activity on (1) maturity of IPSC-MNs (2) activity-dependent mechanisms such as excitotoxicity in C9-ALS.

Methods: We differentiated MNs from IPSCs from C9-ALS patients, isogenic and healthy controls. IPSC-MNs were transduced to express channelrhodopsin (ChR2) and stimulated with 460nm blue light. Light-evoked activity was recorded using a multi-electrode array (MEA). IPSC-MNs were chronically stimulated with high, medium and low-level protocols, then analysed by immunocytochemistry and western blot for markers of MN maturity and toxicity. ChR2-IPSC-MNs and C2C12 muscle were grown in 2D co-cultures and microfluidic chambers and live-imaged for light-evoked muscle contraction.

Results: We confirmed high levels of neuron-specific ChR2 expression in IPSC-MNs. MEA recordings showed robust control of MN activity with light. High level chronic stimulation of C9-ALS IPSC-MNs induced significantly higher levels of cellular stress than in controls. In muscle co-cultures, we confirmed IPSC-MNs retain MN marker expression and found light-evoked muscle twitch confirming functional connectivity.

Conclusions: In this study we have developed and validated a model with optogenetic control of IPSC-MNs. We have used this to demonstrate effects of optogenetic stimulation on maturity, and a selective vulnerability of C9-ALS MNs to high-level activity. Furthermore, we have developed a functional model of the NMJ with optogenetic control of muscle. This novel system provides a platform for further study of activity-dependent pathways to MN and NMJ degeneration in ALS.

43. ALS/FTD-associated C9orf72 C4G2 repeat RNA disrupts phenylalanine tRNA aminoacylation.

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Background: GGGGCC hexanucleotide repeat expansion (HRE) mutation in the C9orf72 gene is the most common genetic cause of ALS and FTD that can reach hundreds to thousands repeats in disease. Transcription of repeats in sense and antisense direction leads to repeat(G4C2)n and (C4G2)n RNA, which can sequester RNA binding proteins and form RNA foci pathognomonic of C9orf72 associated ALS and FTD, predicted to cause RNA toxicity by. The aim of this work was to identify proteins that bind antisense C4G2 RNA transcripts. In our work we show interaction of proteins involved in protein synthesis, cytoskeleton stability and mRNA processing with antisense RNA transcripts. The study focuses on cytoplasmic interaction with Phe-tRNA synthetase (FARS) and its effect on protein synthesis, as disruptions in aminoacyl-tRNA synthetases are increasingly observed in neurodegenerative disorders and can lead to protein misacylation, misfolding and aggregation.

Objectives: Identification of proteins binding to antisense (C4G2)32 RNA transcripts from C9orf72 mutation and determination of how antisense RNA-FARS interaction impact FARS aminoacylation function.

Methods: We performed RNA-pull down assay from mice brain lysates and mass spectrometry to determine proteins that bind to (C4G2)32. Interactions were confirmed using WB, FISH/ICC and RNA-protein PLA. Impact of antisense RNA-FARS interaction was determined using aminoacylation assay and western blots.

Results: We have shown that FARSA and FARSB, interact with (C4G2)32 in RNA-pull down assay from mice brain lysates. The interaction was confirmed using three different assays and interaction between FARSA and (C4G2)32 results in significant decrease of charged tRNAphe in patient lymphoblasts compared to control. Additionally, expression of three phenylalanine-rich proteins was observed to be lower in C9-ALS patient lymphoblasts in comparison to control. The same was not seen for proteins low in phenylalanine.

Conclusion: We found impairment of FARS catalytic function in C9 lymphoblasts, as tRNAphe charging is reduced compared to control. This discovery is important in highlighting the role of aminoacyl-tRNA synthetases in C9orf72 ALS/FTD, as they have been so far implicated in various neurodegenerative diseases.

Friday 3rd June

Session 9: 9.00 to 10.25: Clinical Trials

44. Futility monitoring in clinical trials for amyotrophic lateral sclerosis: saving time, resources and accelerating clinical development.

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Objective: Late-phase clinical trials for neurodegenerative diseases have a low probability of success. Here we introduce an algorithm that optimizes the planning of interim analyses for clinical trials in Amyotrophic Lateral Sclerosis (ALS) to better use the available time and resources, and minimize the exposure of patients to ineffective or harmful drugs.

Methods: A simulation-based algorithm was developed to determine the optimal interim analysis scheme by integrating prior knowledge about the success rate of ALS clinical trials with drug-specific information obtained in early phase studies. Interim schemes were optimized by varying the number and timing of interim analyses, together with their decision rule of when to stop a trial. The algorithm was applied retrospectively to two clinical trials that investigated the efficacy of diaphragm pacing on survival in patients with ALS.

Results: Introduction of an optimized interim analysis scheme would increase the planned number of events by 6.0 to 10.3%, but reduces the expected number of events one actually requires to stop a futile intervention by 40.3 to 44.9%, and reduce the expected trial duration by 32.8 to 36.9%. Retrospective application of the algorithm to two completed trials correctly established lack of efficacy and concluded both studies 15.2 to 22.9 months earlier (reduction of 42.4 to 61.7% in trial duration) and could have reduced the number of randomized patients by 16.2 to 58.1%.

Conclusions: Our algorithm uses prior knowledge to determine the uncertainty of the expected treatment effect in ALS clinical trials and optimizes the planning of interim analyses. Improving futility monitoring in ALS could minimize the exposure of patients to ineffective or harmful treatments, and result in significant efficiency gains.

Key words: Clinical trials; Amyotrophic lateral sclerosis; Group-sequential designs; Futility monitoring; Diaphragm pacing; Frequentist-Bayesian

45. Phase 2 clinical trial of Rapamycin for Amyotrophic Lateral Sclerosis.

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Background: In several in vitro and in vivo studies, Rapamycin has been found to target two pillars of amyotrophic lateral sclerosis (ALS) pathogenesis linked in a vitious auto-maintaining cycle: neuroinflammation and impaired autophagy. Rapamycin, a drug that expands regulatory T cells (Tregs) and stimulates autophagy by inhibiting mTORC1, has never been tested in ALS patients.

Methods: In this multicenter, randomized, double-blind trial, we enrolled participants with probable or definite ALS who had had an onset of symptoms within the previous 18 months. Participants were randomly assigned in a 1:1:1 ratio to receive rapamycin 2 mg/m2/day, rapamycin 1 mg/m2/day or placebo. The primary outcome was the number of patients exhibiting an increase >30% in Tregs from baseline to treatment end. Secondary biological outcomes were the changes from baseline of T,B,NK cell subpopulations, inflammasomes, cytokines, S6 ribosomal protein phosphorylation, neurofilaments, comparing rapamycin and placebo arm. Clinical outcome measures included the rates of decline on the ALSFRS-R total score, the rate of decline in muscle strength, forced vital capacity, the rate and time to nutritional and respiratory procedures, and survival. Safety and quality of life were also assessed.

Results: After screening 70 persons with ALS, 63 were randomly assigned to receive rapamycin or placebo. In intention-to-treat analysis, twice as many patients treated with rapamycin showed an increase in the number of T-reg cells compared to placebo (p=0.24). Treatment with Rapamycin 1 mg/m2/day reduced CD8 T-lymphocytes (p=0.032), IL-18 (p<0.001) and related inflammasome (p=0.023). The mean rate of change in the ALSFRS-R score was −1.20 points per month with the active drug and −1.48 points per month with placebo at treatment end (difference, 0.28 points per month). Patients treated with rapamycin had a better quality of life in the domains related to communication of ALSAQ40.Adverse events were equally represented among treatment arms. Based both on biological and clinical outcome measures, and on safety and plasma dosage stability, rapamycin 1 mg/m2/day resulted the best dosage to be tested in further clinical trials.

Conclusions: A short treatment of 18 weeks with rapamycin showed promising effects on CD8 T-cells, inflammasome, IL18 and quality of life. Longer and larger trials are necessary to evaluate clinical efficacy of Rapamycin in ALS (Funded by ARISLA; NCT03359538).

46. Results from the Phase 1 Trial and Open Label Extension Evaluating BIIB078 in Adults with C9orf72-ALS.

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BIIB078 is an antisense oligonucleotide (ASO) investigated for adults with ALS associated with a mutation of the C9orf72 gene. A Phase 1 multiple ascending dose (MAD) study was performed to evaluate the safety, tolerability, and pharmacokinetics of BIIB078 administered intrathecally in adults with C9orf72-ALS. The primary objective of this study was to evaluate the safety and tolerability of BIIB078. Secondary objectives included evaluation of pharmacokinetics and clinical function. Exploratory objectives included longitudinal evaluation of poly-glycine-alanine (poly-GA) and poly-glycine-proline (poly-GP) peptide levels in cerebrospinal fluid (CSF) indicative of target engagement and neurofilament levels in CSF and plasma indicative of neuronal degeneration. Eligible participants who completed the MAD study could transition to an open label extension (OLE) study.

One hundred six participants were randomized across six dose cohorts (3:1 active:placebo). Adverse events (AEs) were mostly mild to moderate in severity and occurred at a similar rate across BIIB078 and placebo groups. The most common AEs were fall, procedural pain and headache.

At day 85, the upper dose cohorts treated with 60 and 90 mg BIIB078 showed CSF poly-GA reduction from baseline by 42% and 50%, respectively, and CSF poly-GP by 66% and 55%, respectively. These trends were sustained through the end of the 6 month treatment period, suggesting robust target engagement. Across secondary efficacy endpoints including the ALS Functional Rating Scale – Revised (ALSFRS-R), Slow Vital Capacity (SVC), Hand-Held Dynamometry (HHD), and Iowa Oral Pressure Instrument (IOPI), trends towards greater clinical decline were observed with 90 mg BIIB078 (n=18) as compared to the pooled placebo group (n=27). Smaller trends were observed at 60 mg, except for ALSFRS-R, where the trend favoured BIIB078.

At 6 months, participants treated with 90 mg BIIB078 experienced an increase from baseline in both CSF (36%) and plasma NfL (23%) suggestive of increased neuronal degeneration. Increases in neurofilament were also observed with 60 mg BIIB078 (27% in CSF; 9% in plasma).

This Phase 1 study evaluated the hypothesis that C9orf72-ALS is caused by toxicity associated with the repeat expansion containing RNA and the corresponding dipeptides generated from the sense strand. Despite robust reduction of poly-GP and poly-GA, there was no evidence of biological or clinical benefit with BIIB078 administration.

47. Targeting pathological transcriptional variants in C9orf72-associated amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD): Initial results from the ongoing FOCUS-C9 clinical trial

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Background: A hexanucleotide repeat expansion mutation in C9orf72 is the most common genetic cause of ALS and FTD. The expansion leads to RNA foci and dipeptide protein repeats (DPRs), which are believed to drive disease pathology. WVE-004 is an investigational stereopure antisense oligonucleotide designed to selectively target pathological C9orf72 transcriptional variants while sparing C9orf72 protein. FOCUS-C9 (NCT04931862) is an adaptive, first in human clinical trial designed to rapidly optimize dose level and frequency for WVE-004. CSF Poly(GP) is the DPR selected as a biomarker of target engagement in the CNS.

Methods: FOCUS-C9 is an ongoing Phase 1b/2a clinical trial to assess the safety and tolerability of single- and multiple-ascending intrathecal doses of WVE-004 in people with C9-ALS and/or C9-FTD. Twelve patients (ALS: 9; FTD: 3) enrolled into the 10 mg (n = 3), 30 mg (n = 5) and 60 mg (n = 4) cohorts and were randomized to active or placebo. Before each dose escalation, an independent committee reviewed unblinded pharmacokinetic, biomarker, and clinical data to determine the next single dose to be given, whether to start multidose cohorts, and at what frequency.

Results: Significant reductions in poly(GP) were observed across all treatment groups. Compared to placebo, the reduction in the 30 mg treatment group was statistically significant at day 57 (p=0.015) and achieved 34% at day 85 (p=0.011). It appeared that poly(GP) had yet to plateau at this last observed timepoint. At the time of analysis, none of the patients dosed with 60 mg had reached day 85. CSF NfL elevations were observed in some patients in the 30 mg and 60 mg single dose cohorts with no meaningful changes in clinical outcome measures, although the dataset and duration were not sufficient to assess clinical effects. Adverse events (AEs) were balanced across treatment groups, including placebo, and were mostly mild to moderate in intensity. Four patients (including one on placebo) experienced severe and/or serious AEs; three were reported by the investigators to be related to ALS or administration, and one was reported by the investigator to be related to study drug. There were no treatment-associated elevations in CSF white blood cell counts or protein, and no other notable laboratory abnormalities were observed.

Conclusion: Low, single doses of WVE-004 resulted in potent and durable reductions of CSF poly(GP) in patients with C9-associated ALS and FTD. Based on the durability and potency observed in the 30 mg cohort, FOCUS-C9 has been adapted to include additional patients and extend follow-up to identify the maximum reduction of poly(GP) and duration of effect. FOCUS-C9's adaptive trial design successfully provided early indications of target engagement and safety of WVE-004 to enable rapid optimization of dose level and frequency to explore in the multidose phase of the study, and potentially the next phase of development.

48. Evaluating the Efficacy and Safety of Tofersen in Adults with ALS and a SOD1 Mutation: Results from the Phase 3 VALOR Trial and Open-Label Extension.

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VALOR was a Phase 3 trial in which adults with SOD1-ALS were randomized 2:1 to receive tofersen 100 mg (3 doses given ~2 weeks apart, then 5 doses given every 4 weeks) or placebo intrathecally. The primary endpoint was change from baseline to Week 28 in the ALSFRS-R total score. Key secondary endpoints included change from baseline in total SOD1 cerebrospinal fluid (CSF) concentration, plasma neurofilament light chain (NfL) levels, percent predicted slow vital capacity (SVC), handheld dynamometry (HHD) megascore, ventilation-assistance free, and overall survival. Several patient-reported outcomes were evaluated as exploratory endpoints. The primary analysis population comprised the subset of participants who met protocol-defined prognostic enrichment criteria for faster-progressing disease (based on SOD1 mutation type and pre-randomization ALSFRS-R slope) who received ≥1 dose of study treatment (mITT). Participants had the opportunity to continue in an open-label extension (OLE) upon completion of VALOR.One hundred and eight total participants were enrolled (tofersen [n=72], placebo [n=36]). The adjusted mean change from baseline in ALSFRS-R was: tofersen -6.98, placebo -8.14, difference 1.2, p=0.97 (mITT) and tofersen -1.33, placebo -2.73, difference 1.4 (non-mITT). Tofersen administration led to robust reductions in total CSF SOD1 protein and plasma NfL at Week 28 compared to baseline. Trends favoring tofersen were seen across clinical outcome measures of respiratory function, muscle strength, and quality of life. These effects became more apparent with longer-term follow-up in the OLE, particularly with earlier tofersen initiation. Most adverse events were mild to moderate in severity and many were consistent with disease progression or the lumbar puncture procedure. Serious neurologic events, including myelitis (2%), were seen in tofersen-treated participants. VALOR did not achieve statistical significance on the ALSFRS-R at 28 weeks. However, consistent effects were seen across measures of respiratory function, muscle strength, and quality of life. Most AEs were mild or moderate in severity although serious neurologic events, including myelitis, were observed. A new interim data cut of the OLE was performed in January 2022, at which time all participants enrolled in VALOR had the opportunity for 12 months of follow-up. We will debut biomarker, clinical outcome, and survival data from this data cut.

Session 11: 11.50 to 12.50: Disease Models

49. Early reversible structural and functional impairments of excitatory synapses on ALS motoneurons.

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Excessive excitation is hypothesized to cause motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS), but actual proof of hyperexcitation in vivo is missing: how are synaptic inputs to MN affected by the disease, and are they increased or decreased? We demonstrate, by in vivo intracellular MN electrophysiology, that, contrary to expectations, excitatory post-synaptic potentials evoked by electrical or mechanical stimulation of Ia sensory fibers are reduced in MNs of adult presymptomatic mutSOD1 mice. This synaptic impairment correlates with disrupted postsynaptic clustering of Homer1b, Shank, and GluR4 subunits. Moreover, this impairment has a deep impact on the whole MN biology since mechanically-induced Ia inputs translate in a reduced phosphorylation of the CREB transcription factor in MNs. Interestingly, a similar functional impairment is observed in synapses on MN originating from the brainstem descending medial longitudinal fasciculus, indicating a widespread phenomenon. Restoration of excitatory synapses can be achieved by activation of the cAMP/PKA pathway, by either intracellular injection of cAMP or DREADD-Gs stimulation. Furthermore, we reveal, through independent control of signaling and excitability in MN allowed by multiplexed DREADD/PSAM chemogenetics, that PKA-induced restoration of synapses triggers an excitation-dependent decrease in misfolded SOD1 burden and autophagy overload. In turn, increased MN excitability contributes to restoring synaptic structures. Thus, the decrease of excitation to MN is an early but reversible event in ALS. Failure of the postsynaptic site, rather than hyperexcitation, drives disease pathobiochemistry at this stage of the disease evolution.

Fundings: Fondation Thierry Latran (SpinALS and TriALS), ARSLA,Radala Foundation for ALS research, Target ALS, AFM Telethon (Hypertoxic), NIH-NINDS R01NS077863, NCN 2019/35/B/NZ4/02058.

50. Meta-analysis of ALS astrocytes reveals multi-omic signatures of inflammatory reactive states.

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Astrocytes contribute to motor neuron death in amyotrophic lateral sclerosis (ALS), but whether they adopt deleterious features consistent with inflammatory reactive states remains incompletely resolved. To identify inflammatory reactive features in ALS astrocytes, we performed a systematic meta-analysis of all publicly-available ALS astrocyte RNA-sequencing data. We identified RNA-seq human induced pluripotent stem cell (hiPSC)-derived astrocytes carrying SOD1, C9orf72, VCP and FUS gene mutations. Additionally, we found RNA-seq from mouse ALS astrocyte models with Sod1G93A mutation, Tardbp (TDP-43) deletion and Tmem259 (membralin) deletion. In both hiPSC and mouse meta-analyses, ALS astrocytes were characterised by up-regulation of genes involved in extracellular matrix, endoplasmic reticulum stress and the immune response and down-regulation of synaptic integrity, glutamate uptake and other neuronal support processes. We further identify activation of TGFβ, Wnt and hypoxia signalling pathways in both hiPSC and mouse ALS astrocytes. ALS astrocyte gene expression signatures positively correlate with astrocytes treated with proinflammatory TNF, IL-1α and C1q, with significant overlap of differentially expressed genes. By contrasting ALS astrocytes with models of protective astrocytes, including middle cerebral artery occlusion and spinal cord injury, we uncover a cluster of genes changing in opposing directions, which may represent down-regulated homeostatic genes and up-regulated deleterious genes in ALS astrocytes. These observations indicate that ALS astrocytes augment inflammatory processes whilst concomitantly suppressing neuronal supporting mechanisms, thus resembling inflammatory reactive states and offering potential therapeutic targets.

(51) Human iPSC derived neuromuscular assembloid model to study neuromuscular junction degeneration in Amyotrophic lateral sclerosis.

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(5) The European Union's Horizon 2020 research and innovation programme SAND (Secretion and Autophagy in Neurodegenerative Diseases) under the Marie Sklodowska-Curie Actions.

Amyotrophic lateral sclerosis (ALS) is a rapidly progressing, incurable and fatal adult neurodegenerative disease characterised by loss of motor neurons which connects to skeletal muscle (SkM) and controls movement. Patients experience rapid decline of muscle strength, followed by paralysis and death due to respiratory failure owing to loss of synaptic connection between MNs and SkM - the neuromuscular junction (NMJ). Despite only ca. 10% of ALS cases being familial, familial and sporadic patients are clinically and pathologically indistinguishable, including the most commonly affected genes C9orf72 and TARDBP, which supports studying genetic causes of ALS to understand underlying pathomechanisms. C9orf72 mutation is observed in ca. 40% of familial ALS whereas cytoplasmic TDP43 aggregates are widely observed in 97% of all ALS cases. Multiple evidence from animal and patient studies highlights NMJ denervation as an early degenerative event preceding MN loss and symptom onset. However, little is known about the molecular determinants necessary for maintenance of human NMJs or the molecular changes preceding NMJ denervation in ALS. Mechanistic understanding of this will allow to intervene at an early stage of disease and to slow disease progression. Noting that human NMJs are morphologically distinct from other species a human NMJ model is required. Thus, we have adopted a human iPSC derived 3D neuromuscular assembloid model wherein iPSC derived spinal organoids comprising of Islet1/2+/NfH+ MNs were fused to a FastMyHC+/Titin+ primary skeletal muscle organoid. After 30 days post assembly, NfH+ MNs innervated the muscle organoid. More intriguingly, assembloids were positive for Bungarotoxin (Btx) - selectively labelling postsynaptic acetylcholine receptors (AChR) - and colocalizing with NfH+ axons, depicting formation of NMJs. Live imaging of assembloids showed muscle organoid contraction upon stimulation of MNs with glutamate, which was attenuated by addition of curare, a blocker of the AChRs. Taken together, these results show successful generation of a human 3D iPSC derived NMJ model. Future studies will be performed using patient derived stem cells harbouring ALS gene mutations TARDBP and C9orf72 to study their impact on NMJ degeneration. Furthermore, we will better understand the molecular changes at the NMJ in health and disease via an unbiased transcriptomic approach, allowing identification of novel therapeutic targets for NMJ degeneration in ALS.

(52) Alterations in the expression pattern of specific HERV-K copies is associated with Amyotrophic Lateral Sclerosis.

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In the last two decades, retroviral reverse transcriptase activity and overexpression of human endogenous retroviruses (HERVs) have been associated with amyotrophic lateral sclerosis (ALS). However, detection of that increase of HERVs in the central nervous system of ALS patients seems questionable. The aim of this work was to confirm HERV-K overexpression in ALS and to study the role of neuroinflammation in the central nervous system (CNS). First, HERV-K mRNA expression was analysed by real-time quantitative PCR (qPCR) in peripheral blood mononuclear cells (PBMC), and in post-mortem brain samples (brainstem and cortex tissue) from ALS patients and healthy controls. Next, we studied the expression pattern of specific HERV-K copies in PBMC and brain samples by next-generation sequence analysis. Finally, mRNA levels of inflammatory markers were measured in brain samples by qPCR. Our results showed no significant differences in the expression of HERV-K in PBMCs nor in brain samples between ALS patients and control subjects. In contrast, we observed significant differences in the relative expression of specific HERV-K copies in both brainstem and cortex samples between ALS patients and control subjects. Out of 27 HERV-K copies sampled, the relative expression of 17 loci was >1.2 fold changed in samples from ALS patients. The relative expression of three particular HERV-K copies (Chr1-1, Chr3-3 and Chr16-1) significantly changed in brainstem samples from ALS patients compared with controls, showing an increase of >1.5 fold ($p = 0.008$) or a >2 fold decrease ($p = 0.05$) for Chr16-1 and Chr3-3, respectively. Regarding inflammation, we observed decreased mRNA levels of TNF-α, IL-6 and granzyme B (GRZB) in cortex, and a significant increase of NLRP3 in both cortex and brainstem of patients with ALS. In this study, we report ALS-specific overexpression of selected HERV-K copies, and confirm NLRP3 overexpression in ALS brain, highlighting the potential role of particular HERV-K copies in the pathophysiology of ALS.

Abstracts for Poster Presentations

Session 1 - Wednesday

a) Imaging

(a1) Magnetic resonance imaging of the spinal cord provides a marker of the rate of progression in ALS patients

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Background: Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disorder that leads to eventual death(Valsasina, Agosta et al. 2007, Querin, El Mendili et al. 2017). Although neuroimaging seems to be a reliable potential biomarker especially several studies showed significant MRI metrics changes between ALS patients and healthy controls, there is an unmet need for reliable biomarkers, not only for better diagnosis but also to provide a reliable assessment of disease progression (El Mendili, Querin et al. 2019). Therefore, the aim of this study is to measure the change of spinal cord MRI metrics over time based on a prospective, longitudinal, multipoint study.

Methods: This study is an ancillary analysis using data collected from the Paris Center which is part of the PULSE study. PULSE is an ongoing observational and prospective multicentric cohort (Protocol 2013-A00969-36) in ALS patients. We included 40 ALS patients who underwent a structural and diffusion MRI. Magnetic resonance imaging (MRI) scans were acquired on 3T Siemens scanner, and clinical variables were collected over three-time points. Spinal cord toolbox (SCT) was used to treat the structural and diffusion images to compute cross-sectional area (CSA) per-level and DTI parameters (FA, MD, RD, AD) at the lateral corticospinal tract and the dorsal columns at the cervical level. Clinical and demographic data will be then evaluated for correlations with cervical spinal cord imaging findings.

Results: At the inclusion timepoint, MRI damage parameters, including CSA per-level and the DTI parameters at the lateral corticospinal tract and posterior dorsal columns at the cervical level, showed significant differences within the cohorts when we divided them regarding age at onset and site of onset.

A significant difference in the MRI damage parameters was found within subgroups regarding the rate of progression as measured by the ALSFRS.

Conclusion: This study demonstrates different scales of damage, indicated by the damage parameters at the MRI, in ALS patients based on the site of onset and the age at onset. And there was a tendency to show more prominent differences with the use of DTI damage parameters. Our results are in line with the literature on the use of cervical cord MRI as a tool to monitor ALS progression, as this technique is sensitive to degeneration of both UMN and LMN. Analysis of longitudinal data are ongoing.

(a2) Combined microstructural and sodium homeostasis alterations in ALS are widespread in fast progressors: a brain DTI and sodium MRI study

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Background And Purpose: While conventional MRI has limited value in amyotrophic lateral sclerosis (ALS), non-conventional MRI has shown alterations of microstructure using diffusion MRI and recently sodium homeostasis with sodium MRI. We aimed to investigate the topography of brain regions showing combined microstructural and sodium homeostasis alterations in ALS subgroups according to their disease progression rates.

Materials And Methods: Twenty-nine patients with ALS and 24 age-matched healthy controls (HC) were recruited. Clinical assessments included disease duration and the revised ALS functional rating scale (ALSFRS-R). Patients were clinically differentiated into fast (n=13) and slow (n=16) progressors according to their ALFSRS-R progression rate.3T MRI brain protocol included: (1) 1H T1-weighted and diffusion sequence; (2) 23Na density-adapted radial sequence. Quantitative maps of diffusion with fraction anisotropy (FA), mean diffusivity (MD) and total sodium concentration (TSC) were measured. The topography of diffusion and sodium abnormalities were assessed by voxel-wise analyses.

Results: ALSpatients showed significantly higher TSC and lower FA, alongside higher TSC and higher MD, compared to HC, primarily within the corticospinal tracts (CSTs), the corona radiata and the body and genu of the corpus callosum. Fast progressors showed wider spread abnormalities mainly in frontal areas. In slow progressors, only FA measures showed abnormalities when compared to HC, localized in focal regions of the CSTs, the body of corpus callosum, the corona radiata and the thalamic radiation.

Discussion: This study highlighted brain regions with common microstructural and sodium homeostasis disturbances corresponding to relevant regions involved in ALS. Fast progressors showed widespread combined alterations while slow progressors only showed restricted microstructure damage. Recently, increasing evidences emphasized that heterogeneous disease progression rates influence diagnosis, prognosis and might affect the responsiveness to treatment. Therefore, there is an urgent need of patient stratification. Our results confirm previous reports showing that non-conventional and multiparametric MRI technics might contribute to the diagnostic work-up of patients with different clinical profiles.

Conclusion: The present study evidenced widespread combined microstructural and sodium homeostasis brain alterations in fast progressors ALS patient.

(a3) Cross Frequency Coupling Analysis in Amyotrophic Lateral Sclerosis resting-state EEG

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Background: An early motor cortex cortical hyperexcitability characterizes both sporadic (sALS) and familial (fALS) forms of Amyotrophic Lateral Sclerosis (ALS). This dysfunction precedes lower motor neuron signs, suggesting to be a powerful biomarker promoting ALS early diagnosis. Electroencephalography (EEG) can be an approach to detect and monitor cortical dysfunction through investigation of the interaction between neural oscillations at different frequencies, known as Cross-Frequency Coupling (CFC) analysis. Precisely, we studied the Phase-Amplitude Coupling (PAC) between slow and fast oscillations, known to be highly dependent on excitation/inhibition (E/I) balance in the brain cortex (1, 3).Objective: Our aim was to investigate whether PAC is altered in ALS patients and if this alteration can represent an ALS cortical dysfunction biomarker.

Methods: We used high density EEG recording protocol (74 channels, 4kHz sampling rate, bandpass 0.03-1330Hz), consisting in 5 minutes recording with eyes closed and 5 with eyes open, at rest, on 26 sALS patients (median ALSFRS-r score=40) and a gender- and age-matched group of 27 healthy controls. EEG data were pre-processed to delete artefacts using Independent Component Analysis and filters to select EEG signal below 60Hz. We analysed PAC on 5 channels around the cranial vertex (Fz, Cz, Pz, C3, C4 according to the 10-20 system) using the Tort et al. method (2), which measures PAC estimating the mean Modulation Index (MI i.e., mean quantitative estimation of the coupling).

Results and Conclusions: Our results indicate that MI for Theta-(slow) Gamma (4-8Hz vs. 30-60Hz) PAC was significantly decreased in ALS patients compared to controls whether the eyes were closed or opened, especially at the level of Fz, Cz, Pz and C3 channels. We also found a link between MI and disease progression rate, with MI more depressed in fast than slow progressors, suggesting that the faster the progression rate is, the greater the Theta-Gamma PAC reduction gets. Our results suggest a neural uncoupling likely linked to E/I imbalance at cortical level in ALS, and support the hypothesis that the Theta-Gamma PAC MI extracted from EEG may serve as new quantitative biomarker of cortical dysfunction during disease progression. References:1 Canolty RT & Knight RT Trends Cogn Sci 14, 506-515 (2010)2 Tort ABL, et al. J Neurophysiol 104, 1195-1210 (2010)3 Vucic S & Kiernan MC Neurotherapeutics 14, 91-106 (2017)

(a4) Altered resting-state EEG microstates in ALS, associated with distinct sources of brain activity

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Electroencephalography directly measures the brain's electric activity. Previous studies have been able to discriminate ALS patients and healthy controls (HC) based on EEG recordings [1-2]. More specifically, multi-channel analysis of EEG in terms of the constituent microstates (ms), which are transient, reoccurring topographies of EEG, is thought to reflect ongoing mental activity [3]. It is, therefore, a promising method to detect and quantify neural abnormality in ALS. High-density resting-state EEG data from 129 patients and 87 HC were recorded, with up to four follow-up sessions for patients. We identified ms by clustering the EEG signals (1-30Hz) at peak times of global field power (GFP). For patients and HC, each EEG sample (256Hz) was associated with the ms prototype it was most similar to, based on global map dissimilarity. The structures of the ms sequences were then analysed to determine how often they reoccur, how long they last, how they transition and if they retain any memory effect (information theory and Markovianity) [4]. For patients, changes in the properties of the ms over the course of the disease were assessed. Correlations (FDR corrected) between EEG ms properties and clinical scores were evaluated. Finally, the brain sources underlying the ms were estimated based on linearly constrained minimum variance beamformer analyses and a general linear model to match the ms sensor with source space patterns. The extracted ms prototypes were similar, in number and topography, to the ones reported in the literature [3] (HC: 70%, ALS: 64% of the recordings explained). Significantly different duration, global explained variance (GEV) and transition probabilities were observed between patients and HC. There was a significant increase of GFP over time in patients ($p=0.04$). Furthermore, in cognitively impaired patients (ALSci), the GEV of all ms classes correlated with clinical scores progressions ($0.4<|\rho|<0.6$; $0.002<p<0.05$; $0.5<1-\beta<0.9$). This study shows that static and temporal structures of EEG ms are altered in ALS. In addition, ms reveal prospective predictive power over the progression of the symptoms. The distinct brain sources of each ms class also allow us to link altered ms with their corresponding sources. The study confirms the potential of EEG ms as a quantitative marker of motor and cognitive changes in ALS.[1]S. Dukic et al., 2019 [2]B. Nasseroleslami et al., 2019 [3]C. M. Michel and T. Koenig, 2018 [4]F. von Wegner et al., 2017

(a5) Profiling brain morphologic features of motor neuron disease caused by TARDBP mutations: an MRI-based study

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Objective: Mutations in the TARDBP gene are a rare cause of genetic motor neuron disease (MND). Morphologic MRI features of MND patients carrying this mutation have been poorly described in literature. Our objective was to investigate distinctive clinical and MRI features of a relatively sized sample of MND patients carrying TARDBP mutations.

Methods: 11 MND patients carrying a TARDBP mutation were enrolled. 11 patients with sporadic MND (sMND) matched by age, sex, clinical presentation and disease severity were also selected, along with 22 healthy controls. Patients underwent clinical and cognitive evaluations, as well as 3D T1-weighted and diffusion tensor (DT) MRI sequences on a 3 Tesla scanner. GM atrophy was first investigated at a whole-brain level using voxel-based morphometry (VBM). GM volumes of 90 Automated Anatomical Labeling (AAL) regions of interest were also obtained. Lastly, tractography was performed to obtain mean DT MRI metrics of the corticospinal tracts (CST). Clinical, cognitive and MRI features were compared between groups using age- and sex-adjusted ANOVA models, Bonferroni-corrected for multiple comparisons.

Results: Compared with sMND, TARDBP patients showed a trend toward faster disease progression rate ($p=0.056$). Compared with controls, GM volume loss on VBM was greater in TARDBP patients at the level of the right lateral parietal cortex. A significant reduction of GM volumes was found in the left precuneus and right angular gyrus of TARDBP patients, compared to controls ($p=0.002$), whereas sMND showed GM volumes comparable with those of controls. At tractography, the left CST showed increased radD in both sMND ($p=0.043$) and in TARDBP compared to controls, even if the latter did not reach statistical significance ($p=0.059$). The right CST instead showed decreased FA in only in TARDBP patients, compared to controls ($p=0.044$)

Conclusions:TARDBP patients showed a distinctive parietal pattern of cortical atrophy and a greater damage of the CST compared with controls, that sMND patients matched for disease severity and clinical presentation were lacking. This would suggest that TDP-43 pathology may spread more rapidly in TARDBP mutation carriers, causing greater morphologic alterations in both grey and white matter.

Study funding: Italian Ministry of Health (GR-2011-02351217; GR-2013-02357415; RF-2011-02351193), AriSLA (ConnectALS), and European Research Council (StG-2016_714388_NeuroTRACK).

(a6) Structural and functional connectome alterations across King's stages in amyotrophic lateral sclerosis

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Objective: The identification of quantitative and reproducible markers of disease progression in amyotrophic lateral sclerosis (ALS) is of paramount importance for study design and inclusion of homogenous patient cohorts into clinical trials, as there is currently no validated disease-stage biomarker for ALS. Here, we explored the rearrangements of structural and functional connectivity within and among brain networks underlying the clinical spreading of ALS, as described by the King's staging system, in order to suggest objective, continuous measures mirroring disease progression.

Methods: 104 patients with ALS and 61 age- and sex-matched healthy controls underwent clinical and brain magnetic resonance imaging (MRI) on a 3T scanner. Patients were stratified into four groups, according to the King's staging system. No patient had comorbid frontotemporal dementia. Structural and functional connectivity values within and between different anatomical brain regions were obtained using diffusion tensor and resting-state functional MRI data, respectively. Comparisons between groups were performed using age- and sex-adjusted ANOVA models, Bonferroni-corrected for multiple comparisons.

Results: Compared with controls, a significant, progressive reduction of structural connectivity within brain nodes of the sensorimotor network was observed in ALS patients across King's stages 2, 3 and 4 ($p<0.006$). Patients in stages 3 and 4 also showed significant loss of structural connectivity between frontal and sensorimotor regions ($p=0.001$), whereas patients in milder stages were comparable with healthy controls. A significant disruption of functional connectivity between frontal and temporal regions was found only in ALS patients in stage 4 ($p=0.025$).

Conclusions: Brain MRI allows to demonstrate and quantify increasing disruption of structural connectivity involving the sensorimotor and frontal networks in ALS, mirroring disease spreading described by the King's staging system. Frontotemporal functional disconnection characterizes advanced disease stages.

Study funding: Italian Ministry of Health (GR-2011-02351217; GR-2013-02357415; RF-2011-02351193), AriSLA (ConnectALS), and European Research Council (StG-2016_714388_NeuroTRACK).

(a7) Functional connectivity reorganization propagating from disease epicenters in frontotemporal dementia variants

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Objective: This study explored functional connectivity reorganization at increasing topological distance from disease epicenters and its relationship with neurodegeneration in frontotemporal dementia (FTD) presentations.

Methods: Patients with behavioral variant of FTD (bvFTD, n=64), non-fluent (nfvPPA, n=34) or semantic variant of primary progressive aphasia (svPPA, n=36) and 94 healthy controls underwent 3T MRI. The peaks of atrophy of each variant (i.e., disease epicenters) were identified in an independent cohort of 42 path-proven FTD cases and used as seed regions for stepwise functional connectivity (SFC) analyses. SFC rearrangements were compared between patient groups and controls. Correlations between SFC architecture in controls and atrophy patterns in FTD patients were tested.

Results: The identified disease epicenters were the left anterior insula for bvFTD, left supplementary motor area for nfvPPA, and left inferior temporal gyrus (ITG) for svPPA. Compared with controls, bvFTD and nfvPPA patients showed widespread decreased SFC in bilateral cortical regions with direct/intermediate connections with the epicenters, and increased SFC either in circumscribed regions close to the respective seed region or in more distant cortical and posterior cerebellar areas. Across all link-steps, svPPA showed SFC decrease mostly localized in the temporal lobes, with co-occurrent SFC increase in cerebellar regions at intermediate link-steps. Average functional link-step distance from the left ITG in healthy controls correlated with regional grey matter volume in svPPA patients (r=0.29, p=0.03).

Conclusion: Our findings revealed novel insights regarding the topology of functional disconnection across FTD syndromes, holding the promise to be used to model disease progression in future longitudinal studies.

Funding: This study was partially supported by the European Research Council (StG-2016_714388_NeuroTRACK), Foundation Research on Alzheimer Disease and the US National Institutes of Health (R01-DC12519, R01-DC14942, R01-DC010367 and R21-NS94684).

(a8) Disruption of the structural connectivity and of hypothalamic integrity in ALS patients and murine models

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Hypermetabolism is a newly recognized clinical manifestation of ALS, predating disease onset and associated with poor prognosis. The increased metabolic rate has been causally linked to a dysfunction of the hypothalamic energy-balance regulation; however, the nature and the cause of such dysfunction is unknown. We have used retrograde tracing systems in murine ALS models and tract-of-interest (TOI) approaches on MRI-DTI datasets from human ALS patients to address the hypothesis that changes in large-scale projections to the hypothalamus may be associated with the disruption of hypothalamic homeostasis. We found that the hypermetabolic SOD1(G93A) ALS show the selective atrophy of lateral hypothalamus (LHA) and display an alteration of projections from orbitofrontal, insular and motor cortices to LHA, detectable in symptomatic but not in presymptomatic stages. The non-hypermetabolic FUS ALS model display only a selective degeneration of projections from primary and secondary motor cortices to LHA. MRI evidence of hypothalamic atrophy in human patients is mirrored by the observed atrophy of the LHA in SOD1 but not in FUS mice. Furthermore, MRI measures reveal the differential involvement of hypothalamus in ALS clinical subtypes, with reduced atrophy characterizing Primary Lateral Sclerosis patients, which characteristically do not display weight loss and hypermetabolism. Thus, disruption of large-scale cortico-hypothalamic connectivity is a new endophenotype of ALS associated with disturbances of basal metabolism.

(a9) Are short intracortical inhibition (SICI) and intracortical facilitation (ICF) abnormal in ALS patients? – A threshold tracking TMS study

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Background: Threshold-Tracking Transcranial Magnetic Stimulation (TT-TMS) refers to the alteration of magnetic pulse intensity to achieve MEPs of target amplitude. TT-TMS measures exhibit higher reproducibility relative to the traditional fixing pulse intensity approach1. Lower TT-TMS-measured posteroanterior (PA) short intracortical inhibition (SICI, a measure of cortical GABAergic inhibition of upper motor neurones), compared to mimic disease and healthy populations has been proposed as a diagnostic ALS biomarker2. Increase in intracortical facilitation (ICF, physiological underpinnings less clear) has also intermittently been reported in ALS2. Abnormalities reported in SICI and ICF in ALS now require replication in samples from other patient populations.

Objective: To determine if short intracortical inhibition (SICI) and intracortical facilitation (ICF) are abnormal in Irish ALS patients.

Methods: EMG was recorded from dominant-hand APB muscle while fully-automated TT-TMS protocols3 were applied over the contralateral motor cortex using a 50mm figure-of-eight coil with PA and anteroposterior (AP) directions of induced current flow. SICI was recorded using 1ms (PA ALS n: 20, control n: 30) and 3ms (PA ALS n: 22, control n: 31, AP ALS n: 9, control n: 17) ISIs and ICF (PA ALS n: 35, control n: 21) was recorded using a 10ms ISI. Conditioning stimuli were applied at 70% of resting motor threshold.

Results: Mann Whitney U tests indicated no difference in ICF between cohorts (p=0.68), and only marginally lower SICI in ALS when a PA coil orientation was used (p=0.08/0.05 for 1/3ms ISI). Lower SICI in ALS was much more evident when an AP coil orientation was used (p=0.0013, 3ms ISI). Linear mixed modelling was applied to test if the effects of stimulated hemisphere, age, gender or handedness was disguising group differences, however results were similar when these factors were accounted for (p=0.045/0.07 for PA SICI 1/3ms ISI, p=0.52 for ICF, p=7.57*10-4 for AP SICI 1/3ms ISI)

Conclusions: ICF is not abnormal in ALS. While SICI is lower in this ALS patient sample, this disinhibition is only consistent when AP coil orientation is used. When PA coil orientation is used, this measure is not sensitive enough to act alone as a diagnostic biomarker of ALS without consideration of other factors.

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(a10) The effects of background audio-visual processing on the TMS measures of cortical excitability for biomarker research in ALS.

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Background: Threshold-tracking transcranial magnetic stimulation (TT-TMS) measures have been shown to be of diagnostic and prognostic value in ALS[1]. TT-TMS is, therefore, increasingly being used to measure central motor pathophysiology in ALS. These studies require participants to sit still and remain relaxed to consistently maintain very low amplitude EMG in the target muscle for several minutes at a time. However, unlike MRI studies [2], TMS study protocol typically do not allow radio or film to occupy the participant, helping them to relax and prevent potential influence of participants attending to the pulse sound/effect[3].

Aim: To investigate if watching and/or listening to a documentary that does not relate to movement affects commonly-studied TMS measures of motor cortical excitability.

Methods: Data were collected from 10 healthy control volunteers. EMG was recorded from both dominant and non-dominant APB muscles while fully automated TT-TMS was used to measure resting motor threshold (RMT), threshold hunting target (THT), short and long intracortical inhibition (S/LICI) and interhemispheric inhibition (IHI). Stimuli were delivered to the motor cortex contralateral to the dominant hand. In the case of IHI, the conditioning stimuli were delivered to the motor cortex ipsilateral to the dominant hand. Recordings were taken twice with no sensory stimulation, once with visual and auditory stimulation, and once with auditory stimulation, in varied order. Figure-of-eight coils were used for all stimuli. The effects of auditory and/or visual stimulation on baseline EMG amplitudes, number of rejected trials and measures of motor cortical excitability are being investigated by comparison to data collected without distractor stimuli present.

Results: RMT, SICI, LICI and IHI were not found to be significantly altered by auditory ($p>0.16$) or audiovisual ($p>0.46$) input. RMT and IHI ICC values are similar for values compared under each sensory stimulation condition.

Conclusions: We have found no evidence of an effect of auditory or audiovisual stimulation on these common TMS measures.

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(a11) Brain-age predicts survival in ALS

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Objective: Age is the most important single risk factor of sporadic amyotrophic lateral sclerosis (ALS). Neuroimaging together with machine learning allows estimating individuals' brain age. Deviations from normal brain ageing trajectories (so called predicted brain age difference or PAD) were reported for many of neuropsychiatric disorders. While all of them showed increased PAD, there is surprisingly few data on PAD in motor neurodegenerative diseases.

Methods: In this observational study we used previously trained algorithms of 3377 healthy individuals and derived PAD from volumetric MRI of 112 ALS patients and 70 healthy controls. We correlated PAD scores with voxel-based morphometry data and multiple different motoric disease characteristics as well as cognitive/behavioral impairment.

Results: Against our primary hypothesis, there was no higher PAD in the ALS patients per se. None of the motoric characteristics influenced PAD. However, cognitive/behavioral impairment led to significantly increased PAD, while slowly progressive as well as cognitive/behavioral normal ALS patients had even younger brain ages than healthy controls. Of note, the cognitive/behavioral normal ALS patients showed increased cerebellar brain volume as potential resilience factor.

Interpretation: Younger brain age in ALS is able to predict slower disease progression / longer survival, possibly providing a cerebral reserve against cognitive / behavioral impairment and faster disease progression. Brain age analysis pipeline's ease of use might suggest it as novel biomarker for monitoring disease modifying effects. It will be interesting to test whether this cerebral reserve is specific for ALS or is also found in other neurodegenerative diseases with primary motor impairment.

(a12) Preliminary magnetoencephalography findings in active and passive motor task in ALS

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Objectives and aims: Motor neuron loss is the primary cause of disability and death in amyotrophic lateral sclerosis (ALS). Other cortical functions in ALS such as cognitive and sensory processing lack exploration. Magnetoencephalography (MEG) is a non-invasive method for investigating motor, sensory and cognitive cortical processing. The few available studies in ALS using MEG have shown that cortex to muscle connection reported as cortico-muscular coherence is decreased and beta (15-30hz) desynchronization is increased compared with healthy controls.

Previous MEG studies in ALS motor function have focused on active movements, here motor efferent are confounded with the concurrent sensory afferents. Our paradigm offers a means to disentangle these processes with millisecond temporal resolution, relevant to the study of neuronal function.

We here measured the cortical changes during an active and a passive motor task. We hope to confirm some of the previous findings in ALS using MEG and to add to the understanding of the cortical processes in ALS. The primary measure of interest in our MEG paradigm is the Event Related Desynchronization (ERDS) of beta oscillations after movement onset

Methods: Participants underwent one MEG session. Additional electromyograms (EMG) from the flexor digitorum profundus (FDP) and the extensor indicis (EI), and movement of the index finger using an accelerometer were recorded. The session includes; 5x20 active, 5x20 passive movement trials and two 5 minute eyes-open resting state periods. Active trials require the subject to tap the index finger firmly against a light resistance. The passive trial uses a pneumatic muscle to produce precisely-timed linear motion, to mimic the sequence of index finger extension and flexion in the active task.

Status & preliminary results: 11 ALS and 9 control data sets have been collected. MEG and task tolerability is very good. Preliminary analysis reveals overall good MEG data quality, including a low level of head movement.

We observe robust beta-ERD in both active and passive conditions in the 28 sensors above the contralateral pre/post-central gyrus. Preliminary data shows remarkably similar topographical activation patterns for both conditions. Reaction times and acceleration patterns for the active sessions in the two groups are similar. Further investigation of the differences between healthy controls and patients and the active and passive movement tasks are pending.

(a13) Impaired Cortico-muscular Synchrony in ALS during Transient and Sustained Voluntary Movements

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Background: The modulation of cortex-muscle synchrony during different phases of voluntary motor tasks could result from the involvement of different circuits and pathways in the brain and spinal cord, such as spinal interneurons that are affected in ALS. We, therefore, hypothesized that the neural networks engaged during both the transient and sustained phases of voluntary motor activity are affected in ALS and the associated abnormalities in cortex-muscle communication can be quantified by Cortico-muscular coherence (CMC).

Method: EEG (128 channel) and 8 bipolar EMG signals were recorded from 18 ALS and 17 age-matched controls when they performed a simple pincher grip task (at 10% of MVC) using the thumb and index finger of the right hand. The pre-processing of EEG/EMG signals and EEG source reconstruction were implemented using the Fieldtrip toolbox in MATLAB. Three major contralateral anatomical motor brain regions (M1, S1, SMA) and 3 muscles (APB, FDI, FPB) reported showing stronger CMC were chosen for analysis. CMC was calculated to identify the cortex-muscle communication channels and their oscillatory behaviour responsible for motor control during transient and sustained stages of the motor task.

Results and Discussions: CMC results during transient movement showed the presence of significant CMC at multiple frequency bands, including β in both controls and ALS. However, a significant γ (31-97Hz) CMC was consistently observed between motor cortices (M1 and SMA) and all three muscles only in ALS. This could be related to ALS pathophysiology and even present in presymptomatic stages of ALS.CMC results during sustained movement showed the presence of significant β coherence between motor regions and all three muscles in both controls and ALS. CMC at other frequency bands besides β was insignificant in controls. However, δ (2-4Hz), θ(5-7Hz), and α (8-13Hz) CMC were significant in ALS, which could be the result of impaired spinal interneurons inhibitory circuits in ALS.

Conclusions: Our findings suggest that ALS-specific impairments can be detected during different movement conditions. Thus, it is necessary to interrogate the motor networks based on their exact function for designing biomarkers of motor network disruption in ALS.

(a14) The Spinal Cord Lateral Tract Sign in Amyotrophic Lateral Sclerosis: an rAMIRA based MRI sign for Upper Motor Neuron Involvement in a Clinical Setting

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Background: Signs of upper motor neurons (UMN) dysfunction can be elusive and difficult to identify in amyotrophic lateral sclerosis (ALS), but are important for timely diagnosis. One of the core macro-pathological features of ALS, the spinal cord lateral tract (SCLT) sclerosis, was described by Charcot and shaped the name of the disease. Histopathologically, these areas are characterized by axonal loss and diffuse astrocytic gliosis. Averaged Magnetization Inversion Recovery Acquisitions (rAMIRA) is a novel MRI approach enabling spinal cord (SC) gray and white matter (WM) imaging with high contrast in clinically feasible acquisition times at 3 Tesla (T). The aims of the study were to define and validate the SCLT sign and to investigate its sensitivity and specificity in patients with ALS, other lower-motor neuron (LMN) diseases, and healthy controls (HC).

Methods: 38 patients with an established diagnosis of ALS (Gold Coast criteria), 60 HC, 25 patients with post-polio syndrome (PPS, March of Dimes criteria), and 10 patients with 5q-spinal muscular atrophy (SMA) were investigated by axial 2D rAMIRA imaging at the intervertebral disc levels C2/C3 - C5/C6 (3T-PRISMA, Siemens). Clinical and demographic data of all participants were obtained. The SCLT sign was defined and validated in a multi-step process by 4 independent raters in subsets of participants; its sensitivity and specificity in detecting ALS was calculated.

Results: The SCLT sign, defined as evenly spread, uni-or bilateral hyperintensities in the SC WM dorsolaterally to the anterior horns, was present in ALS patients in 50%, 49%, 54% and 46% at the respective levels C2/C3-C5/C6, and in up to 89% of ALS patients with UMN predominance. In the HC, PPS and SMA groups, the sign was present in 3%, 8%, and 0%, respectively, thus rendering a sensitivity of 58% and specificity of 98% at C3/C4 to identify ALS cases. The sign was detectable irrespective of disease duration and persisted on serial imaging over 2 years.

Discussion: The SCLT sign shows a high specificity in distinguishing patients with ALS from HC and patients with other LMN disorders. Further investigations of patients early in the disease course, particularly those without clinical signs of UMN dysfunction, and of patients with other UMN disorders are necessary next steps to estimate the potential of this novel imaging marker to improve the ALS diagnostic process.

(a15) Cervical Spinal Cord Gray Matter Atrophy as an emerging biomarker in Amyotrophic Lateral Sclerosis

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Background: There is an urgent need for valid and reliable imaging biomarkers to reduce the diagnostic delay, to monitor the disease course, and to evaluate drug efficacy in upcoming trials in amyotrophic lateral sclerosis (ALS). The novel AMIRA (Averaged Magnetization Inversion Recovery Acquisitions) method enables high resolution imaging with improved contrast of spinal cord (SC) gray matter (GM) and white matter in clinically feasible acquisition times at 3 Tesla (Weigel & Bieri, 2018; Weigel et al., 2020).The aims of this study were to compare cervical SC GM areas between patients with a diagnosis of ALS and healthy, age- and sex-matched control subjects (HC) and to assess the association of cervical SC GM area and established measures of clinical disability, the revised ALS functional rating scale and of respiratory impairment, the sniff nasal inspiratory pressure (SNIP) in ALS.

Methods: Using axial 2D radial (r)AMIRA imaging at the cervical intervertebral disc level C3/C4 acquired by a 3T PRISMA scanner (Siemens healthineers) and a semi-automated segmentation approach (JIM7, www.xinapse.com), we compared SC GM areas of 36 patients diagnosed with ALS according to the Gold Coast criteria (mean age 61.7yrs, 14 women, with bulbar and spinal onset, mean disease duration 32.9 months) and 36 age- and sex-matched HC. The associations between SC GM area and disability metrics (ALSFRS-R and SNIP) were assessed by multivariable regression analyses with adjustment for age and sex.

Results: SC GM area at the level C3/C4 was significantly reduced in patients with ALS compared to HC (mean GM area in mm2 (SD): ALS 16.6 (2.3); HC 19.65 (2.7); relative reduction 15.4%, $p<0.0001$). In multivariable regression analyses adjusting for age and sex, GM area at C3/C4 explained 36.1% of ALSFRS-R variance ($p=0.0001$) and 32.2% of SNIP variance in patients with ALS, respectively.

Conclusions: Cervical SC GM area in ALS patients shows significant atrophy compared to HC and correlates with established measures of clinical disability and respiratory impairment, namely ALSFRS-R and SNIP. Further longitudinal investigations, particularly of patients early in the disease course, are necessary next steps to evaluate the potential of this novel and easy to assess imaging marker for monitoring and predicting the disease course, and its potential as a surrogate in upcoming drug trials.

(a16) Brain metabolic differences between pure bulbar and pure spinal ALS

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Introduction: Some MRI studies reported that patients with bulbar and spinal onset ALS showed focal cortical changes in the corresponding regions of the motor homunculus. 18F-FDG-PET has been employed to investigate the brain metabolic changes associated with bulbar and spinal onset ALS, with inconsistent findings. We aimed at evaluating the capacity of brain 18F-FDG-PET to disclose the cerebral metabolic features that characterize patients with pure bulbar or spinal motor impairment, since neuroimaging studies focused on this issue are lacking.

Methods: We classified as pure bulbar (PB) the patients with bulbar onset who showed a normal score in the spinal items of the ALSFRS-R at PET. We considered as pure spinal (PS) the patients with spinal onset, displaying a normal score in the bulbar items of the ALSFRS-R at PET. We included 63 PB, 271 PS subjects, and 40 healthy controls (HC). ALS patients underwent brain 18F-FDG-PET at diagnosis. We compared PB and PS ALS patients, and each patient group with HC through the two-sample t-test model of SPM12. Metabolic clusters showing a statistically significant difference between PB and PS patients where tested to evaluate their accuracy in discriminating the two groups. First, we performed a Leave-One-Out Cross-Validation (LOOCV) over the entire dataset. Four classifiers were considered for comparison: Support Vector Machines (SVM), K-Nearest Neighbours, Linear Classifier, Decision Tree. Then, we used a separate test set, composed of 10% of the patients, with the remaining 90% composing the training set.

Results: PB subjects showed a relative hypometabolism compared to PS cases in bilateral precentral gyrus in correspondence with the regions of the motor cortex involved in the control of bulbar function. SVM showed the lowest LOOCV error rate (4.19%). In the hold-out validation SVM showed the lowest error rate on the test set (9.09 ± 2.02%).

Discussion: We found clusters of relative hypometabolism in bilateral motor cortex in PB compared to PS patients, closely overlapping with the somatotopic representation of bulbar functions in the motor homunculus. The metabolism of such regions showed very high capacity to discriminate between PB and PS patients. Our data provide in vivo support for the concept of the focality of ALS onset and strengthen the idea that 18F-FDG-PET can play a role as a biomarker for precision medicine oriented clinical trials.

(a17) Radiological biomarkers of ALS using a clinically-validated web-based analysis MRI platform

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Background: Research studies in ALS showed that diffusion tensor imaging (DTI) allows to detect white matter alterations within the cerebral cortico-spinal tract considered as a proxy of upper motor neuron involvement. As the translation into clinical settings of advanced neuroimaging biomarkers developed in research framework remains a major challenge, BrainQuant, a new web-based platform, provides standardized DTI metrics clinically validated in comatose patients (Velly et al, Lancet Neurol, 2018).

Objectives: To test whether DTI metrics can discriminate patients with ALS (Gold Coast criteria) from normal controls as measured with a clinically-validated and CE marked web-based MRI analysis platform.

Methods: A 3T-MRI with DTI acquisitions was performed in 24 ALS patients and 22 sex- and age-matched controls. MRI images were then transferred and automatically processed by the MRI platform (Brainquant 2.0.6, www.braintale.eu). Outputs were the Mean diffusivity (MD) and Fractional Anisotropy (FA) in two regions of interest selected to encompass the corticospinal tract: the posterior limb of internal capsule (PLIC) and cerebral peduncle (CP). The primary objective was to test whether DTI-metrics were different in ALS patients compared to controls. Secondary objectives were to test whether DTI metrics changes correlated with functional severity (ALSFRS-R) and were abnormal in the subgroup of patients without upper motor neuron (UMN) clinical signs. Statistical analysis was performed with JMP Pro 16.

Results: MD in the PLIC and the CP were increased in ALS patients compared to controls ($p<0.017$ and $p<0.011$ respectively). Decrease of FA did not reach statistical significance ($p=0.104$ and $p=0.147$ respectively). MD in the PLIC was correlated with the ALSFRS-R score) ($p<0.028$, $r2=0.29$). Compared to controls, MD in the CP ($p<0,006$) was increased in the subgroup of patients without clinical UMN signs.

Conclusions: This study shows that a clinically-validated MRI platform can provide DTI metrics proxies of upper motoneuron degeneration in ALS patients with or without clinical signs. It suggests that this tool can be transferred in a clinical setting for diagnosis, decision-making and clinical trials recruitment. The next step is a multicentric evaluation in an ongoing ALS neuroimaging large size cohort (NCT02360891).

(a18) Resting state fmri functional connectome of C9orf72 mutation status

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Resting state functional connectome has not been extensively investigated in ALS spectrum disease, in particular in relationship with patients' genetic status. Here, 19 patients diagnosed with ALS and carrying the C9orf72 mutation (C9orf72+), 19 patients diagnosed with ALS but not affected by C9orf72 hexanucleotide repeat expansion (C9orf72+), and 19 ALS mimicking patients took part in the study. Participants were matched for age and gender. A resting-state fMRI sequence was collected for each participant and processed with a default pipeline. To determine group differences between intrinsic connectivity network nodes, a Region-of-Interest-to-Region-of-Interest network-to-network connectivity approach was performed. Our findings revealed increased connectivity between the language network and the visual network and between the default mode network and the visual network for the C9orf72- group with respect to HC. Moreover, C9orf72+ patients showed increased connectivity between the default mode network and the visual network and the fronto-parietal network and the visual network when compared with HC. Taken together, our results point towards a crucial involvement of extra-motor functions in ALS spectrum disease independently from the C9orf72 mutation status. In particular, the prominent feature is the loss of functional specificity of the visual cortex connectivity with those network underlying specific functional balance between internal (default mode) and external (language and fronto-parietal) awareness.

b) Biomarkers

(b19) Profiling of non-coding RNA as biomarkers in cerebrospinal fluid of amyotrophic lateral sclerosis patients.

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Finding objective biomarkers for the fatal neurodegenerative disease amyotrophic lateral sclerosis or motor neuron disease (ALS/MND) is critical for diagnosis, drug development, clinical trials, and providing insight into disease pathology. Non-coding RNA (ncRNA) transcripts including microRNA, piwi-interacting RNA and transfer RNA are present in human biofluids and are key candidates as biomarkers, supported by our previous work identifying dysregulated ncRNA in serum of ALS patients. To determine if the central nervous system was the source of the dysregulated ncRNA in serum, we sought to identify ncRNA biomarker candidates in cerebrospinal fluid (CSF) which may provide new insight into the disease pathology. Small RNA-seq was undertaken on CSF samples from healthy controls (n=18), disease mimics (n=8), and ALS patients (n=40) in our Oxford Study for Biomarkers of ALS cohort, with RT-qPCR used to confirm their dysregulation. We found a range of ncRNA that were dysregulated in the RNA-seq screen, but these failed to be validated or detected in some cases using RT-qPCR. Additionally, our previously identified serum ncRNA biomarker showed no change in CSF or correlation to serum. This study suggests the CSF may not be the source of dysregulated ncRNA in the serum and highlights the difficulty in identifying ncRNA in CSF as biomarkers for ALS.

(b20) Inflammatory mediators, lipoproteins and apolipoproteins in early diagnosis of Amyotrophic Lateral Sclerosis

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Introduction: There is currently no diagnostic or prognostic biomarker available in clinical practice for Amyotrophic Lateral Sclerosis (ALS). The objective of this study was to monitor a combination of various inflammatory markers, lipids, and apolipoprotein alterations in ALS patients at the time of diagnosis, to assess their role as early diagnostic or prognostic biomarker candidates.

Methods: Protein C reactive, orosomucoid, prealbumin, calprotectin, lipids and apoliproteins were determined in the blood of all subjects (25 ALS patients, 23 controls) as routinely performed in our laboratory. Inflammatory mediators were evaluated by a bead-based multiplex assay. A two-step approach was used for each analytical strategy: univariate analysis followed by multivariate analysis.

Results: Eight features were significantly different between ALS patients and controls, sometimes with important fold changes. The supervised Partial least Squares Discriminant Analysis separated ALS and controls with great accuracy (94 %) and the permutation test was significant ($p < 0.01$), ensuring the robustness of the model. The prediction model leads to a mean sensitivity and specificity of 90 (+/- 10) and 78 (+/- 10) %, respectively, with a mean predictive positive value and negative predictive value of 80 (+/- 8.9) and 89 (+/- 11.8) %, respectively. However, the models did not discriminate subgroups of ALS patients based on ALS characteristics.

Conclusion: This study highlights the usefulness of evaluating a combination of multiple pathways rather than focusing on a single target. These promising results suggest the need for the longitudinal monitoring of these candidates to determine their role in disease evolution.

(b21) CfDNA-miRNA based multiplexed biomarker system for ALS

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Cell-free biomarkers are an unmet need in ALS, both for understanding disease mechanisms and for precision medicine. We have developed methods for profiling miRNAs and cell-free methylated DNA in biofluids. In this work we generated an integrated miRNA –DNA biomarker set via research on 34 patients with ALS and 13 healthy controls. Uniquely relying on a joint analysis of both miRNA and methylated DNA form the same individual, we reveal a panel of 9 miRNAs predictive of disease status (leave one out cross validation receiver operating characteristic curve (ROC) with an AUC of 0.765 (p=0.007). Intriguingly, considering also specific cell free methylated DNA markers improved the model's goodness-of-fit and classification power (AUC=0.79, p=0.002).Circulating microRNAs and methylated DNA can establish a higher-order matrix of performance in biomarker development and should enable unprecedented accuracy in detection of motor neuron and muscle loss in ALS.

(b22) Thiol/disulphide homeostasis in Amyotrophic Lateral Sclerosis

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Thiol groups of proteins, thiol groups of low molecular weight compounds, cysteine residues, and other thiol groups are oxidized by oxidant molecules in the environment and turn into reversible disulfide bond structures. The disulfide bond structures formed can be reduced back to thiol groups, thus maintaining the thiol-disulfide balance. In this study, the status of thiol-disulfide balance in ALS patients were investigated. Serum levels of Native Thiol, Total Thiol, Disulfide was measured and the thiol-disulfide balance and, total antioxidant capacity were investigated in 31 ALS patients, 20 men and 18 women, with a mean age of 55.7±9.5 years. The measured thiol/disulfite values of the patient cohort were compared with the values of 18 patients whose mean age was not statistically different from the patient cohort (51.5±8.7 years, p=0.12). The serums of the individuals in the patient and control groups were taken at the same time and under similar conditions. While 23 of the ALS cases had upper extremity onset, 6 had lower extremity onset and predominantly spinal involvement, 2 had bulbar onset involvement. According to the Revise El Escorial diagnostic criteria, 9 patients were in the definite, 15 probable, and 7 laboratory-supported probable categories. The mean ALSFRS-R of the cases was 38±7, and the disease duration was 22±18 months (3-74 months min-max). The natural thiol level of the ALS group was 475±105 µmol/L, the disulfide level was 20±5 µmol/L, while the natural thiol level was 483±87 µmol/L, and the disulfide level was 22±6 µmol/L, in the control group. There was no statistically significant difference between the two groups (p=0.79 for natural thiol, p=0.26 for disulfide). The disulfide ratios in natural thiol and the total thiol were 4.42%±1.09% and 4.41%±1.11%, respectively, and no significant difference was observed between the two groups. No correlation was found between total thiol-disulfide ratio and ALSFRS-R. It gives the impression of a weak negative correlation with the duration of the disease, with an R2 of 0.16 and a correlation coefficient of -0.39. With these findings, it was determined that the serum thiol/disulfide balance in the ALS cohort was not different from the normal ones, and no detectable change in serum was observed in total thiol/disulfide homeostasis. It seems that the disulfide/total thiol ratio tends to decrease with disease duration.

(b23) "OMICS" Profiling of plasma-derived exosomes: the search of biomarkers for early-stage ALS

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Introduction: An urgent and unmet need in ALS is the identification of reliable biomarkers for this disease. Biomarkers may improve diagnosis, especially in early stages and could improve therapeutics efficiency. Plasma biomarkers or biomarkers from other peripheral tissues easy to be obtained and analyzed in a daily clinical practice have to be developed. Interestingly, brain-derived extracellular vesicles (EVs) can be found in plasma and used as a direct read-out of the status of the central nervous system. EVs have unique membrane composition, regarding lipids and proteins. However, no characterization was done so far for EVs derived from ALS patients. Here, we aimed to characterize the composition of plasma EVs from ALS patients to identify putative biomarkers using omics approaches.

Méthode: We obtained plasma EVs from 10 controls and 10 ALS patients included in the protocol METABOMU (NCT02670226, N°IdRCB: 215-AO1629-40). EVs were purified trough Size Exclusion Chromatography method.

Résultats: Nanoparticle Tracking Analysis (NTA) revealed no differences regarding average size of EVs (Controls: 141.4±9.75nm; ALS: 16.5±13.2nm; mean±SD) nor concentration of particles (Controls: 4.61x1010 particles/mL; ALS: 4.25x1010 particles/mL). Interestingly, ALS- and controls-derived EVs presented different Zeta potential (Controls: -22.53±4.3mV; ALS: -8.01±7.9mv; meand±SD; p=0.001 Unpaired t test). Proteomics analysis were performed by LC-MS/MS followed by TimsTOF Pro Mass Spectrometer. Proteomics analysis revealed 203 proteins significantly different between groups (Padj<0.05). Further analysis with PLGEM algorithm revealed 45 different proteins (p<0.05), 35 being increased in ALS patients in comparison to controls' EVs (for example, serum amyloid proteins and proteins S100-A9 and S100-A7) while other 10 proteins presented higher levels in Controls-EVs (ex. destrin).

Conclusion: Western blot, metabolomics and lipidomics analysis are currently being performed to confirm the differences suggested so far between Controls- and ALS-derived plasma EVs. We will further validate the application of the identified molecules or profile of molecules from EVs as putative biomarkers for ALS diagnosis.

(b24) isomiRs - a novel family of molecular biomarkers for ALS prognostication

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microRNAs are endogenous, non-protein coding, small RNAs. With Fratta and Malaspina labs we have reported the value of plasma microRNA-181 (miR-181) in ALS prognostication (Nature Neuroscience 2021). However, until recently, we were unable to analyze a much larger variety of microRNA isoforms, called isomiRs. Here, we develop a new bioinformatics technique for analysis of isomiRs. We reveal a novel panel of isomiRs that are candidate ALS biomarkers with value in disease prognostication. Intriguingly, some of the isomiRs perform better as predictors than their canonical miRNA counterparts. The study features a conceptual, first-of-its kind, evaluation of a new family of molecular biomarkers in any disease. We hope that isomiRs will allow a more accurate ALS patient prognostication and promote clinical development.

(b25) Neurofilaments can differentiate ALS subgroups and ALS from common diagnostic mimics

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A problem in the early management of ALS is the heterogeneity in clinical presentation but also that in particular early ALS disease share many clinical features with a number of alternative diagnoses. Earlier studies have revealed that a substantial number of patients initially carries an alternative diagnosis. There is thus a great demand for better diagnostic accuracy in ALS. ALS patients also show diverse inter- and intragroup variations in terms of disease duration, making prognostication difficult. In a study cohort consisting of 234 ALS patients, 44 ALS mimics and 9 healthy controls, we retrospectively investigated the diagnostic accuracy in terms of differentiating ALS patients from ALS mimics and the prognostic performance in ALS patients for cerebrospinal fluid (CSF) NFL, CSF pNFH and plasma NFL. Furthermore, we investigated differences between ALS onset types and genotype groups. Receiver operating characteristics (ROC) analyses showed highest area under the curve (AUC) for CSF pNFH in differentiating ALS patients from ALS mimics. Bulbar onset ALS patients showed significantly higher plasma NFL levels and shorter disease duration compared to spinal onset ALS patients. ALS patients with C9orf72HRE mutations had significantly higher plasma NFL levels and shorter disease duration compared to ALS patients with SOD1 mutations. All three biomarkers correlated significantly negatively with survival after symptom onset and significantly positively to ALSFRS-R progression rate. Plasma NFL showed highest AUC in differentiating ALS patients with short versus long survival time. In summary, we report minimal differences for plasma NFL compared to CSF NFL and CSF pNFH in differentiating ALS patients from ALS mimics and for estimating prognosis in ALS. Plasma NFL can differentiate ALS onset types and genotype groups.

(b26) Deep proteomics of cerebrospinal fluid for biomarker identification in ALS

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Unbiased proteomics of human biofluids is a powerful tool for the identification of changes in protein abundance that aid in disease diagnosis and investigation of the underlying pathophysiological processes, and has been used successfully to identify markers of Amyotrophic Lateral Sclerosis (ALS) in cerebrospinal fluid (CSF). However, in biofluids highly abundant single proteins mask detection of other less abundant proteins, but which may have more valuable diagnostic and prognostic use. New approaches which increase proteomic depth therefore hold great promise for clinical biomarker discovery.

We compared proteomic profiling of CSF using a simplified preanalytical sample processing method coupled with multiple mass spectrometry data acquisition methods for label free quantitation: data-dependent acquisition (DDA) and BoxCar with either ion-trap or orbitrap as mass analysers, as well as library-free data-independent acquisition (DIA). Three-fold higher proteomic depth was achieved with DIA, and quantification was highly reproducible (Pearson correlation R = 0.97-0.98). This optimised method was used to compare 50 µl samples of CSF from 40 ALS patients, 15 healthy controls and 7 disease mimics. We identified a total of 1790 protein groups, 7 of which were significantly upregulated in ALS patient compared with healthy control participants (FDR-adjusted p <0.05), including Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1), Chitotriosidase 1 (CHIT1), Chitinase 3-like protein 1, and Low affinity immunoglobulin gamma Fc region receptor III-A (FCG3A). Quantified UCHL1 (R= 0.498, p=0.001), CHIT1 (R = 0.409, p=0.009) and FCG3A (R =0.353, p=0.025) also showed strong positive correlation with disease progression rate. A further group of 20 upregulated proteins were identified that did not exceed the stringent FDR threshold, including previously described changes in Neurofilament heavy polypeptide, Chitinase 3-like protein 2, Apolipoprotein B and Macrophage-capping protein, illustrating the consistency of the method.

This demonstrates that DIA proteomics with simple pre-processing produces high proteomic depth with robust quantitation from small sample volumes of CSF suitable for biomarker discovery. Applying this to a cohort of ALS patient samples identified several candidates for further analysis.

(b27) Frepeaters as an index of disease progression in ALS

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Objectives: We studied the value of repeater F-waves (Freps) analysis in variably affected nerves to assess alterations of motor neuron pool in amyotrophic lateral sclerosis (ALS).

Methods: Forty consecutive F-waves were recorded from the ulnar nerve in 25 ALS patients with normal compound muscle action potential (CMAP) measurements (Group A), 25 ALS patients with abnormal CMAP measurements (Group B) and 50 healthy control subjects. Data were imported into an automated computerized system (F Wave Analyzer), which identifies Freps and groups them. Freps frequency variables and F-wave latencies were compared between the study groups.

Results: F-wave persistence was significantly lower in Group B compared to Group A (62.6±27.9% vs 86.6±17.7%, P<0.005). Minimum F-wave latencies in Group A were significantly prolonged compared to healthy (27.94±2.17msec vs 25.39±2.17msec, P<0.005), but shorter compared to Group B (30.49± 3.25, P=0.006). Occurrence of Freps per stimuli (Freps persistence= 100xFreps/40stimuli) was significantly increased in both ALS groups compared to the control group (P ≤ 0.005); however the frequencies of Freps per number of total F-waves (Index Total Freps =100xFreps/total number of F-waves) was significantly higher in Group B compared to Group A (71.9±20.9 vs 44.8 ± 19.4, P<0.005).

Discussion: These findings indicate that an increased number of Freps is an early sign of pathology, being present in muscles with normal standard motor conduction studies. Disease progression, demonstrated by abnormal CMAP parameters, was characterized by the preservation of increased total Freps accompanied by a reduction of F non-repeaters.

Conclusion: The early appearance of Freps reflects the hyperexcitability on motorneurons (MNs), while the ensuing reduction of F non-repeaters implies loss of MNs during the course of the disease.

(b28) The role of plasma CHI3L1 levels in Amyotrophic Lateral Sclerosis

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Background and aim: motor neuron diseases (MND) are neurodegenerative diseases characterised by complex and heterogeneous pathological mechanisms. Biomarkers could help in defining patients' prognosis and stratification. Recently, besides neurofilaments, chitinases seem to be a promising family of biomarker. They correlate with neuroinflammation status and they include CHIT1, CHI3L1 and CHI3L2. In one study CHI3L1 CSF levels have been correlated with cognitive impairment. Since blood samples are easy and less invasive to obtain compared to CSF, we wanted to evaluate CHI3L1 plasma levels in MND, MND mimics and healthy controls (HCs).

Methods: sandwich ELISA was used to quantify plasma CHI3L1 from 44 MND (including 8 ALS/FTD), 7 HSP, 9 MND mimics (including myelopathy, radiculopathy, axonal neuropathies) and 19 HCs. ALSFRSr, MRC, spirometry, genetic tests, disease progression rate (PR), blood examinations, neuropsychological tests (MMSE, ECAS, TMT-A, TMT-B, RAVLT, ROCF, FAB, Digit Span, FRSBE). We analysed data using Kruskal-Wallis, ANCOVA and Cox regression analysis.

Results: CHI3L1 plasma levels result to be different between groups ($p= 0.029$), in particular Bonferroni's correction shows that MND mimics have higher levels of CHI3L1 compared to MND and HCs. No difference between HSP, MND and HCs ($p>0.05$). Differences are confirmed co-variating for age and sex ($p= 0.022$). A sub-group analysis of MND patients (divided in PLS, ALS and PMA) do not show any difference in CHI3L1 levels. Moreover, CHI3L1 do not correlate to ALSFRSr, MRC, FVC, FEV1, PR and blood examination, except for red blood cells and for haemoglobin (respectively, p 0,05).

Discussion: CHI3L1 plasma levels result to be increased in acute myelopathy, radiculopathy and neuropathies, compared to MND, HSP and HCs. This is consistent with the increase of CHI3L1 in neuroinflammatory processes. Contrarily to CHI3L1 CSF levels, CHI3L1 plasma levels are not able to differentiate between ALS and HCs and do not correlate with neuropsychological impairment. Further multicentre studies, including a huge number of patients and testing together other fluid biomarkers, are needed to better explain the role of CHI3L1 in diagnosis and prognosis in MND and, also, in neuropathies

(b29) Underscoring the role of fatty acid elongation in ALS

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive degenerative motor neuron disease with no effective treatment and no accepted prognostic biomarker in clinical practice or clinical trial development. Analysis of lipid metabolism has produced descriptive biomarkers of disease with additional information on the biological processes affected in a wide range of psychiatric and neurodegenerative disorders, including Alzheimer's and Parkinson's disease. Clinical and experimental studies have already shown that lipids also play an important role in the neurodegenerative process of ALS, and blood cholesterol levels have been proposed to predict survival. Some lipid-lowering strategies have been tried in the disease with promising results. In a previous study in which we performed a comprehensive analysis of the blood lipidomic profile of ALS patients, we reported imbalances in long-chain fatty acids that suggested specific alterations in the utilization or production of such biomolecules. Subsequently, through the specific analysis of the relative amount of the different fatty acids in the lipid fraction in a follow-up cohort and the application of complex computational methods, we were able to identify the participation of the very long chain fatty acid elongation (ELOVL) in ALS disease progression. A recent publication has revealed the role of very long chain saturated fatty acids in the toxicity exerted by glia in neurodegenerative processes, postulating the inhibition of elongation as a first-class therapy. In our hands we have observed that both genetic and pharmacological inhibition with a potent, selective and orally bioavailable inhibitor of ELOVL consistently improves neuromuscular phenotypes and life expectancy in fruit flies with deficiency of TDP-43.

c) Genomics

(c30) "When the odds are down to the toss of a coin…": pre-symptomatic genetic testing amongst people at an increased risk of inherited MND.

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Background: Pre-symptomatic genetic testing is available to some people who are at an increased risk of developing inherited MND and is generally offered where a gene variant has been identified in an affected relative. However, calls for genetic testing to be routinely available for people with MND means that more people could face such decisions in the coming years. This study aims to explore the experiences of individuals around pre-symptomatic genetic testing, including how people engage with such choices and the reasons and factors involved.

Methods: This study draws on 35 semi-structured interviews, carried out as part of a study on family experiences of inherited MND. We focus on the experiences of people living at an increased risk of MND, including those who had had pre-symptomatic genetic testing and those who had not had testing (yet). Interviews were analysed thematically, using a method of constant comparison.

Results: We present findings on how people engage with choices around genetic testing, highlighting that what is often described as a "decision" is not always experienced as such; whilst some people weighed up pros and cons, others had an immediate and self-evident sense of whether they wanted to pursue testing or not. Views and decisions sometimes changed over time and with changing circumstances. Secondly, this study looks at the motivations and factors involved in choices around testing, highlighting the many considerations at play, based on people's past experiences, present-day considerations, and future goals.

Discussion: We highlight choices around genetic testing as multi-faceted and complex, made in the context of individual beliefs and goals, life stages and circumstances, and family responsibilities and relationships. Understanding the factors and considerations involved in pre-symptomatic genetic testing is important for healthcare professionals when supporting individuals and families at an increased risk of developing inherited MND.

Acknowledgements: We wish to thank the participants who took part in the interview study for sharing their experiences. We are grateful for the support of the MND Association, our funders for this study, and the Institute of Applied Health Sciences, University of Aberdeen, who fund Jade Howard's PhD studentship.

(c31) Common genetic polymorphisms of inflammation and oxidative stress genes modify ALS

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Background: Inflammation and oxidative stress are recognized as important contributors to amyotrophic lateral sclerosis (ALS) disease pathogenesis. Our aim was to evaluate the impact of selected single-nucleotide polymorphisms in genes involved in inflammation and oxidative stress on ALS susceptibility and modification.

Methods: 185 ALS patients and 324 healthy controls were genotyped for nine polymorphisms in seven antioxidant and inflammatory genes using competitive allele-specific PCR. Logistic regression, nonparametric tests and survival analysis were used in statistical analysis.

Results: Investigated polymorphisms were not associated with ALS susceptibility. Carriers of at least one polymorphic SOD2 rs4880 T or IL1B rs1071676 C allele more often had bulbar ALS onset (P=0.036 and P=0.039, respectively). IL1B rs1071676 was also associated with a higher rate of disease progression (P=0.015). After adjustment for clinical parameters, carriers of two polymorphic IL1B rs1071676 C alleles had shorter survival (HR=5.02, 95% CI=1.92-13.16, P=0.001), while carriers of at least one polymorphic CAT rs1001179 T allele had longer survival (HR=0.68, 95% CI=0.47-0.99, P=0.046).

Conclusion: We found that selected common variants of the antioxidant and inflammatory genes modify ALS disease. These types of studies are important to identify specific patient populations that may be responsive to immune or antioxidant system - based therapies.

(c32) TBK1 p.E696K mutation causes autophagolysosomal dysfunction and ALS/FTD-like symptoms but not inflammation in mice

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TANK-binding kinase 1 (TBK1) is a Ser/Thr kinase regulating selective autophagy and innate immunity. Heterozygous mutations in TBK1 cause genetic ALS and FTD in men. While almost all cases of TBK1-linked, dominantly inherited ALS are caused by loss-of-function mutations, few instances of missense variants with proven pathogenicity are known with E696K variant being one of them. Here, we generated and characterized mice with a constitutive, global hetero- and homozygous knock-in (KI) of the E696K variant. While mice with a full KO of Tbk1 do not display motor deficits or neurodegeneration even at a high age, mice with a specific Tbk1 point mutation develop an age- and gene dose-dependent motor phenotype, behavioral deficits, neuromuscular junction denervation and histological evidence of autophagy impairment including accumulation of dysmorphic and enlarged lysosomes. Homozygous deletion of TBK1 results in TNF-alpha hypersensitivity, RIPK-dependent liver necroptosis and immune cell infiltration in multiple organs. This immune phenotype was completely absent in the mice with a homozygous KI of E696K. Thus, the missense mutation in TBK1 caused an ALS/FTD-related phenotype in mice, possibly due to a toxic gain of function. The observed autophagy defects combined with the absence of autoimmunity and RIPK-dependent necroptosis supports the conclusion that autophagy- rather than immune-linked functions of TBK1 are most relevant for ALS/FTD causation.

(c33)Genetic Analysis of ALS in Norway ("GAIN")

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Knowledge of genetic causes of ALS is becoming increasingly important as new treatments are emerging. In Europe, disease-causing genetic variants have been identified in 40–70% of familial ALS patients and approximately in 5% of sporadic ALS patients, but these percentages vary from region to region. The study "Genetic Analysis of ALS in Norway" (GAIN) was started in 2019 to collect information on genetic variants in Norwegian persons with ALS. All 17 neurological hospital departments in Norway recruit patients. Clinical and genetic data on familial and sporadic ALS patients in this population-based cohort were gathered during a 2-year period. Genetic analysis of the samples included expansion analysis of C9orf72 and exome sequencing targeting 30 known ALS-linked genes. The variants were classified using ACMG criteria, genotype-phenotype correlations and bioinformatics tools. So far 279 ALS patients have been included. Of these, 11.5% had one or several family members affected with ALS, whereas 88.5% had no known family history of ALS. A disease causing variant was identified in 11.1% of the participants, 58.1% of whom had a known family history of ALS, and 41.9% were considered sporadic. The most common genetic cause was the C9orf72 expansion, followed by SOD1 and TBK1. Our results show that restricting genetic testing to patients with a family history of ALS would exclude a large considerable proportion of patients from participation in gene-targeted medical trials.

(c34) Pathway-level perturbations link the pre-symptomatic synapse in CLN3 disease, spinal muscular atrophy, and ALS.

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Tandem mass tagging-based proteomic profiling of pre-symptomatic SMA (spinal muscular atrophy – a childhood MND), ALS type 8 (amyotrophic lateral sclerosis - a late adult onset MND), and CLN3 (a juvenile dementia) rodent models through parameters of regional vulnerability and pathological gene "dose" identified 200 conserved candidates altered in a dose-dependent manner with respect to their distinct causative mutations. Predictive in silico analyses confirmed that these individual protein alterations interact hierarchically to mediate dysregulation in a broader cellular context, including changes in a number of metabolic pathways. Strikingly, both genetic and pharmacological manipulation of the majority of conserved candidates, regulates degeneration across multiple in vivo disease models. Investigation within the synaptic proteomes of differentially vulnerable regions in post mortem brains reveals that these regulatory pathways are also activated in a regional-vulnerability-dependent manner in spontaneous ALS with predictive mechanistic regulation of lipid homeostasis. These results provide evidence for shared pathogenic mechanisms promoting a common degenerative signature that link otherwise unrelated degenerative diseases affecting every stage of the human lifecourse, including genetic and spontaneous ALS.

(c35) ATXN2 as genetic risk factor in Spanish population

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Background: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia form a clinical, pathological, and genetic continuum. The aim of this study is to evaluate the association of several intermediate "CAG" repeats the ATXN2 gene as a risk factor for ALS and/or FTD.

Methods: 757 patients were analyzed (620 ALS patients and 137 FTD patients) as 362 age- and sex-matched controls from 3 referral centres from 3 different regions of Spain: Hospital La Fe (Valencia), Hospital de Bellvitge (Barcelona) and Hospital 12 de Octubre (Madrid). The sequencing methods were PCR with labeled primer for the detection of alleles of different sizes and in cases where only one size was obtained, repeat-primed-PCR was performed to discern possible masked long alleles.

Results: In the ALS group, a higher number of patients (55) were found to harbor intermediate repeats (≥ 27) of the CAG triplet compared to controls (10) (O.R.= 2.739; C.I. 95% 1.263-5.619; p = 0.0097). However, the same association was not found for FTD patients (O.R.= 1.252; C.I. 95% 0.4821-3.471; p = 0.6548).

Discussion: Our outcomes in the Spaniard population are similar to those carried out in other countries such as France, Canada or Italy. However, they are different from studies in other countries with a greater threshold number of CAG triplet repeats in the ATXN2 gene (> 30 repeats). In this case the p-value obtained (< 0.01) for intermediate repeats (≥ 27) is statistically significant, and slightly lower comparing to that obtained with the directly higher number of repeats (≥ 28; p= 0.018).As in our results, there are several studies in which a higher association with an intermediate number of repeats has been seen in FALS cases (Daoud et al., 2011; Lee et al., 2011; Lattante et al., 2014).Keywords: ATXN2, risk factor, ALS, FTD

Acknowledgements: This work was supported by grants PI17/00491 from the Instituto de Salud Carlos III (ISCIII), the support of Comunidad de Madrid (B2017/BMD-3813 "ELA-Madrid"), CM-Fundación H12O agreement (BOCM 142 – 17/09/2019 pg. 105), and Plataforma de afectados de ELA.

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(c36) Assessing the impact of C9orf72 DNA methylation using CRISPR/Cas9-targeted Nanopore sequencing

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The heritability of amyotrophic lateral sclerosis (ALS) is estimated to be 40 to 50%. Many of the genetic factors involved in the pathogenesis of ALS remain incompletely understood. A hexanucleotide repeat expansion in C9orf72 is the most common genetic aberration in familial ALS. Previous studies have shown that for the majority of ALS patients carrying a C9orf72 repeat expansion, elevated DNA methylation occurs within the repeat, and for up to 36% in the CpG island 5' of the repeat. DNA methylation in the C9orf72 repeat and surrounding CpG islands is a possible disease modifier of ALS, although there is still conflicting evidence about the direction of its effect. To investigate whether DNA methylation in and around the repeat is associated with repeat length and various ALS disease characteristics, we selected patients with early versus late onset, fast versus slow disease progression and high versus low penetrance in the family. We performed amplification-free long-read Nanopore sequencing on DNA isolated from the blood of these patients with CRISPR/Cas9-targeted enrichment of the C9orf72 gene. With this approach, we have reached average coverage of 40-330x across the full gene. Analysis of repeat length on a single-read level suggests intraindividual repeat length instability of large repeat expansions. We will determine methylation status within the repeat and surrounding CpG islands in the same sequencing reads and correlate them with repeat length on a single-read level. In addition, we will compare methylation status between patients with different disease characteristics. These results could provide grounds for analysis in a larger patient cohort, which could point towards an important modifier role for DNA methylation in and around the C9orf72 hexanucleotide repeat.

(c37) Investigating the role of stress granule composition on C9ORF72-related ALS

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One of the challenges of our ageing population is the increasing prevalence of age-related diseases such as amyotrophic lateral sclerosis (ALS). ALS is characterized by the gradual degeneration of motor neurons and is fatal 2-5 years after its diagnosis. GGGGCC hexanucleotide (G4C2) expansion in the C9ORF72 gene is the most common genetic cause of familial cases. Different mechanisms have been proposed regarding how G4C2 expansion contributes to the pathology of ALS. One of those mechanisms is that the expanded G4C2 repeats are translated in sense and anti-sense manner through repeat-associated non-ATG (RAN) translation, giving rise to 5 different dipeptide repeats (DPRs), namely GR, PR, GA, AP and GP. Among them, poly-GA is known to be aggregation-prone whereas poly-PR and poly-GR phase separate and additionally disrupt nucleocytoplasmic transport and stress granule (SG) dynamics. In this project, we would like to gain further insights into the link between DPR-protein aggregation and neurodegeneration. To achieve this goal, we will use a trimodal approach, induced pluripotent cells (iPSCs) from ALS patients with an expansion in their C9ORF72 gene, motor neurons derived from iPSCs (MNs) and C. elegans models expressing dipeptides and G4C2 repeats. Our preliminary results demonstrated the differences in stress granule dynamics in both iPSCs and MNs. Analysis of the composition of SGs in MNs revealed common loss-of-function and gain-of-function interactors. Screening of these candidates using a targeted RNAi approach in ALS C. elegans models revealed that while some candidates only affect the insoluble DPR levels in G4C2 repeats, other candidates had an impact on both DPR and SG aggregates. The effects of the knock-down on neurotoxicity will be further assessed by motility assays, and genetic manipulation of our cell lines. We believe that our project can lead to defining novel mechanisms for C9-ALS pathology.

(c38) A five-years collection of ALS-related mutations in patients from an Italian Center for Motor Neuron Diseases

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder involving upper and lower motor neurons. Although it is mostly sporadic, ALS pathogenic mutations are recognizable especially in those patients who had an early disease onset and a positive family history for neurodegenerative disorders. More than 50 genes have been put in relation with ALS so far, among which SOD1, C9Orf72, FUS, ATXN2 and TARDBP are the most frequent. Because of their pleiotropic behavior and variable penetrance, cases of unexpected positivity are often detected because of the widespread use of genetic tests performed in association with the availability of gene therapy. Here we describe the distribution of sporadic and genetic forms of ALS in patients followed by a large Center for Motor Neuron Diseases in Central Italy.174 patients with ALS are currently in charge in our Center for Motor Neuron Diseases at Santa Chiara Hospital, Pisa, Italy. Among these, 92 patients performed genetic tests for ALS related genes (NGS panel analysis, C9Orf72 and ATXN2 expansion) between January 2017 and March 2022.Among the 17 patients with a positive genetic test, 14 had pathogenic mutations and 3 had a Variant of Uncertain Significant Mutation. Among the former, seven patients had a mutation in C9orf72, three in FUS, three in SOD1 and one in TBK1. Among patients with C9Orf72 mutation, one had a late disease onset and no familiar track record, while one had a clinical phenotype consistent with a Primary Lateral Sclerosis. Among patients with FUS mutations, one had a late disease onset and no family history, while the other had an early disease onset, a long disease duration and no family history for neurodegenerative disorders, although a sister died young for cerebral malignancy. Among SOD1 mutations, one patient had a new mutation never described before but predicted to be pathogenic, while one had a very long disease duration and is still alive after more than 15 years. The patient with TBK1 mutation had an early disease onset and no family history. The spread of genetic analysis due to gene therapy has highlighted the multifaceted possible phenotypes linked to pleiotropy and to variable penetrance of the involved genes, often revealing unexpected positivity and increasing the amount of genetic ALS. The analysis of large panels of patients may increase our knowledge of pathogenetic mechanisms and contribute in discovering new genes involved in disease development

(c39) Unravelling genetic modifiers of ALS caused by mutations in FUS and TDP-43

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An essential characteristic of ALS is that patients have a remarkable variability in disease presentation, such as onset, progression and survival, suggesting that important disease-modifying genes exist, which if identified, will serve as novel therapeutic targets. Our lab generated a Drosophila FUS model, in which expression of FUS in the fly's motor neurons leads to an eclosion defect. Using this fly model, we performed a whole-genome unbiased screen, to identify candidate modifiers of FUS toxicity.

Two of the identified candidate modifiers were mts (PP2A) and sgg (GSK3).

Both, genetic and pharmacological inhibition of PP2A and GSK3 in flies, rescues toxicity. PP2A acts as an upstream inhibitor of GSK3, since modifying the activity of PP2A in fly tissue and human cells affects GSK3 inhibitory phosphorylation. Inhibition of PP2A and GSK3 in Drosophila TDP-43 models also rescues toxicity, suggesting a broader applicability of our findings.

Axonal transport deficits are common to many ALS-associated iPSC motor neuron models, suggesting a shared downstream mechanism of toxicity. Mitochondrial transport is mediated by the motor protein kinesin, a substrate of GSK3. Interestingly, pharmacological inhibition of PP2A and GSK3 rescue axonal transport deficits of FUS iPSC-derived motor neurons. Moreover, we have found that overexpression of kinesin rescues FUS-induced toxicity in Drosophila too, suggesting that GSK3 and PP2A inhibition may affect toxicity by regulating axonal transport directly.

It has previously been suggested that GSK3 may become hyperactive in ALS patients. Consistent with a pathologically increased GSK3 activity, we have observed reduced GSK3 inhibitory phosphorylation in our Drosophila models. Currently, we are testing if the same applies in iPSC motor neurons from patients.

Altogether, we found a previously unexplored mechanistic link between PP2A, GSK3 and kinesin in FUS and TDP-43 models, linking two previously described ALS phenotypes, GSK3 hyperactivity and axonal transport deficits.

(c40) ALS/FTD-associated C9orf72 C4G2 repeat RNA disrupts phenylalanine tRNA aminoacylation

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Background: GGGGCC hexanucleotide repeat expansion (HRE) mutation in the C9orf72 gene is the most common genetic cause of ALS and FTD that can reach hundreds to thousands repeats in disease. Transcription of repeats in sense and antisense direction leads to repeat(G4C2)n and (C4G2)n RNA, which can sequester RNA binding proteins and form RNA foci pathognomonic of C9orf72 associated ALS and FTD, predicted to cause RNA toxicity by. The aim of this work was to identify proteins that bind antisense C4G2 RNA transcripts. In our work we show interaction of proteins involved in protein synthesis, cytoskeleton stability and mRNA processing with antisense RNA transcripts. The study focuses on cytoplasmic interaction with Phe-tRNA synthetase (FARS) and its effect on protein synthesis, as disruptions in aminoacyl-tRNA synthetases are increasingly observed in neurodegenerative disorders and can lead to protein misacylation, misfolding and aggregation.

Objectives: Identification of proteins binding to antisense (C4G2)32 RNA transcripts from C9orf72 mutation and determination of how antisense RNA-FARS interaction impact FARS aminoacylation function.

Methods: We performed RNA-pull down assay from mice brain lysates and mass spectrometry to determine proteins that bind to (C4G2)32. Interactions were confirmed using WB, FISH/ICC and RNA-protein PLA. Impact of antisense RNA-FARS interaction was determined using aminoacylation assay and western blots.

Results: We have shown that FARSA and FARSB, interact with (C4G2)32 in RNA-pull down assay from mice brain lysates. The interaction was confirmed using three different assays and interaction between FARSA and (C4G2)32 results in significant decrease of charged tRNAphe in patient lymphoblasts compared to control. Additionally, expression of three phenylalanine-rich proteins was observed to be lower in C9-ALS patient lymphoblasts in comparison to control. The same was not seen for proteins low in phenylalanine.

Conclusion: We found impairment of FARS catalytic function in C9 lymphoblasts, as tRNAphe charging is reduced compared to control. This discovery is important in highlighting the role of aminoacyl-tRNA synthetases in C9orf72 ALS/FTD, as they have been so far implicated in various neurodegenerative diseases.

(c41) Characterizing SOD1 mutations in Spain. The impact of genotype, age, and sex in the natural history of the disease

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Introduction: The aim of this study is to describe the frequency and distribution of SOD1 mutations in Spain, and to explore those factors contributing to their phenotype and prognosis.

Methods: Seventeen centres from 14 regions in Spain shared data on the frequency and characteristics of amyotrophic lateral sclerosis (ALS) patients carrying pathogenic or likely pathogenic SOD1 variants. The relationship between demographic, clinical and genetic variables was explored using plots and multivariable models.

Results: In 144 patients (from 88 families), 29 mutations (26 missense, 2 deletion/insertion and 1 frameshift) were found in all 5 exons of SOD1, including 7 novel mutations. 2.6% of ALS patients (including 17.7% familial and 1.3% sporadic) were estimated to carry SOD1 mutations. Its frequency varied considerably between regions, due to founder events. The most frequent mutation was p.Gly38Arg

(n = 58), followed by p.Glu22Gly (n = 11), p.Asn140His (n = 10), and the novel p.Leu120Val (n = 10). Most mutations were characterized by a protracted course, and some of them, by atypical phenotypes suggestive of spinal muscular atrophy, or motor and/or sensory neuropathy. Older age of onset was independently associated with faster disease progression (exp(Estimate) = 1.03 [0.01, 0.05], p = 0.001) and poorer survival (HR = 1.05 [1.01, 1.08], p = 0.007), regardless of the underlying mutation. Female sex was independently associated to faster disease progression (exp(Estimate) = 2.1 [1.23, 3.65], p = 0.012) in patients carrying the p.Gly38Arg mutation, resulting in shorter survival compared with male carriers (236 vs 301 months).

Conclusions: The data presented here may help to evaluate the efficacy of SOD1 targeted treatments in both clinical trials and clinical practice, and to expand the number of patients that might benefit from these treatments.

(c42) Genome-wide assessment of genetic modifiers in ALS progression

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Our latest and largest genome-wide association study in amyotrophic lateral sclerosis (ALS) to date, including over 29,612 ALS patients, identified 15 SNPs significantly related to ALS risk . This study shed light on central pathophysiological mechanisms in ALS by identifying numerous ALS risk factors and biological processes with a notable lack of neuroinflammatory processes. The identified SNPs, however, showed overall little effect on ALS progression individually or when used as constructed polygenic risk scores (PRS). This could imply different biological mechanisms are involved in ALS susceptibility compared to ALS progression which could have extensive therapeutic consequences. Studying the genetics of disease progression is way more challenging than comparing cases versus controls. Collecting and harmonizing clinical data in thousands of subjects requires the dedication of many clinical researchers around the globe. To start to unravel the genetic architecture of ALS progression we set up a first study that included 6,349 whole-genome-sequenced patients with ALS, included in Project MinE, where we collected and harmonized clinical data. We adopted a cox proportional hazards model in a mixed model framework correcting for population structure, relatedness and added site of onset, country, platform, sex, and PC1-20 as covariates. This analysis identified two significant low-frequency variants on chromosomes 11 and 14 just passing genome-wide significance, not seen before in our case control GWAS, and confirmed previously identified signals for C9orf72 and UNC13A (although not genome-wide significant). This motivates further increasing sample size for future analyses. Therefore, we are currently collecting clinical data for over 10,000 ALS patients for which we already have genotype data available. With this increased sample-size we aim to identify the genetic architecture of disease progression and more specifically to identify SNPs and biological processes associated with ALS progression. Combined with our repeat expansion imputation panel under development, this could lead to the identification of new therapeutic targets specifically related to ALS progression.

(c43) Mutation in ALS associated KIF5A C-terminus promotes protein aggregation

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the loss of upper and lower motor neurons. Patients suffering from this pathology are clinically characterized by progressive muscle weakness, followed by death mainly due to respiratory failure, within 2 to 5 years from the onset of symptoms. Despite the underlying mechanisms responsible for the selective loss of motor neurons being still unclear, several genes with heterogeneous functions have been causally linked to ALS. Recent genomic studies have shown one such gene to be causative of motor neuron disease, is KIF5A, which encodes for a kinesin involved in protein transport. The KIF5A mutations identified in ALS patients are predicted to affect the splicing of exon 27, leading to a frameshift mutation affecting the cargo binding domain and leading to an aberrant protein product. Overexpression of this mutant form of KIF5A triggers the accumulation of cytotoxic protein aggregates and induces apoptosis in vitro. Similar cytotoxic aggregates were also identified in human KIF5a-mutant motor neurons. Our data indicate that protein aggregation and impaired degradation might represent a crucial alteration characterizing the patho-biochemistry of KIF5A ALS cases.

(c44) Targeting C9orf72 repeat-expanded RNAs with antisense FANA oligonucleotides.

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Massive expansion of the 5'-GGGGCC-3'/3'-CCCCGG-5' sequence within the chromosome 9 open reading frame 72 (C9orf72) gene has been associated with familial amyotrophic lateral sclerosis (ALS).1,2 Both the mutant intronic and antisense RNAs have been implicated in this disease. Indeed, C9orf72 repeat-containing RNAs accumulate in aberrant nuclear foci1,3 that may sequester RNA-binding proteins (RBPs) leading to a loss of function.4 Additionally, non-ATG translation gives rise to dipeptide-repeat proteins (DRP) which contribute to toxicity and cell damage.5 This makes targeting of C9orf72 repeat-containing RNAs a very promising strategy to combat ALS.

Antisense therapy allows the degradation of an RNA through the activation of RNase H upon hybridization of the targeted RNA with a complementary antisense oligonucleotide (ASO). Likewise, and depending on the chemical composition of the ASO, the formation of the RNA:ASO hybrid can lead to translation arrest by steric hindrance of the ribosome. Different ASOs targeting C9orf72 have shown success in rescuing phenotypic defect in different patient-derived models.6 We have designed and synthesized a collection of ASOs containing 2'-fluorine modifications in the sugar ring, targeting both the G- and C-rich C9orf72 repeated RNAs. Here we present results on the in vitro activity of these ASOs. We found that most of the ASOs are able to fold into secondary structures which does not preclude the formation of very stable duplex with the target RNA. Moreover, we tested the ASOs in ALS cell models and observed that some of them are efficient in reducing RNA foci and C9orf72 expression.

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(c45) SARM1 variants in a cohort of Italian ALS patients

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Axonal degeneration is an early stage of several neurological disorders. Damaged axons are cleared via an intrinsic self-destruction pathway, known as Wallerian degeneration, in which the activation of the central executioner SARM1, a NAD+ hydrolase, has a crucial role. SARM1 TIR domain is responsible for enzymatic activity, whereas the auto-inhibitory ARM domain maintains the protein in an inactive state in healthy neurons. Two recent studies demonstrated that variants in the ARM domain, that cause constitutive hyperactivation of SARM1 NADase activity, are more frequent in patients with amyotrophic lateral sclerosis (ALS) compared to controls. These results support an emerging role for SARM1 mutations in ALS and led us to investigate the presence and impact of SARM1 variants in a cohort of Italian patients with motor neuron disease (MND).

In this study, approved by the local ethic committee, we sequenced a cohort of 364 unrelated Italian MND patients (207 males and 157 females), including ALS (n=278), primary lateral sclerosis (PLS, n=28) and lower motor neuron disease (LMND, n=58). Our in-house control cohort was represented by 369 non-neurological patients. We screened the patients using a next-generation sequencing panel and, after filtering, variants were confirmed with Sanger sequencing.

We detected and confirmed six different SARM1 rare variants (Minor Allele Frequency, MAF<0.01) in 10 unrelated patients (0.27% of the cohort). All of these variants were missense, were found in heterozygosity and were classified as variants of uncertain significance (VUS), according to the ACMG classification. Only one was also present in the control cohort. Two patients had the same variant, that was also reported in two patients and in zero controls from previous studies and might cause a gain of protein function. One patient carried two variants, including a novel variant. Five patients shared a missense variant which was absent in our control cohort; however a previous study reported it with a similar frequency in patients and controls. None of the patients with SARM1 variants had a concomitant variant in major ALS-related genes. MND patients carrying SARM1 variants did not significantly differ for sex distribution, age at onset, site of onset or survival from the other patients of the cohort. However, we observed that survival was significantly shorter for patients carrying variants located in the ARM domain of the protein, when compared to the rest of MND

(c46) Comparative analysis of the structural dynamics of Superoxide dismutase 1 (SOD1) variants associated with Amyotrophic Lateral Sclerosis.

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Mutations in the Superoxide Dismutase 1 (SOD1) gene are linked to both familial and sporadic Amyotrophic Lateral Sclerosis (ALS). SOD1 is a metalloenzyme, which exists as a homodimer and catalyzes the conversion of superoxide O2- free radical present in the cytoplasm to hydrogen peroxide. SOD1 protein structure is characterized by the presence of an immunoglobin fold, electrostatic loop (ESL) and a metal binding loop (MBL). At present, more than 200 SOD1 variants have been found in ALS patients. In most cases, the basis of these variants being attributed to ALS is simply that they are rare and found in SOD1. Neither of these is sufficient for such a statement to be made. The p.D91A variant, for example, reaches polymorphic frequency in parts of Scandinavia, and yet has been convincingly shown to be causative of ALS. Moreover, the clinical phenotype of people with SOD1 ALS is highly heterogenic e.g. A4V variant is associated with a rapid disease progression whilst others like H46R with slower forms of disease. The aim of our study is to identify structural properties of wild type SOD1 and its mutants, that can help us explain their phenotypic variability and quantify their effect on the risk of ALS. We did this by characterizing the differences in the dynamic behaviour SOD1 of the wt-SOD1 and in presence of different missense mutations using molecular dynamics (MD) simulations. We performed MD simulations over 100 ns for wild type SOD1 and 12 missense mutations (A4V, C57S, D124V, D125H, G37R, G85R, G93A, H46R, I113T, L38V, T2D, T54R) in SOD1. We observed that the difference in structural features such as number of hydrogen bonds, conformational flexibility of the ESL, might be able to explain diverse clinical outcomes of different variants. Our results could be used to predict the effect of novel SOD1 variants as they are found in patients and classify the 200+ already found.

(c47) HnRNP A1B, a splice variant of HNRNPA1 dependent on TDP-43, has a novel function in cytoskeleton-dependent transport

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The development of an effective treatment for amyotrophic lateral sclerosis (ALS) is urgent and dependent on a better understanding of the molecular mechanisms underlying the pathology. TDP-43 mislocalization is observed in the majority of ALS cases and we have discovered that TDP-43 nuclear depletion drives the accumulation of an alternatively spliced variant of heterogeneous nuclear ribonucleoprotein A1 (HNRNPA1), termed hnRNP A1B. hnRNP A1B differs by the inclusion of the 7B exon that elongates its intrinsically disordered region by 52 aa. hnRNP A1 is well studied and is a major player in RNA metabolism, but surprisingly little is known about the splice variant hnRNP A1B. HNRNPA1 is mutated in rare cases of familial ALS and some mutations are in the 7B exon and thus uniquely impact the hnRNP A1B isoform. Their contribution to disease pathogenesis is not clearly understood due to the lack of information about hnRNP A1B function, but they are known to drive aggregation. We aim to investigate the expression and function of hnRNP A1B to understand its contribution to physiology and pathology. Using a custom antibody specific for hnRNP A1B, we have found that hnRNP A1B expression is more restricted to the central nervous system (CNS) compared to hnRNP A1. Also, hnRNP A1B expression becomes progressively restricted to the motor neurons in the spinal cord ventral horn, compared to hnRNP A1 which is more broadly distributed. We also uncovered that at steady state, hnRNP A1B is present in the nucleus and in the soma of motor neurons, compared to hnRNP A1 which is only nuclear. In addition, hnRNP A1B forms puncta in neuronal processes. To characterize the role of hnRNP A1B in the soma of motor neurons, we have performed IP-MS from mouse spinal cord. The interactome of hnRNP A1B is enriched in cytoskeletal motor proteins suggesting of a role in transport. We have demonstrated that hnRNP A1B granules in neuronal process contain messenger RNA (mRNA) and nascent proteins which collectively support the role for hnRNP A1B as an RNA adaptor in mRNA transport and a regulator of local translation. Our results demonstrate that both isoforms of HNRNPA1 are differentially expressed across tissues and have distinct localization profiles, suggesting that the two isoforms may have specific subcellular functions. Future work will examine the respective contribution of each HNRNPA1 isoform, and their disease-associated mutations, may uniquely contribute to disease pathogenesis

(c48) Reprogrammed astrocytes from a C9-ALS family with variable penetrance display differential C9orf72 pathology and motor neuron toxicity in co-culture

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Hexanucleotide repeat expansions in C9orf72 (C9-HRE) are the most common genetic risk factor for amyotrophic lateral sclerosis (ALS). The triggers for the switch between the asymptomatic and symptomatic disease state, which emerges with age, are poorly understood. Our group has shown that induced neural progenitor cells (iNPCs) and their astrocyte derivatives (iNPC-As) retain the ageing features of donor fibroblasts (Gatto et al., 2021). Here we describe a C9-HRE+ family with an asymptomatic father and discordant twins. The affected twin presented with ALS at age 37, with a biopsy collected at symptom-onset and a second, six years later, at disease end-stage.We aimed to generate iNPC-As from the fibroblasts of these individuals to establish if we replicate the family's variable penetrance, understand the pathological features, and discern if this pathology correlates with disease progression. Fibroblasts were directly converted as previously described (Meyer et al,. 2014). C9orf72 pathology was assessed using fluorescent in situ hybridisation for RNA foci and MSD ELISA for dipeptide repeat proteins (DPR). Toxicity was determined by survival after 48-hour co-culture with murine HB9-GFP motor neurons. We first evaluated C9-HRE length by Oxford Nanopore sequencing, the father carrying 70 repeats and 800 in both twins. We next assessed presence of RNA foci. While in induced astrocytes we could not detect antisense foci above background signal, nuclear sense foci were observed in 37% of the affected twin's cells at symptom-onset, but only 1% at end-stage, 3% of unaffected twin's cells and <1% in the father (Healthy Control vs Twin Af. Early P = <0.0001). Interestingly, only the affected twin at symptom-onset had significant production of poly-GP DPRs, ablated in end-stage astrocytes (HC vs Twin Af. Early P = <0.0001). Contrastingly, the astrocytes from the affected twin at symptom-onset were not toxic when co-cultured with motor neurons; however, survival was significantly reduced when co-cultured with end-stage astrocytes (HC vs Twin Af. Late P = 0.0036).While RNA foci and DPR detection do not seem to correlate with disease stage in the affected twin, these parameters might reflect the overall lack of correlation between C9-specific pathology and neurodegeneration that has been reported in post-mortem tissue. On the contrary, increasing astrocyte toxicity is an important finding to elucidate the mechanisms underlying C9-ALS disease progression.

(c49) ALSoD: An updated resource linking clinical research and bioinformatics tools in ALS

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The ALS Online Database is a source of information on genetic variants that have been identified in people with ALS, mainly from published literature. Genetic research and bioinformatics tools have advanced considerably since ALSoD was first developed, as well as genetic screening becoming increasingly available as a clinical tool. Clinicians and researchers need a resource that will provide variant interpretation, linking published literature, summary statistics from large genetic consortia and population statistics on genetic variants. Literature searches for papers researching genetic variants in ALS from 2015 onwards were performed and information about the variants were added to ALSOD. Data were updated to include variant HGVS and genomic location for annotation with other databases including the gnomAD v2.1.1 non-neurological cohort, the latest summary statistics from the Project MinE consortium and pathogenicity scores. ALSoD has information on variants from 154 genes with over 1,100 variants that were identified in people with ALS from 906 papers. Clinical data from over 1200 people can be used to interrogate genotype-phenotype correlations in a subset of the 150 genes. 20 genes are considered to have strong enough evidence to be causal of ALS. New features of ALSoD include cleaned variant descriptions, clearer signposting of research papers, manually written evidence summaries and interactive genotype-phenotype graphs where data from people with ALS variants are available for interrogation by web users. Linkage to other bioinformatics datasets will be displayed on individual variant pages that are under development.

(c50) Use of Next Generation Sequencing to Elucidate the Genetics of Monomelic Amyotrophy (MMA) in Bangladesh and UK patients

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Introduction: Brachial monomelic amyotrophy (MMA), a rare neurological condition that can mimic motor neuron disease (MND), is characterized by juvenile muscular atrophy of the distal upper extremities. Approximately1,500 MMA patients are reported globally with the majority in Asian populations. To date, mutations in only two genes (KIAA1377 and C5orf42) have been linked to increased risk for MMA. Clinical phenotypic similarities between amyotrophic lateral sclerosis (ALS) and MMA suggests the two diseases may be part of a clinical spectrum.

Objectives: This study aims to screen MMA patients from Bangladesh and the UK for mutations in genes associated with motor system disorders, to determine whether MMA shares a genetic architecture with other disorders including ALS.

Methodology: 153 risk genes within the Sheffield Neurogenetic Motor Disorder NGS Panel were sequenced in DNA extracted from MMA patients (11 Bangladesh and 8 UK) blood samples. Sequencing data were analyzed using a purpose-built pipeline for variant-calling and comparison with the ClinVar database of genotype-phenotype relationships.

Findings: Overall 49 heterozygous candidate pathogenic variants with a Combined Annotation Dependent Depletion (CADD) score>15 were identified within MMA patients. No MMA patient carried a clinically validated pathogenic variant within any gene-based on comparison with ClinVar. However, 4 variants of uncertain significance (VUS) were present within MMA patient samples and were rare in population-matched control databases: SETX c.1504C>T;(p.Arg502Trp) was present in 2 Bangladeshi patients and MAG c.1849C>G;(p.Leu617Val) was present in 2 UK patients. Two other VUS were identified within OPTN, a known ALS gene: a novel missense variant OPTN c.1466A>G;(p.Lys489Arg) and a non-coding variant OPTN c.1402-7C>T; these mutations occurred in one Bangladesh and one UK MMA patient, respectively.

Conclusions: Our genetic study of MMA did not identify any variant shown to be pathogenic in another motor system disorder. However, we identified three risk genes where multiple MMA patients carried a variant that was absent or very rare in control populations. One of these genes, OPTN, is strongly associated with ALS, suggesting some overlap in the genetic architecture of the two diseases. Our data suggest that the genetic basis of MMA is different between Asian and European populations. Further work is needed to validate the pathogenicity of the variants identified.

(c51) MutaPipe – A Bioinformatics Pipeline to Identify High Quality Mutant and Wildtype PDB Structures for ALS Proteins

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Motor Neuron Diseases (MNDs), including Amyotrophic Lateral Sclerosis (ALS), are incurable conditions that result in progressive degeneration and death of neurons. Genetic factors are an important cause of MND, with variants in >25 genes having strong evidence, and weaker evidence available for variants in >120 genes. Our scientific understanding of ALS pathology has been greatly advanced by the continuous identification of novel disease-associated variants/genes, however, the exact molecular mechanisms linking any genetic variant to ALS aetiology remain unknown for all variants in all identified genes to date. Hence, the systematic study of how disease-associated genetic variants subsequently influence protein structure and/or dynamics will be of extremely high value in our research aiming to elucidate the molecular underpinnings of ALS aetiology. While a growing number of experimentally solved protein structures harbouring well-known disease-associated mutations and/or a range of other variants are continuously deposited in the worldwide Protein Data Bank (PDB), it remains challenging to identify the most suitable structures for all available wildtype and mutant protein structures associated with ALS. To facilitate this crucial first step of selecting/identifying suitable template structures for structural analysis, we have developed MutaPipe, a fast and efficient bioinformatics pipeline to screen the PDB for genes associated with ALS and identify the highest quality protein structure for each unique sequence associated with a given gene of interest. Additionally, whenever corresponding data is available, variants will be annotated using information on variant pathogenicity from ClinVar. MutaPipe allows researchers to efficiently screen the PDB for the most suitable template structures for a specific WT or mutant gene/protein which can be used for further in silico analysis including homology modeling, mutagenesis experiments and molecular dynamics simulations.

(c52) GEOexplorer: an R/Bioconductor package for gene expression analysis and visualization

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Background: Over the past three decades there have been numerous molecular biology developments that have led to an explosion in the number of gene expression studies being performed. Many of these gene expression studies publish their data to the public database GEO, making them freely available. By analysing gene expression datasets, researchers can identify genes that are differentially expressed between two groups. This can provide insights that lead to the development of new tests and treatments for diseases. Despite the wide availability of gene expression datasets, analysing them is difficult for several reasons. These reasons include the fact that most methods for performing gene expression analysis require programming proficiency.

Results: We developed the GEOexplorer software package to overcome several of the difficulties in performing gene expression analysis. GEOexplorer was therefore developed as a web application, that can perform interactive and reproducible microarray gene expression analysis, while producing a wealth of interactive visualisations to facilitate result exploration. GEOexplorer is implemented in R using the Shiny framework and is fully integrated with the existing core structures of the Bioconductor project. Users can perform the essential steps of exploratory data analysis and differential gene expression analysis intuitively and generate a broad spectrum of publication ready outputs.

Example: We have used GEOexplorer to analyse a publicly available ALS dataset and identified several differentially expressed genes.

Conclusion: GEOexplorer is distributed as an R package in the Bioconductor project (http://bioconductor.org/packages/GEOexplorer/). GEOexplorer provides a solution for performing interactive and reproducible analyses of microarray gene expression data, empowering life scientists to perform exploratory data analysis and differential gene expression analysis on GEO microarray datasets.

(c53) Mitochondrial function determines severity but not risk of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Selective vulnerability of energy-intensive motor neurons (MNs) has fostered speculation that mitochondrial function is a determinant of ALS. Previously, the position of mitochondrial function in the pathogenic cascade leading to neurotoxicity has been unclear. We separated upstream genetic determinants of mitochondrial function, including genetic variation within the mitochondrial genome or autosomes; from downstream changeable factors including mitochondrial copy number (mtCN) and MN gene expression. We discovered that functionally validated mitochondrial haplotypes are a determinant of ALS survival but not ALS risk. Loss-of-function genetic variants within, and reduced MN expression of, ACADM and DNA2 lead to shorter ALS survival; both genes impact mitochondrial function. MtCN responds dynamically to the onset of ALS independent of mitochondrial haplotype, and is also significantly correlated with disease severity. We conclude that mitochondrial function impacts ALS progression but not risk; our findings have therapeutic implications.

(c54) A Knowledge-Based Machine Learning Approach to Gene Prioritisation in Amyotrophic Lateral Sclerosis

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Introduction: As a result of the advent of high-throughput technologies, there has been rapid progress in our understanding of the genetics underlying biological processes. Despite such advances, the genetic landscape of human diseases has only marginally been disclosed. Amyotrophic Lateral Sclerosis represents this scenario well. In ALS several rare disruptive gene variants have been associated with the disease and are responsible for about 15% of all cases. Although our knowledge of the genetic landscape of this disease is improving, it remains limited. Machine learning models trained on the available protein–protein interaction and phenotype-genotype association data can use our current knowledge of the disease genetics for the prediction of novel candidate genes.

Objectives: Our objective is to exploit the available biological information from publicly available datasets and our current knowledge of the disease genetics for the prediction of novel candidate genes, and to instigate the relevance of the predicted genes in ALS.

Method: We developed a knowledge-based machine learning method for this purpose. We trained our model on protein–protein interaction data from IntAct, gene function annotation from Gene Ontology, and known disease-gene associations from DisGeNet. These three databases are the default setting in DGLinker. Using several sets of known ALS genes from public databases and a manual review as input, we generated a list of new candidate genes for each input set [1].

We also developed DGLinker [2], a webserver that implements and generalizes our machine learning method, making it publicly and easily accessible for the prediction of candidate genes associated with any target human disease. DGLinker has a user-friendly interface that allows non-expert users to exploit biomedical information from a wide range of biological and phenotypic databases, and/or to upload their own data, to generate a knowledge-graph and use machine learning to predict new disease-associated genes. The webserver includes tools to explore and interpret the results and generates publication-ready figures.

Results: We investigated the relevance of the predicted genes in ALS by using the available summary statistics from the largest ALS genome-wide association study and by performing functional and phenotype enrichment analysis. In total 651 genes were predicted. The predictions were enriched for genes associated with biological processes known to be affected by the ALS pathogenesis, and with

other neurodegenerative diseases for which evidence of phenotypic and genetic overlap with ALS exist. Using ALS genes from ClinVar and our manual review as input, the predicted sets were enriched for ALS-associated genes (ClinVar p = 0.038 and manual review p = 0.060) when used for gene prioritization in a genome-wide association study. As our predictions were based on data released in 2019, in March 2021 we retrospectively tested the validity of our prediction by assessing if any of the genes that were discovered to be associated with ALS between the end of 2019 and 2021 were predicted by our method. Five out of seven discovered genes were present among our predictions (p = 0.012).

Conclusions: The genetics of ALS has proven challenging, and even though great progress has been made by generating and analysing large multi-omics datasets, the causes of ALS in most patients (~85%) remain unexplained. Using machine learning models to leverage our current knowledge of ALS and other diseases could allow us to accelerate our progress in the understanding of the genetic causes of ALS and lead towards new avenues of treatment. Our method is available via DGLinker at this web address https://dglinker.rosalind.kcl.ac.uk. The webserver is free and open to all users without the need for registration.

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(c54a) CAV1 and CAV2 are over-expressed in ALS patients and mutations in CAV1 and CAV2 enhancers are associated with longer survival

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Background: Caveolin-1 and Caveolin-2 are proteins associated with intercellular neurotrophic signalling. Converging evidence links CAV1/CAV2 genes to ALS. In mice, CAV1 is associated with increased neuronal survival and protection from cognitive decline. In humans, CAV1 coding regions are enriched for ALS-associated variation, and disease-associated variants have been identified within CAV1/CAV2 enhancers, which reduce CAV expression and lead to disruption of membrane lipid rafts.

Methods: Using the largest available ALS DNA whole-genome sequencing and post-mortem RNA sequencingdatasets (5987 Project MinE samples and 365 tissue samples, respectively), and iPSC-derived motor neurons from 55 ALS cases and 15 controls from AnswerALS, we investigated the role of CAV1/CAV2 expression and enhancer variants in the ALS phenotype. Firstly, we conducted differential expression analyses between ALS cases and controls for CAV1/CAV2 genes across six brain tissues and three independent datasets. We subsequently analysed gene expression in iPSC-derived motor neurons to ascertain whether expression differences were specifically within neuronal cells. Next, we ran survival and age-of-onset analyses among carriers of CAV1/CAV2 mutations compared to non-carriers and examined correlations between CAV1/CAV2 expression and disease progression (ALSFRS).

Results: CAV1/CAV2 expression was consistently higher in ALS patients across RNAseq differential expression analyses, with significant results in the primary motor cortex ($p = 0.010$), lateral motor cortex ($p = 0.029$), and cerebellum ($p = 0.004$). Consistent with this, higher expression was observed for patients within the iPSC-derived motor neuron expression analysis. Increased survival was found among carriers of CAV1/CAV2 enhancer mutations, with a median increase of 345 days, and CAV2 expression associated with disease progression.

Discussion: These results add to an increasing body of evidence linking CAV1/CAV2 genes to ALS. We propose that carriers of CAV1/CAV2 enhancer mutations may be conceptualised as an ALS subtype, who present a less severe ALS phenotype with longer survival duration. Differential expression of CAV1/CAV2 genes may be compensatory or represent a causal pathway. Evidence of the beneficial role of CAV1/CAV2 may point to a compensatory mechanism, where increases in CAV2 expression are commensurate with disease progression rate, but further investigation is needed to elucidate this mechanism.

d) Disease models

(d55)Sleep and orexinergic pathway alterations in mice models of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive motor neuron disease inexorably leading to an early death. Sleep disturbances have been described and appear at a later stage of the disease. Those disturbances can be triggered by muscle cramps, spasticity, or restless legs syndrome, all leading to increased wakefulness. However, sleep changes were rarely studied in the context of ALS. We used two murine models of ALS, Superoxide Dismutase 1 G86R (Sod1G86R) and Fused in Sarcoma (FusΔNLS), which both represent 25% of the familial cases of ALS, to underpin the structural and cellular mechanism involved. A recent pathological study in ALS patients observed decreased neurons immunoreactive for orexin, a neuropeptide highly involved in sleep regulation. In both Sod1G86R and FusΔNLS mice, there was no change in the number of Orexin-positive neurons in the lateral hypothalamus. However, electroencephalograms showed an increase in wakefulness and a decrease in rapid eye movement (REM) episodes in both mouse models before the onset of major motor troubles, while Suvorexant® - a dual Orexin receptor antagonist - was able to increase the REM episodes and decrease the wakefulness in both Sod1G86R and FusΔNLS mice. Thus, our results show that two mouse models of ALS models display an impaired sleep pattern, that is, at least partially, dependent upon orexinergic neurons. This study is a starting point for a better comprehension of sleep defects observed in ALS patients and the potential benefit from pharmacological manipulation.

(d56) Blocking the β6/β7 loop epitope of misfolded SOD1 strongly delays disease onset and extends survival in a mouse model of ALS.

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The most attractive strategies to mitigate toxicity of misfolded SOD1 in animal models of ALS through the occlusion of SOD1 expression, are indiscriminative and result in the elimination of essential enzymatic antioxidant activity of SOD1 in CNS. Here, we used scFv-SE21 intrabody to block the beta 6/beta 7 loop epitope exposed exclusively in misfolded SOD1, as an alternative strategy to alleviate toxicity of misfolded SOD1 without affecting properly folded SOD1 species. Expressed in CNS of ALS hSOD1-G37R transgenic mice, scFv-SE21 intrabody reduced the accumulation of soluble and aggregated misfolded SOD1, decreased glia activation and neuroinflammation, rescued spinal MNs, and delayed disease onset and extended survival by 90 days. Our results stress the importance of the beta 6/beta 7 loop epitope in the pathogenesis of misfolded SOD1, and indicate that its blocking may constitute a therapeutic strategy, which minimizes potential adverse effects of treatment by preserving enzymatic activity of SOD1 essential to neuron physiology.

(d57) Subcommissural organ-spondin-derived peptide (NX210c) improves motor function and prolongs survival in the SOD1G93A mouse model of ALS

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Objectives: NX210c is a short cyclic peptide derived from the thrombospondin repeats of the subcommissural organ-spondin that displays in vitro beneficial effects on several aspects of ALS pathogenesis such as on blood-brain barrier permeability and neuronal death induced by glutamate excitotoxicity or oxidative stress. Therefore, the aim of this study was to evaluate the therapeutic effect of NX210c in the SOD1G93A mouse model of ALS.

Methods: Female SOD mice were treated daily with intraperitoneal injections of vehicle or different doses of NX210c (2.5, 5 or 10 mg/kg) from 90 days old. The static rods test was performed every other week to evaluate motor deficits. Briefly, mice were placed with their back facing the clamped end of the rod. The orientation time to turn back and the travel time to walk the 60 cm back to the edge of the rod were recorded (= total time). The smaller the rod diameter, the harder the task. The clinical score (see ALS Therapy Development Institute guidelines) was evaluated twice a week to determine overall survival.

Results: The orientation, travel and total times during the static rods test were higher in SOD mice compared with WT mice from 16 weeks old until disease end-stage ($p<0.001$). A dose-dependent improvement of motor performances was observed in SOD mice treated with NX210c. More particularly, the peptide at 10 mg/kg reduced ALS-induced increased orientation and travel times from 16 weeks old (WT: 1.3s and 3.2s, vehicle SOD: 24.5s and 26.6s and 5.4s, NX210c SOD: 2.6s and 4.8s for orientation and travel times, respectively; p0.05), the median survival of SOD mice treated with NX210c at 10 mg/kg was increased by 11 days compared with that of vehicle-treated SOD mice (vehicle SOD: 143d, NX210c SOD: 154d, $p<0.01$).

Conclusions: Overall, NX210c is a new promising drug candidate and its solid preclinical package (mechanism of action, proof of concept in ALS) should support the clinical development of NX210c in ALS patients. In addition, we have gathered several proofs of concept showing that NX210c restores cognitive functions (Le Douce et al., 2021), which may represent a supplementary beneficial effect of NX210c for ALS patients, since 35% of them suffer from cognitive or behavioral impairments, with an additional 15% having FTD.

(d58) Blocking the pathogenic β6/β7-loop epitope of misfolded SOD1: a strategy for ALS disease treatment

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There is emerging evidence that the misfolded form of superoxide dismutase 1 (SOD1) may represent a common pathogenic pathway in both familial and sporadic cases of amyotrophic lateral sclerosis (ALS). To reduce misfolded SOD1 species in the nervous system, we have tested an immunogenic therapeutic approach based on adeno-associated virus (AAV) serotype-derived capsid variant AAV-PHP.eB delivery of two variants encoding a secretable and cytosolic single-chain variable fragment (scFv). The scFv, which was developed in our lab, is made of the variable heavy and light chain regions of a monoclonal antibody that binds specifically to misfolded forms of SOD1 via the pathogenic β6/β7-loop epitope. The AAV-PHP.eB encoding the scFv antibody or EGFP as a control were delivered via a single intrathecal spinal cord (SC) injection to mutant SOD1G37R mice at P0, before accumulation of any misfolded species of SOD1. This single injection resulted in sustained expression of both forms of scFv in the SC. We demonstrated that the cytosolic scFv variant, but not the secreted one, delayed disease onset and extended survival of SOD1G37R mice for about 90 days. Moreover, expression of this intrabody in the SC was able to reduce the accumulation of soluble and aggregated misfolded SOD1 throughout the disease course. In addition, it was able to rescue spinal motor neurons and to reduce the neuroinflammation in the tissue.The administration of the intrabody at P0 is a preventative strategy and not applicable for the treatment of symptomatic ALS patients. Therefore, we administered the vector encoding for either intra-scFv or EGFP as a control approximately at disease onset (230 days) via stereotaxic intraventricular (ICV) injection to the brain. This single injection resulted in sustained expression of scFv in the SC of the mice. As compared to control animals, the stereotaxic injection of AAV-PHP.eB-intra-scFv vector had no effect on disease progression and mice survival. We postulate that it could stem from the inability of scFv intrabody, at the level of motor neuron expression achievable by the stereotaxic injection to the brain, to counterbalance the pool of misfolded SOD1 species that are already abundant at such advanced age. Our results propose that immunotherapy based on intrathecal injection of AAV-PHP.eB encoding a cytosolic scFv against misfolded SOD1 should be considered as a potential treatment for ALS, especially for individuals carrying SOD1 mutations.

(d59) Comparison of neuromuscular junction pathology in four mouse models of spinal muscular atrophy indicates distinct patterns of selective vulnerability

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Spinal Muscular Atrophy (SMA) is a childhood motor neuron disease characterised by the loss of lower motor neurons (MNs). Breakdown of the neuromuscular junctions (NMJs) is an early pathological event in SMA. However, not all MNs are equally vulnerable, with some subpopulations of motor neurons consistently spared from disease whilst others are lost. A thorough understanding of the basis of this selective vulnerability will give critical insight into the factors which drive MN pathology and help identify novel neuroprotective strategies. We have conducted an extensive analysis of selective vulnerability in the Smn2B/- mouse model of SMA, performed a comparison of selective vulnerability patterns between four mouse models of SMA, and compared published RNAseq data to gain insight into molecular mechanisms which regulate selective vulnerability. We have performed a body wide analysis of NMJ pathology in 20 muscles from the Smn2B/- mouse model of SMA. In the Smn2B/- mouse model, we have identified an increased NMJ loss in muscles from the core, neck, proximal hind limb and proximal forelimb, with marked sparing of the distal limbs and head. MN cell body loss was also more profound in the thoracic and upper lumbar regions compared to lower lumbar levels. We have subsequently used this data and published work to compare patterns of selective vulnerability between 4 commonly used mouse models of SMA. We show that although each mouse model of SMA shows a similar range in vulnerability, each mouse model of SMA has a distinct pattern of selective vulnerability. Finally, in order to gain insight into the molecular mechanisms which regulate selective vulnerability, we have compared published RNAseq data acquired from differentially vulnerable MNs from 2 different mouse models of SMA. The comparison reveals an enrichment for transcripts falling within the gene ontology terms ‘protein localisation to cell periphery’ and ‘ribonucleoprotein complex biogenesis’ in selectively resistant MNs. Collectively, this work demonstrates that the patterns of selective vulnerability in the Smn2B/- mouse model show remarkable similarity to patients with SMA, but there are drastic differences in patterns of selective vulnerability across four mouse models of SMA which is critical to consider during experimental design. We further show that differences in patterns of selective vulnerability can be used to gain insight into the molecular basis for MN vulnerability in SMA.

(d60) Multi-omics from hiPSC-derived motor neurons and patient biopsies identifies mutational signatures and potential transcriptional biomarkers for ALS

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Objectives: Amyotrophic lateral sclerosis (ALS) is a rare disease with heterogenous progression rates ranging from slow to rapid progression. Studies aiming to characterize the factors associated with the progression rate have focused on survival but few concerned the functional decline trajectory [1].

Methods: Using PROACT database (8,569 patients), we select spinal and bulbar patients with at least a baseline and a second follow-up visit. We randomly select spinal patients to get two balanced groups of 1,380 patients. The following steps were performed: 1) We built a multimodal ALS course map that grasped long-term disease progression in a mixed-effects fashion [2] with Leaspy. We used 6 features: the four subscores of ALSFRS-R, forced vital capacity (FVC) and BMI. 2) We extracted the progression rate and onset age, and the relative progression of each feature of each patient from the parameters of the model. 3) We also computed from the disease course, the conversion age to a progression threshold for each feature: 8 points for ALSFRS-R subscores in reference to FT9 score, 18.5 for the BMI and 2.43 litres for FVC. Finally, we compared the distributions of the extracted parameters and conversion ages using independent t-test.

Results: We found that ALS starts 2.84 years later for bulbar patients, but progresses 1.36 times faster. We observed, for bulbar patients, that ALSFRS-R fine and gross motor progress 3.7 and 4.4 months later than the spinal patients but ALSFRS-R bulbar progression starts almost 8 months before. Bulbar patients reached endpoint thresholds define above, in average after spinal patients for: ALSFRS-R fine motor (8 points, 49.7 months), gross motor (44 months), bulbar (7.5 months) and respiratory (23.3 months), FVC (24 months).

Conclusions: We build a modelling framework for describing ALS subtypes effect on functional endpoints from multimodal screening assessments. This model also allows describing and predicting individual progression which can pave the way to discriminate fast and slow progressor for stratification of clinical trial. This methodology could also be applied in clinical trial to compare treated and placebo arms.

References[1] Grollemund, Vincent, et al. Frontiers in neuroscience 13 (2019): 135.[2] Schiratti, Jean-Baptiste, et al. The Journal of Machine Learning Research 18.1 (2017): 4840-4872.

(d61) New approach based on synergic stimulation of cell membrane receptors targeting muscle tissue for ALS treatment.

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This work is based on the functional coupling of integrins, boron (B) transporter (NaBC1) and growth factor receptors (GFRs) after their activation. In our previous work, we have demonstrated that active-NaBC1, co-localise with integrins and GFRs producing a functional cluster that synergistically enhances biochemical signals and crosstalk mechanisms, accelerating muscle repair after an injury and restoring dystrophic phenotypes in vivo in muscular dystrophies with different aetiological origin. Thus, we aimed to study the effects of B in ALS mouse model targeting muscle. We have engineered and characterised injectable alginate-based hydrogels with controlled local B-release. Selected compositions were injected in both quadriceps at 10, 12, 14 and 16 weeks old B6SJL-Tg(SOD1-G93A)1Gur/J ALS male and female mice randomly distributed between different groups. We have followed changes in body weight and motor impairment in behavioural four limb hanging test along 12 weeks. Results showed increased body weight and hang time of the B-treated mice compared with non-treated control group. After euthanasia at 17 weeks old, tissues were analysed by histological and immunofluorescence techniques. The histoarchitecture of quadriceps muscle from B-treated mice, although still displaying ALS pathology, resulted in a significant decrease in size variability among muscle fibres. The evaluation of muscle pathology by analysing muscle inflammatory cell recruitment by toluidine blue staining revealed that B-treated mice presented a strong decrease in the density of infiltrated mast cells as well as the degranulating mast cell number, suggesting an inhibition of mast cell migration and activation. Further, mast cells were mainly perivascular located and not in the close proximity to nerve fibres, an indication of extensive nerve inflammation characteristic of SOD1 mouse model. Immunofluorescence analysis showed that B treatment significantly decreased muscle type 1 slow myofibers, suggesting the prevention of the loss of type 2 fast myofibers, which drastically decrease during ALS. Further, Pax7+ cells were significantly increased in ALS B-treated mice compared with control, indicating a major number of active satellite cells involved in muscle regeneration and repair. Altogether, our findings show that active-NaBC1 promotes ALS muscle repair and prevents muscle and nerve inflammation, suggesting a novel mode of action to explore new therapies for ALS treatment.

(d62) A novel RT-QuIC method for prion-like SOD1 aggregation associated with ALS pathogenesis

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During ALS disease propagation the ubiquitous enzyme superoxide dismutase 1 (SOD1) can misfold and aggregate. Strong evidence supports the theory, that SOD1 aggregation can drive ALS pathogenesis in a prion-like template assisted manner. This leads to accumulation of differently structured aggregates, referred to as strains. In our laboratory we have identified two distinctive pathological strains, A and B in transgenic (tg) mouse models expressing mutant human SOD1 (hSOD1) using an in-house method, called binary-epitope mapping (BEM). These neurotoxic aggregates have a defined structure composed of a tightly packed core and loose ends extruding on the outside (Bergh et al., PNAS, 2015). Data obtained from inoculations into the ventral horn of tg mice, suggests a seeding-nucleation mechanism behind SOD1 aggregation in vivo (Bidhendi et al., The Journal of Clinical Investigation, 2016). The collection of pathological strains from patient tissue and tg mice in high quantities remains to be a great challenge due to the degeneration of motor neurons during disease progression. Previous efforts to produce these aggregates in vitro resulted in structural differences when compared to in vivo aggregates. This is also observed for other neurodegenerative conditions, which are associated with protein inclusions, such as Parkinson or Alzheimer. Thus, we developed a novel Real-time quaking induced conversation protocol (RT-QuIC) to produce disease relevant strain A and B aggregates in vitro. Aggregates isolated from tg mice are used as seeds binding unfolded, recombinant hSOD1 and induce the proteins to adopt pathological conformations. This leads to accumulation of misfolded SOD1 and to the exponential growth of fibrils. These fibrils fragment and serve as templates, which initiate further aggregation. We found that seeding with strain A aggregates results in the formation of aggregates with the same structure as the in vivo template, which confirms that we are able to mimic the aggregation process. By combining in vitro SOD1 aggregation with our unique BEM method for structural characterization, we are able to produce pure, strain specific aggregates in larger quantities. These aggregates can be used for inoculations studies to prove neurotoxicity or structural analysis. Additionally, our method might be used to develop a diagnostic or a screening platform for aggregation inhibitors.

(d63) Modelling cortical changes in human C9ORF72-ALS brain organoids

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Brain imaging studies show widespread cortical thinning in C9ORF72-ALS (C9ALS) patients and in carriers of the C9ORF72 repeat expansion, both in- and outside the motor cortex. The mechanisms underlying this change remain poorly understood. To fill this gap, we have developed a brain organoid model to study human cortex morphology and composition in C9ALS patients. The brain organoids are derived from induced pluripotent stem cells (iPSC) of C9ALS patients and healthy controls, and generated using cerebral and forebrain-specific protocols. Here, we present several cellular phenotypes. First, C9ALS iPSC and organoids show a 30% reduction of C9ORF72 protein as compared to control, but gene and protein expression of neuronal cytoskeletal markers is preserved in C9ALS organoids (as determined by quantitative PCR and immunostaining). To analyze whether specific cell types are affected, single cell RNA sequencing was performed on 3 C9ALS and 3 healthy control organoid sample sets. These data unveiled several cellular changes. For example, fewer deep-layer neurons were detected in C9ALS organoids, which was confirmed by immunostaining for CTIP2. This effect was absent in cell lines in which the C9ORF72 repeat expansion was removed using CRISPR/Cas9 editing. Our data indicate that changes in specific cell populations may contribute to the cortical thinning observed in C9ALS patients and repeat carriers. In future studies, we aim to determine whether the observed cellular phenotypes are also present in organoids derived from presymptomatic carriers of the C9ORF72 repeat expansion to explore the potential of using organoids for presymptomatic screening. Finally, the 3D organization of the C9ALS organoids will be assessed using tissue-clearing and 3D fluorescence imaging.

(d64) Characterization of macrophage migration inhibitory factor as a therapeutic target for amyotrophic lateral sclerosis

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Mutations in superoxide dismutase 1 (SOD1) cause amyotrophic lateral sclerosis (ALS), a neurodegenerative disease characterized by the loss of upper and lower motor neurons (MNs). Transgenic mice expressing mutant SOD1 develop paralysis and accumulate misfolded SOD1 onto the cytoplasmic faces of intracellular organelles. We have shown previously that macrophage migration inhibitory factor (MIF) is able to directly inhibit mutant-SOD1 misfolding and binding to intracellular membranes. Furthermore, our previous studies revealed that MIF protein level is extremely low in the spinal MNs, implicating low chaperone activity as a component of selective vulnerability of MNs to mutant SOD1 misfolding and toxicity. Interestingly, MIF low protein levels do not correlate with its mRNA levels, suggesting MIF instability in MNs. Also, we showed that overexpressing MIF using injection of adeno-associated viral (AAV) vectors in the spinal cord of mutant SOD1G93A and loxSOD1G37R newborn mice, elevated MIF mRNA and protein levels in the spinal cord and significantly delayed disease onset and prolonged survival compared with their GFP-injected or non-injected littermates. For more therapeutic relevance, we injected MIF to mutant loxSOD1G37R at the symptomatic stage of the disease, when disease diagnosis can be established. To this end we utilized an AAV PHP.eB virus that has the ability to penetrate the blood-brain barrier (BBB), following peripheral injection at the symptomatic phase. Our data show that loxSOD1G37R mice injected with MIF demonstrated a significant extension of lifespan, improvement of motor function and delay in disease progression compared to their GFP-injected or non-injected littermates. 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.

(d65) Investigating sporadic ALS using hiPSC-derived cells

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disorder due to selective loss of motor neurons (MNs). While in 10% of ALS patients the disease is inherited, 90% of ALS cases have no family history. Genetic variants in sporadic ALS (sALS) are not infrequent, while the modelling is difficult as the potential mutations are always unidentified. With the help of induced pluripotent stem cell (iPSC) technology, we can include full genetic information in patients to research sALS in vitro. The aim of this study is to investigate ALS using iPSC-derived cells from patients, using five sALS lines and five control lines. We were able the differentiate these iPSCs into MNs with the same efficiency using Tuj-1, ChAT and ISL-1 as markers. No apoptotic cell death was observed. We also didn't observe a clear mislocalisation or aggregation of TDP-43 in the sALS motor neurons. However, a slightly higher cytoplasmic over nuclear TDP-43 ratio was seen in the sALS. We could demonstrate a defective neurite outgrowth, characterised by a shorter length and less branching, as well as neurite transport defect compared with control. Our results are in line with ALS being a multi-step disease and sporadic cases might take more steps to become symptomatic. sALS MNs generally present mild phenotype and we are inducing DNA damage to help trigger disease related characteristics.

(d66) Integrated multi-omics approach reveals ALS mutation-independent synaptic dysfunction in hiPSC-derived motor neurons

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Familial ALS is caused by various genetic mutations via varying pathomechanisms, such as protein mislocalization and aggregation, autophagy impairment, and organellar dysfunction, which consistently lead to a selective upper and lower motor neuronal loss, indicating a probable confluence of phenotypes at the molecular level. Over the years, synaptic disruption has emerged as one of the key players implicated in the hypo-/hyper-excitability phenotype of progressive motor neuron degeneration. Therefore, we sought to elucidate the role of synaptic machinery in ALS pathophysiology by integrating high-throughput proteomic, RNA-Seq and WGBS (whole-genome bisulphite sequencing) techniques. In our study, we first analysed the proteome of synaptic fractions isolated from hiPSC-derived motor neurons from patients with C9orf72, SOD1, FUS and TARDBP mutations, which showed a significant down-regulation of proteins involved in pre-synaptic vesicle release, irrespective of the mutations. Similarly, transcriptomic analysis also showed significantly down-regulated synaptic terms across mutations. Subsequent integration with methylomic data in turn revealed an associated hyper-methylation of synaptic gene promoters, albeit not in C9orf72-neurons, possibly suggesting a distinctive pathway of synaptic gene control in this particular mutation. In summary, using an integrated multi-omics analysis in a top-down approach, we identified an underlying mutation-independent synaptic phenotype at the genetic, transcriptomic and proteomic levels, thereby providing a crucial insight into how different mutations and divergent pathomechanisms over time can still converge into a singular presentation of the disease.

(d67) Y526 phosphorylation of FUS in ALS and FTD

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Fused in Sarcoma (FUS), a normally nuclear DNA/RNA-binding protein, forms abnormal cytoplasmic inclusions in the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD). In ALS, these inclusions are associated with mutant FUS, whereas in FTD they consist of mutation-free FUS. As Tyr526 phosphorylation inhibits nuclear import of FUS, our aim in this study was to determine the extent of Tyr526-FUS phosphorylation in vitro and in vivo in mouse brain and human postmortem brain tissue. Tyr526 phosphorylation of FUS by Src-family kinases indicated impaired nucleocytoplasmic distribution and aggregation of FUS in cell models, and pronounced phospho-Tyr526 co-localization with pSrc/pAbl was detected in mouse brain. Brain region-specific phospho-Tyr526 FUS co-localization with active pSrc/pAbl kinases in mice pointed to preferential involvement of cAbl in cytoplasmic phospho-Tyr526 FUS mislocalization in cortical neurons. Final analysis of the detail patterns of active cAbl kinase and phospho-Tyr526 FUS in the neurons of human post-mortem frontal cortex brain tissue demonstrated the increased cytoplasmic phospho-Tyr526 FUS distribution in the cortical neurons of ALS/FTD patients as compared to FTD. Considering the overlapping patterns of cAbl activity and phospho-Tyr526 FUS distribution in cortical neurons, we propose that cAbl kinase is involved in mediating cytoplasmic toxic FUS mislocalization in FTD and ALS/FTD patients, likely leading to differences in disease progression.

(d68) The p97-Nploc4 ATPase complex plays a role in muscle atrophy during cancer and amyotrophic lateral sclerosis

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The p97 complex participates in the degradation of muscle proteins during atrophy upon fasting or denervation interacting with different protein adaptors. We investigated whether and how it might also be involved in muscle wasting in cancer, where loss of appetite occurs, or amyotrophic lateral sclerosis (ALS), where motoneuron death causes muscle denervation and fatal paralysis. As cancer cachexia models we used mice bearing colon adenocarcinoma C26, human renal carcinoma RXF393 or Lewis Lung Carcinoma (LLC), with breast cancer 4T1-injected mice as controls. As ALS models we employed 129/SvHsd mice carrying the mutation G93A in human SOD1. The expression of p97 and its adaptors was analyzed in their muscles by qPCR and WB. We electroporated plasmids into muscles or treated mice with disulfiram (DSF) to test the effects of inhibiting p97 and nuclear protein localization protein 4 (Nploc4), one of its adaptors, on atrophy. The mRNA levels of p97 were induced by 1.5 to 2-fold in tibialis anterior (TA) of all the cachectic models but not in the non-cachectic 4T1 tumor-bearing mice. Similarly, p97 was high both in mRNA and protein in muscles from 17 week-old SOD1G93A mice. Electroporation of a shRNA for murine p97 into mouse muscle reduced the fiber atrophy caused by C26 and ALS. When we interrogated a microarray we had previously generated for the expression of p97 adaptors, we found Derl1, Herpud1, Nploc4, Rnf31 and Hsp90ab1 induced in cachectic TA from C26-mice (FC>1.2, adj.p≤0.05). By qPCR, we validated their inductions in TA of cachectic and ALS models and selected Nploc4 as the one also induced at the protein level by 1.5-fold. Electroporation of a Crispr/Cas9 vector against Nploc4 into muscle reduced the fiber atrophy caused by C26 and ALS. Since DSF uncouples p97 from Nploc4, we treated atrophying myotubes with DSF, and found accumulated polyubiquitinated proteins and reduced degradation of long-lived proteins by 35%, including actin. DSF halves Nploc4 in the soluble muscle fraction and given to C26-bearing mice limited the body and muscle weight loss, with no effect on tumor growth. Overall, cancer cachexia and ALS seem to display similar mechanisms of muscle wasting at least at the catabolic level. The p97-Nploc4 complex appears to have a crucial role in muscle atrophy during these disorders and disrupting this complex might serve as a novel drug strategy.

(d69) C21orf2 mutations found in ALS disrupt primary cilia function

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Amyotrophic lateral sclerosis (ALS) is a devastating progressive neurodegenerative disease that affects 1 in 400 people. Almost 40 genes have been associated with ALS, currently explaining about 15% of the ALS risk. These genes tend to cluster in certain disease pathways such as protein quality control, RNA metabolism and axonal function. Despite these advances, adequate treatments for ALS patients are still missing. In this study, we investigate the role of a newly discovered ALS gene, C21orf2, in ALS pathology. We show that C21orf2 is localized to the basal body of the primary cilium and plays an important role in ciliogenesis in vitro and in vivo. Knock down of C21orf2 also lowers cilia frequency and length in human iPSC-derived spinal motor neurons (sMNs). Furthermore, we show that intraflagellar transport is impaired, causing primary cilia to fail in transducing extracellular signals essential in the sonic hedgehog pathway. ALS-associated mutations in C21orf2 lead to loss of binding to centrosomal proteins, loss of proper localization at the basal body and hereby prevent C21orf2 from carrying out its normal function in primary cilia by loss-of-function. Finally, we confirm that sMNs derived from iPSCs from ALS patients with C21orf2 mutations display similar cilia dysfunction and have disturbed sonic hedgehog signaling. Collectively, our data reveal impaired cilia homeostasis as a novel disease mechanism at play in ALS, opening new avenues for further research.

(d70) Combined epigenetic drugs elicit neuroprotective effect on sex dimorphic features in ALS mice

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that affects upper and lower motor neurons (MN). There is no cure and current treatments fail to slow the progression of the disease. Transcriptional dysfunction, which involves a defect in histone homeostasis has recently been implicated in MN degeneration. Epigenetic modulation in the acetylation state of NF-kB RelA and histone 3 (H3) protein, involved in the development of neurodegeneration, is a drugable target for the class-I histone deacetylases (HDAC) inhibitors, valproate, and the AMP-activated kinase (AMPK)-sirtuin 1 pathway activator, resveratrol.

Aim: In this study, we demonstrated that the combination of valproate and resveratrol can restore the acetylation state of RelA in the SOD1(G93A) murine model of ALS. We also investigated the sexually dimorphic development of the disease, as well as the sex-sensibility to the treatment administered.

Method: Animals where subjected to behavioural tests to examine motor function. They were sacrificed at the end stage, and immunohistochemistry and molecular analysis were carried out on the spinal cord of the mice

Results: The combined drugs, which rescued RelA and the histone 3 acetylation state, reduced the motor deficit and the disease pathology associated with motor neuron loss and microglial reactivity, Brain-Derived Neurotrophic Factor (BDNF) and B-cell lymphoma-extra large (Bcl-xL) level decline. Specifically, the treatment administered at 50 days of life, postponed the time of onset in the male by 22 days, but not significantly in females. Nevertheless, in females, the drugs significantly reduced symptom severity of the later phase of the disease and prolonged the mice's survival. Only minor beneficial effects were produced in the latter stage in males.

Conclusion: Overall, this study shows a beneficial and sexually dimorphic response to valproate and resveratrol treatment in ALS mice.

(d71) Inhibition of class I histone deacetylases ameliorates TDP-43 pathology in experimental models of ALS

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TDP-43 pathology is a neuropathological hallmark in most ALS and FTD patients. Alterations in TDP-43 trafficking lead to cytoplasmic mislocalization and aggregation, fragmentation and hyperphosphorylation of TDP-43.We demonstrated that Cyclophilin A (PPIA), a foldase and molecular chaperone, is a functional interacting partner of TDP-43 and regulates its trafficking. The absence of PPIA increases TDP-43 pathology and exacerbates disease progression in a mouse model of ALS. Moreover, we found that the PPIA/TDP-43 interaction depends on PPIA Lys-acetylation (acetyl-PPIA). Indeed, we detected low acetyl-PPIA and a reduced PPIA/TDP interaction in PBMCs of sporadic ALS patients, which also display TDP-43 pathology. We hypothesize that a reduction in Lys-acetylation levels of PPIA could affect nuclear-cytoplasmic shuttling of TDP-43 and lead to TDP-43 pathology. The removal of acetyl groups is mediated by histone deacetylases (HDACs), whose inhibitors (HDACis) are promising therapeutic molecules for neurodegenerative disorders. Here, we investigate the effect of HDACis on TDP-43 pathology models, focusing on HDACis that target single HDACs or some specific classes. First, we performed HDACis screening in a cellular model of TDP-43 pathology, HEK293 cells where TDP-43 mislocalization, fragmentation and aggregation are induced by a chronic nutrient starvation protocol. We found that the inhibition of class I HDACs is effective in reverting TDP-43 pathology in a dose dependent manner. Moreover, it seems to increase acetyl-PPIA levels. Finally, we treated the homozygous TDP-43 Thy1/Thy1 mouse model of ALS with the selected HDACi to evaluate its effect on TDP-43 pathology. This TDP-43 mouse model presents an aggressive ALS phenotype, characterized by early and progressive loss of motor function and motor neuron degeneration. Interestingly, TDP-43 mice show an increase of TDP-43 and PPIA mislocalization and aggregation in lumbar spinal cord tissue. After one week of treatment, TDP-43 mice present a reduction in neurodegeneration and neuroinflammation, as well as an attenuated TDP-43 pathology in comparison with vehicle-treated ones. In conclusion, we demonstrated that the selected class I HDACi reverts TDP-43 pathology both in our cellular model and in TDP-43 mice, where it exerts a neuroprotective effect, suggesting that it could be a potential therapeutic approach for ALS.

(d72) Cytoplasmic dynein defects cause reduced muscle strength and motor coordination, aberrant neuromuscular junction formation and TDP-43 mislocalisation

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Cytoplasmic dynein is the major motor driving retrograde transport and its function is particularly important in motor neurons (MNs) due to their highly polarised structure. The mouse legs at odd angles (Loa) mutation in dynein cytoplasmic 1 heavy chain 1 (Dync1h1) causes defective axonal transport, neuronal migration defects and proprioceptive and MN degeneration. Using this line, we generated mice with decreasing levels of functional dynein in cholinergic MNs: wildtype (Dync1h1+/+), hemizygous wildtype (Dync1h1-/+), heterozygous mutant (Dync1h1+/Loa), and hemizygous mutant (Dync1h1-/Loa).

Longitudinal assessment over 12 months indicated a decrease in body weight in heterozygous mutants and an even greater decrease in hemizygous mutants. The same pattern was observed in their muscle strength (inverted screen test) and motor coordination (rotarod test). Hemizygous mutants showed a steeper decline in muscle strength between 1 and 4 months, compared to heterozygous mutants. Hemizygous wildtypes did not differ from wildtypes.

At 12 months, the motor endplates of the neuromuscular junctions (NMJs) of the hind limb lumbrical muscles were increasingly smaller with decreasing levels of functional dynein. A similar pattern was found in 2-month-old mice, apart from hemizygous wildtypes showing a significant increase, compared to wildtypes. Young hemizygous mutants showed a decrease in endplate compactness compared to all other groups. Interestingly, at 12 months, this decrease was only significant compared to the wildtypes, suggesting that, as well as defective dynein function, aging has a role in NMJ anatomical changes. Moreover, there was a significant loss of endplate innervation in hemizygous wildtypes and an even greater loss in hemizygous mutants at 2 and 12 months, whereas this loss was only evident in the heterozygous mutants at 2 months.

Interestingly, hemizygous wildtypes showed an increase in cytoplasmic TDP-43 protein in lumbar spinal cord MNs at 12 months. In addition, hemizygous wildtypes and heterozygous mutants showed a decrease in the levels of the autophagy protein p62/SQSTM1 in these MNs.

Since impaired axonal transport, MNJ disassembly and muscle denervation are early pathological events in ALS, our findings suggest a critical role for impaired dynein function in these processes. In addition, our results suggest that the mislocalisation of TDP-43 protein commonly observed in ALS patients may be partly due to reduced dynein function.

(d73) Mapping PDH1-mediated neuroprotection in mutant SOD1 ALS mice at single-cell resolution.

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A growing body of evidence shows disturbances in energy metabolism in amyotrophic lateral sclerosis (ALS). The oxygen-sensing prolyl hydroxylase domain proteins (PHDs) regulate cellular metabolism and play a role in oxidative stress response. Interestingly, selective inhibition of the PHD isoenzyme 1 (PHD1) protects cortical neurons via metabolic rewiring in a model for stroke. As the major source of oxidative stress, which is known to be elevated in ALS, energy metabolism forms a promising target for ALS research. Therefore, this project aims to investigate the effect of targeting cellular metabolism, via selective PHD1 ablation, in ALS. We demonstrated that genetic deletion of PHD1 improves muscle innervation and motor neuron integrity and extends the lifespan of SOD1-G93A mice with 10 days, increasing the disease duration with 40%. Using single-nuclei RNAseq (snRNAseq) on the lumbar spinal cord of early symptomatic mice, we were able to identify the different cell types present in the spinal cord of SOD1-G93A mice with and without PHD1. Gene ontology analysis of the differentially expressed genes showed that pathways related to oxidative metabolism are downregulated upon PHD1 deletion in several of these cell types, including motor neurons. A decrease of oxidative phosphorylation at the electron transport chain, which is the main site of mitochondrial reactive oxygen species (ROS) production, decreases oxidative stress levels. Further in-depth analysis on the differentially expressed genes in these different cell types should reveal how all players are involved in the mode of action of this ALS-mitigating strategy. In conclusion, our data indicate that neuroprotection by deletion of PHD1 in SOD1 ALS is mediated by decreased oxidative stress through metabolic modulation.

(d74) Glycogen modulates lifespan in a mouse model of amyotrophic lateral sclerosis

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Metabolic alterations have been described in amyotrophic lateral sclerosis (ALS). Motor neurons depend on glia energy metabolism to survive. Glycogen is a soluble polymer of glucose that is synthetized by glycogen synthase (GS). Nervous tissue contains low glycogen levels under homeostatic conditions, while aberrant glycogen deposition occurs in several pathologic conditions, such as Lafora Disease (LD). Abnormal astrocytic glycogen accumulation underlies astrogliosis and inflammation in a mouse model of LD. An astrocyte-specific knockout of GS prevents astrogliosis, microglial activation, and cerebral metabolic changes in these LD mice. Interestingly, glycogen is also increased in the spinal cord from ALS donors and from the SOD1G93A ALS mouse model. Nevertheless, the role of glycogen in the disease is unknown. The present work demonstrates an increase in glycogen in the spinal cord and brainstem, beginning at the pre-symptomatic stage in the SOD1G93A mouse model. Interestingly, this increase is associated with reactive astrocytes. To study the role of the accumulation of glycogen in ALS, we combined the SOD1G93A mouse model with a GS knockout. SOD1G93A mice heterozygous for GS (SOD1G93A GS+/-), and thus with a lower expression of GS, showed an increase in survival time compared to SOD1G93A. We also analyzed the expression of genes encoding mediators of the inflammatory response. C3, Ccl2, and Cxcl10 increased in SOD1G93A progressively from onset to end-stage, suggesting a possible role in disease progression. SOD1G93A GS1+/- mice showed lower levels of Cxcl10 at end-stage, compatible with a less marked pro-inflammatory response. In line with the previous results, the overexpression of an activator of GS shortened lifespan in SOD1G93A mice. In samples from patients with sporadic ALS (n=8), glycogen content in the spinal cord was highly variable between individuals and not different from control samples in general. Interestingly, two ALS patients exhibited high nuclear glycogen accumulation in motor neurons and glia. These results suggest that a subset of ALS patients might show disturbances in glycogen deposition. These results point to a pathogenic role of glycogen accumulation in ALS and suggest glycogen metabolism as a novel therapeutic target for this devastating disease.

(d75) Dysregulation of spinal interneuron subpopulations in the SOD1G93A ALS mouse model

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ALS is a neurodegenerative disease characterized by the progressive loss of motor neurons and premature death. Given their central role in the disease, major focus has been directed to motor neuron intrinsic properties as a cause for degeneration. However, our previous data demonstrated that V1 inhibitory interneurons degenerate and lose their connectivity to vulnerable motor neurons, possibly playing a role in disease initiation. V1 interneurons are a heterogeneous population that can be identified by specific marker co-expression. Using in situ multiplexing techniques, we have performed a comprehensive study on the expression of several markers in the SOD1G93A mouse model with the aim to elucidate the contribution of specific interneuron subpopulations to ALS pathology. We first validated the expression of 24 interneuron markers, typically described at embryonic stages, in adult mouse tissue using in situ sequencing. Then, we quantified transcript expression at three different timepoints using RNAscope HiPlexUp, an in situ hybridization quantitative technique that allows for co-detection of multiple transcripts. Our data show downregulation of different V1 interneuron clade-defining transcription factors starting at early stages of disease (from postnatal day 63) and preceding motor neuron degeneration. Notable is the early dysregulation of V1 Renshaw cells, defined by the Calbindin 1 marker. Additionally, we investigated other interneuron populations. Here, we describe substantial downregulation of the Chx10 transcription factor, marker for the excitatory V2a interneuron subpopulation, at later stages of disease (from postnatal day 112). Overall, our study evidences a pronounced dysregulation of interneuron motor networks throughout ALS progression and identifies several potential sources for non-autonomous motor neuron degeneration.

(d76) An interaction between synapsin and C9orf72 regulates excitatory synapses and is impaired in ALS/FTD.

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Dysfunction and degeneration of synapses is a common feature of amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD). A GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene is the main genetic cause of ALS/FTD (C9ALS/FTD). The repeat expansion leads to reduced expression of the C9orf72 protein. How C9orf72 haploinsufficiency contributes to disease has not been resolved. Here we identify the synapsin family of synaptic vesicle proteins, the most abundant group of synaptic phosphoproteins, as novel interactors of C9orf72 at synapses and show that C9orf72 plays a cell-autonomous role in the regulation of excitatory synapses. We mapped the interaction of C9orf72 and synapsin to the N-terminal longin domain of C9orf72 and the conserved C-domain of synapsin and show interaction of endogenous proteins in synapses. Functionally, C9orf72 deficiency reduced the number of excitatory synapses and decreased synapsin levels at remaining synapses in vitro in hippocampal neuron cultures and in vivo in the hippocampal mossy fibre system of C9orf72 knockout mice. Consistent with synaptic dysfunction, electrophysiological recordings identified impaired excitatory neurotransmission and network function in hippocampal neuron cultures with reduced C9orf72 expression, which correlated with a severe depletion of synaptic vesicles from excitatory synapses in the hippocampus of C9orf72 knockout mice. Finally, neuropathological analysis of post-mortem sections of C9ALS/FTD patient hippocampus with C9orf72 haploinsufficiency revealed a marked reduction in synapsin, indicating that disruption of the interaction between C9orf72 and synapsin may contribute to ALS/FTD pathobiology. Thus, our data show that C9orf72 plays a cell-autonomous role in the regulation of neurotransmission at excitatory synapses by interaction with synapsin and modulation of synaptic vesicle pools, and identify a novel role for C9orf72 haploinsufficiency in synaptic dysfunction in C9ALS/FTD.

(d77) Evaluation of M102, a dual NRF2 and HSF1 activator, as a Novel Therapeutic in Amyotrophic Lateral Sclerosis (ALS)

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ALS is a devastating neurodegenerative disease for which new therapies are urgently needed. We have identified M102, a small molecule new chemical entity (NCE) which activates the NFE2-related factor 2-antioxidant response element (NRF2-ARE) pathway, as a promising drug candidate for the treatment of ALS. More recently, we have identified that M102 can also activate the heat shock factor 1 (HSF1) signalling pathway, activating transcription of heat shock element (HSE) associated genes that upregulate molecular chaperones to improve proteotoxic stress. Here we investigate the pharmacology and efficacy of M102 in two mouse models of ALS, TDP-43Q331K and SOD1G93A.

TDP-43Q331K transgenic mice were dosed subcutaneously with vehicle or M102 (2.5mg/kg twice daily, or 5mg/kg once daily) from 25 days of age. One cohort was dosed until 6 months (n=14/group), while a smaller cohort were dosed until 3 months (n=6/group). SOD1G93A transgenic mice were dosed orally with vehicle or M102 (5mg/kg, 12.5mg/kg, or 25mg/kg once daily) from 25 days of age until 90 days of age (n=8 per group). For both studies behavioural tests for motor function (rotarod, gait analysis) and muscle function (electrophysiology) were measured at set time points. Body weights were recorded daily and at the end of the study, brain and spinal cord were collected for immunohistochemistry and qPCR analysis.

In the TDP-43Q331K 6-month cohort, a significant decrease in body weight was observed for the 2.5mg/kg M102 dosing group compared to the vehicle group, suggesting a beneficial effect of M102. In addition, improvements in compound muscle action potential (CMAP) amplitude and gait parameters were observed in M102 dosing groups at 6 months of age when compared to the vehicle group. Tissue analysis showed significant upregulation in NRF2 target genes in the cortex at 3 months of age and upregulation in HSF targets at 3 and 6 months of age in M102 dosed groups. In the SOD1G93A a significant improvement in mouse weight was observed in the M102 groups when compared to vehicle as well as a significant increase in CMAP amplitude at 90 days of age. Together these data point to M102 as a promising therapeutic approach in ALS as there is improvement to ALS phenotypes in multiple preclinical models. Manufacturing scale up and preclinical toxicology to support clinical trials in ALS are currently underway.

(d78) Peroxisomal lipid synthesis can be a disease-modifier factor in motor neurons from ALS patients

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Recent studies reveal that nuclear membrane lipids, including peroxisome-derived plasmalogens, are decreased in ALS. To evaluate the causes of this defect, we studied changes in essential proteins involved in lipid synthesis in MN derived from induced pluripotent stem cells from patients with the disease and controls by using a set of inhibitors and pharmacological agonists of the TGF-β, Wnt, SHH, and RA signaling pathways. These MNs are maintained in culture through the activation of FGF, BDNF, and CNTF, and they were subsequently induced with two ALS-related types of stress: H2O2 and polyinosinic: polycytidylic acid (poly-IC). We performed an immunofluorescence assay to study proteins involved in cellular stress (TDP-43 and G3BP1) and the peroxisome-mediated lipid synthesis (AGPS and SCP-2). The results demonstrate that MNs derived from ALS patients increase cytosolic (but not nuclear) aggregates of TDP-43 and G3BP1 in both types of stress. We observed an increase of TDP-43 aggregates only with the treatment with poly-IC for the control line. These stressors caused diminished levels of AGPS, a key enzyme in plasmalogen synthesis, and increased values of SCP-2 (a peroxisomal transporter of lipid peroxides). These results were partially reproduced in the G93A mice, showing decreased AGPS expression in MN from lumbar spinal cords. Further, samples from the spinal cord from ALS donors showed increased SCP-2 mRNA levels, though changes at the protein levels were less evident. Our results point to the potential pathogenic role of intracellular membrane lipids' altered composition involving peroxisomal located enzymes.

(d79) Insights for the identification of a molecular signature for ALS exploiting integrated miRNA profiling of iPSC-derived motor neurons and exosomes

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder characterized by progressive degeneration of motor neurons (MNs). Most cases are sporadic, whereas 10% are familial. The pathological mechanisms underlying the disease are partially understood, but it is increasingly being recognized that alterations in RNA metabolism and deregulation of microRNA (miRNA) expression occur in ALS. In this study, we performed miRNA expression profile analysis of iPSC-derived MNs and related exosomes from familial patients and healthy subjects. We identified dysregulation of miR-34a, miR-335 and miR-625-3p expression in both MNs and exosomes. These miRNAs regulate genes and pathways which correlate with disease pathogenesis, suggesting that studying miRNAs deregulation can contribute to deeply investigate the molecular mechanisms underlying the disease. We also assayed the expression profile of these miRNAs in the cerebrospinal fluid (CSF) of familial (fALS) and sporadic patients (sALS) and we identified a significant dysregulation of miR-34a-3p and miR-625-3p levels in ALS compared to controls. Taken together, all these findings suggest that miRNAs analysis simultaneously performed in different human biological samples could represent a promising molecular tool to understand the etiopathogenesis of ALS and to develop new potential miRNA-based strategies in this new propitious therapeutic era.

(d80) Synaptic degeneration is P53 independent in scenarios of disease, injury and development in mice

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P53 is widely appreciated for its role in apoptosis, however its role at the synapse and in the axon remains unclear. P53 has been implicated at early stages of neuronal death, with local actions suggested to contribute to dysfunction and degeneration of synapses, however further work is required to understand how P53 is involved in different neurodegenerative contexts. Here, we aimed to assess whether reductions in P53 can reduce synaptic loss in a range of different neurodegenerative scenarios, with the hypothesis that P53 reduction will delay synaptic loss. To test this, we utilised an inducible, P53-floxed mouse model which undergoes Cre-LoxP-mediated recombination to express significantly reduced levels of P53 following postnatal treatment with tamoxifen. Using this P53-deficient model, we assessed levels of synaptic loss under different conditions: after nerve injury caused by axonal transection; during synapse elimination that occurs as part of normal development; and finally, during synaptic withdrawal, often referred to as die-back, caused by disease. Firstly, to investigate whether P53 reductions can delay degeneration after injury, we introduced nerve-muscle preparations from mice with reduced P53 to an ex vivo model of peripheral nerve injury and assessed levels of synaptic loss after 24 hours. To investigate the role of P53 in a developmental context, we quantified levels of poly- and mono-innervation when P53 is reduced, as an indicator of neuromuscular maturity. Finally, by crossing the inducible P53-floxed model with a mouse model of Spinal Muscular Atrophy (SMA), we assessed whether reductions in P53 could alter the extent of synaptic loss in a disease scenario. Overall, our data indicate a lack of association with P53 in all contexts examined, with no reductions in synaptic loss seen after injury or in a disease scenario. The rate of normal, developmental pruning of synapses was similarly unaffected. Collectively, our data support that synaptic and axonal degeneration in these scenarios are P53-independent.

(d81) Convergent pathomechanisms underlying selective death of motoneurons in ALS using patients-derived iPSCs

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The heterogeneity of the genetic causes leading to ALS represents one of the major limiting factors to the identification of effective therapeutic strategies. Indeed, ALS-related genes are involved in several and different biological processes such as RNA metabolism, autophagy, DNA damage repair and protein transport. This puzzling context moved the research efforts toward the development of gene-specific therapies (such as antisense oligonucleotides), which seem to represent a valuable strategy rather than focusing on the identification of a common therapy for the majority of the ALS cases. Nevertheless, all the pathological alterations linked to different ALS genes eventually lead to motor neuron loss, indicating the presence of commonly shared pathomechanisms that are still unknown. In this study, we aimed at identifying molecular and biological alterations shared by familial cases of ALS with different genetic backgrounds. To this end, we analyzed the phospho-proteome of hiPSC-derived motor neurons carrying mutations in the C9orf72, TBK1, FUS, TARDBP and SOD1 genes with the final goal of highlighting a common malactivation of precise biological pathways in ALS. We found increased phosphorylation levels of different c-Jun N-terminal Kinase (JNK) targets, which are involved in apoptotic signaling as well as in synaptic functions. Indeed, the pre-synaptic localization of JNK suggested that this kinase might contribute to the impaired release of synaptic vesicles through an aberrant interaction with SNARE proteins. Thus, our data highlighted a potential pathological link between Jun-mediated cell death and synaptic impairment. This strengthens the theory that restoration of synaptic composition and activity might represent a valid neuroprotective entry point for rescuing MN loss in a broad spectrum of ALS.

(d82) Using optogenetics to model activity-dependent neurodegeneration in amyotrophic lateral sclerosis

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Background: The hallmark of amyotrophic lateral sclerosis (ALS) is degeneration of motor neurons (MNs). There is increasing evidence that hyperexcitability or excitotoxicity mechanisms may underlie the selective vulnerability of MNs in ALS. To determine the relationship between activity and MN degeneration requires a system in which activity can be controlled. Here we present a novel in vitro model with optogenetic control of induced pluripotent stem cell (IPSC)-derived MN firing. We use this to explore the impact of activity on maturity of IPSC-MNs and activity-related disease mechanisms in C9orf72 ALS (C9-ALS). Further we generate 2D and 3D "disease in a dish" co-culture systems using optogenetic MNs and muscle to model the neuromuscular junction (NMJ) in health and disease.

Aims: To establish optogenetic control of IPSC-MNs alone and in co-culture with muscle and use this model to study effects of activity on (1) maturity of IPSC-MNs (2) activity-dependent mechanisms such as excitotoxicity in C9-ALS.

Methods: We differentiated MNs from IPSCs from C9-ALS patients, isogenic and healthy controls. IPSC-MNs were transduced to express channelrhodopsin (ChR2) and stimulated with 460nm blue light. Light-evoked activity was recorded using a multi-electrode array (MEA). IPSC-MNs were chronically stimulated with high, medium and low-level protocols, then analysed by immunocytochemistry and western blot for markers of MN maturity and toxicity. ChR2-IPSC-MNs and C2C12 muscle were grown in 2D co-cultures and microfluidic chambers and live-imaged for light-evoked muscle contraction.

Results: We confirmed high levels of neuron-specific ChR2 expression in IPSC-MNs. MEA recordings showed robust control of MN activity with light. High level chronic stimulation of C9-ALS IPSC-MNs induced significantly higher levels of cellular stress than in controls. In muscle co-cultures, we confirmed IPSC-MNs retain MN marker expression and found light-evoked muscle twitch confirming functional connectivity.

Conclusions: In this study we have developed and validated a model with optogenetic control of IPSC-MNs. We have used this to demonstrate effects of optogenetic stimulation on maturity, and a selective vulnerability of C9-ALS MNs to high-level activity. Furthermore, we have developed a functional model of the NMJ with optogenetic control of muscle. This novel system provides a platform for further study of activity-dependent pathways to MN and NMJ degeneration in ALS.

(d83) Misfolded SOD1 aggregates are shaped by disease associated mutations and their microenvironment

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Mutations in the human Superoxide dismutase 1 (hSOD1) gene is a well-established cause of the motor neuron disease ALS. Patients and transgenic (Tg) ALS model mice carrying mutant variants develop hSOD1 aggregates in the CNS. We have identified two hSOD1 aggregate strains, which both transmit spreading template-directed aggregation and premature fatal paralysis when inoculated into adult transgenic mice. This prion-like spread of aggregation could be a primary disease mechanism in SOD1-induced ALS.

Explorations of pathogenesis and development of therapeutics should be conducted in models that replicate aggregate structures forming in the CNS. To date SOD1 aggregation has been studied extensively both in cultured cells and under various conditions in vitro. To determine how the structure of aggregates formed in these model systems relate to disease associated aggregates in the CNS, we used a binary epitope-mapping assay to examine aggregates of hSOD1 variants G93A, G85R, A4V, D90A, and G127X formed in vitro, in four different cell lines and in the CNS of Tg mice. We found considerable variability between replicate sets of in vitro generated aggregates. In contrast, there was high similarity between replicates of a given hSOD1 mutant in a given cell line, but pronounced variations between different hSOD1 mutants and different cell lines. The aggregates formed in vitro or in cultured cells did not replicate the aggregate strains that arise in the CNS. Our findings suggest that the distinct aggregate morphologies in the CNS could result from the specific microenvironment combined with stringent quality control and a second-order selection by spreading ability.

(d84) Ghrelin-based therapies for ALS – understanding mechanisms of action

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Ghrelin is an endogenous growth hormone secretagogue and neuropeptide that enhances food intake and appetite, and signals via the GHSR1 receptor. Patients with ALS have markedly reduced circulating levels of ghrelin, despite having a lower body mass index than age matched controls. This suggests that impaired ghrelin signalling could contribute to disease progression, with ghrelin having been found to counteract neuro-inflammation and exert neuroprotective effects on motor neurons. Furthermore, the endogenous adipokine hormone leptin, which suppresses food intake and appetite, has also been found to exhibit neuroprotective effects and, while historically detrimental to ALS progression, could have an additive protective effect when co-administered with ghrelin. To investigate the neuroprotective capabilities of these hormones against the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2), SH-SY5Y neuroblastoma cell cultures were utilised. Further experiments utilised the NSC-34 motor neuron like and BV-2 microglial cell lines to assess the effects of these hormones on neuroinflammatory cytokines (TNF-α). Assays were then undertaken to assess metabolic function and cell viability, MTT and crystal violet (CV) respectively, further cell imaging and ELISA assays were undertaken to investigate receptor and inflammatory marker expression. In SH-SY5Y cells, treatment with either hormone led to an increase in metabolic function when compared to H_2O_2 alone with significant changes in viability being observed in CV assays for ghrelin. Co-treatment results suggested a decrease in mitochondrial function and a small but non-significant increase in cell viability over ghrelin alone. For NSC-34 cells, ghrelin provided significant protection against cell death and restored mitochondrial function in TNF-α induced cell death and in co-culture with active BV-2 cells. No significant change was observed when treated with leptin or a coadministration of both hormones. These results would suggest that ghrelin exerts neuroprotective effects individually; however, there is insubstantial evidence to support an additive effect for treatment with both hormones.

(d85) Investigating impaired methylglyoxal detoxification as a potential mechanism of astrocyte toxicity in Amyotrophic Lateral Sclerosis (ALS/MND)

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Rationale and Hypothesis: Astrocytes play a crucial role in supporting normal motor neuron function, but become toxic in ALS through various mechanisms. Our work in C9orf72-ALS patient-derived astrocytes (iAstrocytes) identified a potential defect in the glyoxalase system, the major pathway for detoxification of methylglyoxal (MGO), a highly reactive by-product of glycolysis which damages nucleic acids and proteins. We observed reduced GLO-1 levels, the rate-limiting enzyme in the glyoxalase pathway. Furthermore, the cytoprotective NRF2 pathway, which is activated by MGO and promotes it's breakdown by the glyoxalase system, may also be dysfunctional in ALS. An impaired ability to safely eliminate MGO due to reduced GLO-1 levels and NRF2 dysfunction, may contribute to astrocyte toxicity in ALS through accumulation of damaged molecules termed advanced glycation end products (AGEs).

We hypothesised that ALS iAstrocytes would exhibit elevated MGO levels and AGE formation due to disrupted glycolysis and impaired MGO detoxification, exacerbated by a dysfunctional NRF2 pathway.

Objectives and Methodology: We performed GC-MS to assess whether glycolysis was disrupted in ALS iAstrocytes. Moreover, to determine whether ALS iAstrocytes can efficiently detoxify MGO compared to healthy controls, we measured MGO levels, AGE formation, and NRF2 pathway activation in untreated iAstrocytes, and iAstrocytes challenged with CBR-470-1, which increases MGO production via inhibiting the glycolytic enzyme PGK1.

Results: Preliminary GC-MS analysis revealed decreased metabolite levels in late glycolysis in ALS iAstrocytes, indicating a build-up of metabolites upstream, previously shown to increase MGO formation. However, cellular MGO levels did not appear elevated in untreated ALS iAstrocytes, despite no upregulation of alternative compensatory detoxification pathways. Nevertheless, AGE levels were higher in ALS iAstrocytes and increased following CBR-470-1 treatment. ALS iAstrocytes showed muted CBR-470-1-induced NRF2 activation compared to controls, despite a significant induction of the pathway. To characterise this pathway further, we will measure glyoxalase pathway activity, and glutathione levels - a crucial NRF2-regulated antioxidant and GLO-1 co-factor.

(d86) Aging-dependent activity impairments of human C9orf72-mutant motor neurons are accompanied by aberrant transcriptional programs

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by dysfunction and subsequent loss of cortical and spinal motor neurons (MN). Previous studies demonstrated that alterations in synapses and neuronal activity are part of the underlying pathomechanisms in both in vitro and in vivo models, but their contribution to neuronal death is still under debate. Specifically, the influence of hyper- or hypoactivity on cellular disease progression is highly controversial since both phenotypes have been described and found to be harmful in ALS MN.In this study, we employed high definition multielectrode array (HD-MEA) techniques to longitudinally monitor the electrophysiological properties of hiPSC-derived C9orf72-mutant MN. We found an early hyperactivity of ALSC9orf72 MN, which drastically decreased upon aging while neurodegeneration started to occur. In accordance with previous findings of synaptic alterations in ALS MN, we could also observe a generally reduced network synchroneity in ALSC9orf72 MN cultures. Next, we performed a longitudinal transcriptomic analysis to elucidate the molecular causes triggering the loss of activity properties in mutant neurons. Consistent with our HD-MEA findings, we observed an up-regulation of synaptic transcripts in ALSC9orf72 MN at the earlier time point, which was followed by a significant reduction over time. By administration of the SK channel inhibitor Apamin, which has previously been shown to be neuroprotective in ALS MN, we were able to achieve beneficial effects on an electrophysiological as well as transcriptional level. Altogether, this study suggests phenomena of synaptic maturation as possible explanation for contradicting evidence on electrophysiological alterations in ALSC9orf72 MN, provides an insight into the longitudinal development of their neuronal activity and links these functional changes to aging-dependent transcriptional programs.

(d87) RIPK1 is elevated in ALS patient spinal cords and RIPK1 kinase inhibition delays ALS disease progression in the SOD1G93A mouse model

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Background: Receptor interacting protein kinase 1 (RIPK1) mediates inflammatory and cell death signalling. Loss of function mutations in OPTN and TBK1 can lead to amyotrophic lateral sclerosis (ALS) and cause hyperactivation of RIPK1 kinase activity, suggesting abnormal RIPK1 activation in the central nervous system (CNS) contributes to the pathophysiology of ALS. Previous studies have shown increased RIPK1 expression in SOD1G93A mouse models and ALS patient-derived spinal cord samples, but the role of RIPK1 kinase activity in these pre-clinical and clinical samples remains to be further elucidated.

Objectives: 1) To determine the efficacy of a CNS-penetrant RIPK1 inhibitor (RIPK1i) in an ALS mouse model. 2) To assess RIPK1 kinase activation (phospho-RIPK1) and expression in post-mortem spinal cord samples from ALS patients relative to control.

Methods: SOD1G93A mice were monitored to characterize symptom onset and disease progression using C57BL6 mice as control. A total of 35 mice (25 SOD1G93A mice and 10 C57BL6) were divided into four groups: Group 1 (C57BL6 control, n=5) received vehicle, Group 2 (C57BL6 control, n=5) received RIPK1i, Group 3 (SOD1G93A, n=12) received vehicle and Group 4 (SOD1G93A, n=13) received RIPK1i. Symptom onset, survival and RIPK1 target engagement were assessed, and motor impairment and locomotor activity were measured by wire hang and open field test. Meso Scale Discovery technology was used to measure the levels of activated (phospho-RIPK1) and total RIPK1 in post-mortem spinal cord samples from sporadic ALS patients (n=41) and non-neurological controls (n=25) obtained from Target ALS, Massachusetts General Hospital, and University of Pittsburgh biobanks.

Results: RIPK1 inhibition in the SOD1G93A mice significantly delayed the onset of motor impairment and demonstrated target engagement in the spinal cord. In human specimens, phospho-RIPK1 and total RIPK1 expression were significantly increased in post-mortem spinal cord samples from sporadic ALS patients compared to control.

Conclusions: Inhibition of RIPK1 kinase activity was effective in delaying symptom onset and progressive motor impairment in SOD1G93A mice. Both RIPK1 expression and activation were significantly elevated in post-mortem spinal cord samples from sporadic ALS patients relative to control. This study supports the relevance of RIPK1 kinase activation for ALS progression in humans.

(d88) Muscle stem cell and neuromuscular junction crosstalk in an ALS animal model

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Neuromuscular junction (NMJ) dismantlement and muscle atrophy are key pathological hallmarks of Sod1G93A mice, which is the most used Amyotrophic Lateral Sclerosis (ALS) animal model that presents an early disease onset and fast progression of the disease. Despite the etiopathogenesis of ALS is still uncertain, growing evidence guide toward non-cell autonomous mechanisms that trigger NMJ and motoneuron (MN) degeneration. Indeed, NMJ dismantlement is associated to the result of the insufficient capacity of Terminal Schwann Cells (TSCs) to restore synaptic reinnervation and consequent nerve retraction. Meanwhile, alterations in the postsynaptic endplate and early metabolic changes in muscle have also been observed. Furthermore, it is known that muscle stem cells are essential for NMJ restoration and maintenance. Since muscle metabolic/trophic alterations and changes in synaptic transmission are present prior to symptom onset, we assume that there might be an important dynamic crosstalk between muscle stem cells and NMJ in early stages of the disease that needs to be studied.

After characterizing the NMJ during disease progression in Sod1G93A mice, we observe an increase in satellite cell number, NMJ denervation and TSC miscoverage appear by symptomatic age mainly in fast-twitch muscles. These symptoms are aggravated in end-stage mice and are also observed in slow-twitch muscles. Although this work needs to be further completed, we believe that muscle stem cell-NMJ crosstalk is an interesting insight that could be of important relevance in the backward-signaling process from the muscle to the distal axon. This view would highlight the skeletal muscle as key target in ALS disease.

(d89) Differential effects following the cannabinoid CB1 or CB2 receptor activation in a frontotemporal dementia-related TDP-43 mouse model

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Cannabinoids are a group of compounds that have attracted interest in recent years for their ability to modulate the so-called endocannabinoid system (ECS) and exert beneficial effects in several neurological disorders. One of the most relevant and promising therapeutic implications is the improvement of neuronal homeostasis, integrity and survival and their anti-inflammatory effects. While the therapeutic potential of cannabinoids in amyotrophic lateral sclerosis has been more extensively explored with promising results, less data are available in frontotemporal dementia (FTD), the other side of the ALS-FTD spectrum. Thus, the main objective of this study was to explore the therapeutic effects of the pharmacological activation of CB1 or CB2 receptors in TDP-43-related FTD. To this end, we used FTD mice that overexpress TDP-43 in the forebrain under the control of α-CaMKII promoter, then showing marked symptoms of dementia (provided by CKJ Shen, Taiwan; see Tsai et al., J Exp Med 2010). They were daily administered (i.p.) with the selective CB1 agonist ACEA (1.5 mg/kg) or the CB2 agonist HU-308 (5 mg/kg) from pre-symptomatic phases (PND45) to symptomatic stages (PND90). Our data proved a delay in the appearance of the cognitive deterioration, after the treatment of FTD transgenic mice with HU-308 or ACEA. However, at PND90 the effects of HU-308 were attenuated, whereas those obtained with ACEA persisted to a greater extent. Paradoxically, the relevance of CB1 receptor activation in improving cognitive impairment contrasts with the results obtained in immunohistochemical analyses. Whereas a preservation of pyramidal neurons of the prefrontal cortex (PFC) and the CA1 layer of the hippocampus (labelled with Ctip2 and NeuN immunostaining, respectively) was evident after HU-308 treatment, ACEA effects were less pronounced in the PFC. Moreover, a marked reduction in the immunoreactivity for the microglia marker Iba-1 was detected in the PFC and CA1 layer of hippocampus after the treatment with HU-308, but not with ACEA, and the same happened with the astroglial marker GFAP in the CA1 and dentate gyrus of the hippocampus. In summary, our data confirm the potential of modulating the ECS as a therapy against TDP-43-induced neuropathology in FTD, but, given the difference found with ACEA and HU-308 in relation with their behavioural and histopathological effects, we assume that both CB1 and CB2 receptors should be activated to obtain the best therapeutic effects.

(d90) The role of FUS in axonal translation for motor neuron homeostasis and degeneration

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Spinal motor neurons are highly polarized cells with axons that can extend up to one meter in length to reach their muscle targets. Muscle denervation is one of the earliest hallmarks of Amyotrophic lateral sclerosis (ALS), which leads to muscle atrophy and ultimately paralysis. The mechanisms underlying the early demise of neuromuscular junctions (NMJs) remains poorly understood. Yet targeting this early event may provide a valuable therapeutic strategy to combat ALS.

FUS (FUsed in Sarcoma), whose mutations account for 5% of the familial ALS forms, is an RNA binding protein involved in a wide range of cellular processes including DNA damage repair, RNA stability, splicing and transport. FUS is known to bind more than 2500 RNA targets and has been linked to local translation. While it is predominantly found in the nucleus, FUS is indeed also present in dendrites, axons and at the NMJ.

Previous work from our lab identified suppression of local, axonal protein synthesis prior to development of ALS-like disease in a humanized ALS mouse model expressing the human FUS gene harboring an ALS-causing mutation. This was accompanied by increased accumulation of ALS-causing FUS mutants in axons, suggesting gain of toxic mechanisms. Our results raised the possibility that impaired local translation may contribute to the age-dependent neurodegeneration associated with mutant FUS expression.

To gain insight into the compartment-specific mechanisms of toxicity, in particular in the axons, we exploited primary spinal cord motor neuron cultures using microfluidic devices to separate axons from their cell bodies. Using antisense oligonucleotides (ASOs), FUS protein levels were successfully reduced by ~80%, allowing us to unravel the FUS-specific axonal targets by mapping the transcriptomic changes (using SMART-seq2) under physiological versus FUS-depleted conditions. In order to identify the transcriptomic alterations caused by ALS-causing mutation in FUS, we also applied the same approach using spinal cord motor neurons isolated from mice expressing FUS(R521H). Ultimately my work will pose the question, so far untested in a compartment specific manner, as to whether FUS causes toxicity uniquely through gain of toxicity or if loss of FUS function also contributes to axonal degeneration. Finally, we will assess whether and how newly identified FUS-dependent axonal targets are locally translated to gain insight into ALS pathological mechanisms.

(d91) Histone Deacetylase Inhibition Regulates Lipid Homeostasis in a Mouse Model of Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) patients suffer from the lack of effective treatments and urgently need the development of new therapeutic strategies. While the aetiology is still incompletely understood, it has become clear that lipid homeostasis perturbations act as a major contributor to ALS susceptibility, disease progression and survival [1]. Recently, we demonstrated that FUS mice (PrP-hFUS-WT3) present metabolic alterations in the spinal cord at pre-symptomatic and symptomatic stages. Using ACY-738, a potent HDAC inhibitor that can cross the blood-brain barrier, we showed that HDAC inhibition restores metabolic defects and subsequently ameliorates disease phenotype and prolongs the life span of FUS mice [2]. Here, we investigated the specific effects of HDAC inhibition on lipid metabolism using untargeted lipidomic analysis coupled with transcriptomic analysis in the spinal cord of FUS mice [3]. We demonstrated that symptomatic FUS mice recapitulate significant lipid alterations found in ALS patients and the SOD1 mouse model. Glycerophospholipids, sphingolipids and cholesterol esters mainly were affected, with markedly fatty acids remodelling. Strikingly, HDAC inhibition mitigated lipid homeostasis defects and selectively targeted glycerophospholipid metabolism at the gene and metabolite level. Moreover, HDAC inhibition decreased the accumulation of cholesterol esters. Therefore, our data suggest that HDAC inhibition is a potential new therapeutic strategy to modulate lipid metabolism in ALS. Our data highlight the Prp-hFUS-WT3 as a valuable model to investigate lipid alterations in ALS and revealed that ACY-738, a potent and selective HDAC inhibitor that can cross the blood-brain barrier, can act as a lipid-modifying drug. Specific lipid alterations could serve as effective biomarkers and potent indicators for the effectiveness of new HDAC inhibitors, adding a stepping stone to developing new innovative treatments for ALS.

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(d92) Human iPSC-derived motor neurons and spinal cord organoids to model C9orf72 ALS

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GGGGCC repeat expansions in C9ORF72 gene are the most common identified cause of Amyotrophic lateral sclerosis (ALS), but their pathogenic processes are still unknown. Moreover, no therapy is able to effectively slow, halt or reverse disease progression.

Therefore, expanding our knowledge on ALS pathophysiology is imperative to develop novel treatment strategies. Induced pluripotent stem cells (iPSC)-derived 2D and 3D cultures represent a reliable disease models to unraveling C9ALS pathogenesis, and test disease-modifying therapies.

We reprogrammed iPSCs from C9ALS patients and isogenic lines and differentiated them into MNs using a 14-days protocol. We investigated the phenotype of the C9ALS lines compared to controls, evaluating cells survival, DNA damage pathways, defects in axonal elongation and identifying disease hallmarks. Moreover, we generated spinal-cord organoids (SCOs) from C9ALS and isogenic iPSCs using a free-floating protocol promoting neural caudalization and ventralization obtaining mini-spinal cord displaying different co-existing neuronal subpopulations. Mass spectrometry and single cells RNA seq analysis revealed aberrant proteins expression and different cluster presence and gene expression in C9ALS SCOs compared to isogenic. We treated C9ALS-MNs and SCOs with morpholino antisense oligonucleotides (MO) against C9Orf72 repeat expansion, demonstrating the modification of the pathological phenotype in both models.

Our results suggest that patient specific iPSC-derived MNs and SCOs are a valuable system for modeling features of C9ALS pathology, investigating C9ALS pathomechanisms, and testing possible new treatments in vitro. Moreover, we demonstrated that Morpholino-mediated approaches represent a promising therapeutic strategy.

(d93) Characterisation of TDP-43 expression in the Thy1-hTDP-43 ALS mouse model

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that results from the loss of upper and lower motor neurons. Despite a heterogeneous, complex genetic background, one aspect that connects 97% of ALS patients is the presence of abnormal protein aggregates in motor neurons with TDP-43 as the main constituent. Moreover, mutations in the TARDBP gene are causative in 4% of familial cases and 1% of sporadic ALS patients. This indicates that TDP-43 is central to ALS pathogenesis.

To investigate this key aspect of ALS, we used the Thy1-hTDP-43 mouse model, a severe model of ALS with a lifespan of 20-25 days. Here, we have quantified TDP-43 protein expression over the lifespan, facilitating comparison with other disease phenotypes, such as motor neuron cell death and NMJ denervation. Using human-specific versus pan-mammalian antibodies for TDP-43, we compared the expression of pathological hTDP-43 and endogenous mouse TDP-43 in the spinal cord and brain, via western blotting. To document cell death, blinded motor neuron counts were performed at pre-symptomatic (P8), early-symptomatic (P15) and symptomatic (P17) timepoints, as well as at disease end-stage (P19/20).

Our results demonstrate a significant overexpression of pathological hTDP-43 pre-symptomatically, leading to an 8-fold increase in TDP-43 expression at end-stage. Interestingly, non-symptomatic heterozygous littermates also showed overexpression of hTDP-43 up to P15, where expression then plateaued at a two-fold increase compared to wild-types for up to 9 months. Motor neuron counts showed that there was a significant overall loss of motor neurons in hTDP-43+/+ mice at end-stage (N=3 mice per genotype, n=18 spinal cord slices analysed per genotype, P-value=0.02). This detailed analysis of the time course of TDP-43 overexpression will allow us to investigate the order of pathological events in the Thy1-hTDP-43 model, including the timing of neuromuscular junction denervation compared to motor neuron cell death.

(d94) 3D Bioprinting as a promising approach for the differentiation of stem cells in the study of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease (NDD) that affects upper and lower motor neurons in many different compartments of the nervous systems leading to their degeneration. The typical symptoms are muscle atrophy, weakness and spasticity. As other NDDs, ALS can be manifested in two forms, the sporadic (sALS) and the familial one (fALS). sALS patients account for the 90% of the cases. Unfortunately, the only treatments available are symptomatic ones. Due to the lack of a cure, it is important to find a good model. Two important innovations of recent years are 3D Bioprinting and induced pluripotent stem cells (iPSCs). Bioprinting allows the inclusion and the growth of cells in a more physiological model for the study of the interactions between cells and environment. Moreover, iPSCs can be obtained from patients and differentiated in many different types of cells and are fundamental for the advance of personalized medicine. Aim of this work was the development of a protocol of 3D stem cells differentiation to demonstrate the robustness of this tool for the study of ALS pathogenesis. We first reprogrammed iPSCs from peripheral blood mononuclear cells (PBMCs) of a healthy subject and an ALS patient, and we differentiated them in neural stem cells (NSCs) in 2D. NSCs were then included in Cellink Bioink, a commercial hydrogel composed of cellulose nanofibrils and alginate, and printed in 3D. Included cells were differentiated first in motor neuron progenitors (MNPs) and finally in motor neurons (MNs). During the differentiation, cells were tested for viability and characterized by confocal microscopy and RT-qPCR. Moreover, we tested if included cells maintain their electrophysiological characteristics after the printing. NSCs show a good viability and proliferation rate during the differentiation process. Moreover, we confirmed the good differentiation in 3D using specific markers of every differentiation step. Finally, we found that Mouse Motor Neuron-Like Hybrid Cells (NSC34) included and printed in the hydrogel maintain their ability to produce potential actions when opportunely stimulated.In conclusion, we confirmed that 3D Bioprinting is a potential tool for the study of ALS

(d95) Assessing impaired mitostasis as common denominator underlying motor neuron degeneration in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by the loss of upper and lower motor neurons. Due to the heterogeneity in factors leading to ALS, the affected pathways causing motor neuron death remain unknown. We hypothesize that impaired mitochondrial homeostasis (mitostasis) is a common underlying process resulting in selective motor neuron degeneration. Therefore, it is our goal to assess the therapeutic potential of targeting impaired mitostasis. We have first identified mitostatic defects in multiple neuroblastoma models carrying three different fALS associated mutations: SOD1G93A, VCPR191Q and TBK1R357* respectively. This will later on be used to perform a high throughput screen to identify compounds able to rescue the characterised mitochondrial phenotype and the consecutive motor neuron degeneration. We found a depolarization of the mitochondrial membrane potential in the SOD1G93A, VCPR191Q and TBK1R357* models. We confirmed these data in isolated mitochondria from the respective cell lines and we confirmed that these changes were not caused by alterations in mitochondrial mass as indicated by TOM20 staining. Using high-resolution respirometry, we also found an increased mitochondrial respiration in the cells carrying the SOD1G93A and VCPR191Q mutations. These findings were supported by metabolic measurements using the Omnilog assay. The observed increase in oligomycin dependent respiration indicates mitochondrial proton leak or uncoupling as the cause of the observed mitochondrial depolarization. Finally, the mitochondrial membrane potential could be partially restored during compound screens in both SOD1G93A and VCPR191Q cell lines. In conclusion, we found common defects in mitochondrial homeostasis in our models for familial ALS. In combination with the partial rescue of our depolarization phenotype using different compounds, this suggests that mitostatic defects are a promising target for new therapeutic strategies in ALS.

(d96) Characterization of motor neuron organoids derived from sALS patients

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Amyotrophic lateral sclerosis (ALS) is a non-cell autonomous disorder as many cell types contribute to motor neurons death. The lack of effective treatments is probably due to the absence of a realistic model that can recapitulate pathogenic mechanisms. Cerebral organoids are pluripotent stem cell-derived self-organizing structures that recapitulate brain development and allow in vitro generation of the tissues. We developed a new method for the generation of motor neuron organoids (MNOs) that can be used for the study of pathogenic mechanisms in ALS. Aim of the work was to characterize a 3D organoid model for the study of ALS pathogenesis. We started from iPSCs obtained from healthy controls and sALS patients. We differentiated iPSCs into neural stem cells (NSCs). NSCs were dissociated using StemPro Accutase and a cell strainer. Then, NSCs were plated on low-attachment plates and were cultured in floating conditions using an orbital shaker. NSCs were differentiated in these conditions into motor neuron progenitors (MNPs), immature motor neurons (MNs) and finally mature MNs. We then characterized cells by phase-contrast and confocal microscopy. We found that brain organoids derived from sALS patients were smaller and with irregular morphology compared to healthy controls. Using the GFAP marker, we found that sALS organoids have a thicker glial layer compared to healthy controls. We also found that healthy controls organoids show longer neurites compared to sALS organoids. Finally, we found a diverse composition of cell populations. Indeed, healthy controls organoids show a higher amount of differentiated cells compared to sALS organoids. By RNAseq, we found a ten-fold increase of deregulated gene in MNOs respect to 2D cell models. Moreover, in ALS MNOs we found an extensive deregulation of genes involved axon guidance, axonogenesis, and extracellular matrix organization when compared to control. Our data suggest that brain organoids represent a promising tool for the investigation of pathogenic mechanisms of ALS. In fact, we found typical pathological hallmarks of the pathology, such as the presence of gliosis, the smaller length of neurites, decreased level of mature MNs, and deregulation of genes implicated in ALS. In conclusion, brain organoids represent a promising tool for the investigation of pathogenic mechanisms of ALS.

(d97) Assessing FUS liquid-liquid phase separation and toxicity in Drosophila ALS models

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Mutations in the gene fused in sarcoma (FUS) are a cause of around 5% of cases of familial ALS. In mutation carriers FUS mislocalises to the cytoplasm where it aggregates. In addition, FUS aggregates in around 10% of cases of frontotemporal dementia (FTD). It has been found that recombinant FUS protein can undergoes liquid-liquid phase separation (LLPS) in vitro, a process that precedes FUS aggregation. FUS LLPS is mediated by an interaction between N-terminal tyrosine residues and C-terminal arginine residues in the FUS protein. We have previously generated Drosophila models of FUS-opathies by overexpressing either wild-type or ALS-mutant FUS protein in Drosophila neurons. This results in neuron loss, reduced eclosion and shortened lifespan. Deletion of the N-terminal tyrosine-rich region of the protein or the C-terminal arginine-rich region prevents toxicity, suggesting that LLPS of FUS may be involved. We have now generated Drosophila models that express human FUS carrying amino acid substitutions which prevent LLPS in vitro. Substitutions that replace either arginine (R>K, R>G) or tyrosine residues (Y>F, Y>S) prevent toxicity in Drosophila, supporting the hypothesis that LLPS of FUS is required for toxicity. Further substitutions which limit (Q>A, Q>G) or enhance (G>A) the aggregation of FUS in vitro decrease and enhance toxicity respectively. The substitutions or deletions in FUS that suppress LLPS result in a lower abundance of FUS in tissue, suggesting that LLPS limits the clearance of FUS by neurons. However, even when correcting for differences in abundance, LLPS mutants suppress toxicity. Wild-type human FUS protein localises to the nucleus in Drosophila, we do not observe mislocalisation induced by the toxicity-preventing substitution mutations. Using CRISPR/Cas9 we generated Drosophila where the endogenous ortholog of G3BP1 (rin) is epitope tagged. Using these lines we fail to find evidence of cytoplasmic stress granule induction in FUS expressing flies. Surprisingly, we find that the majority of FUS in Drosophila brain tissue appears to be associated with chromatin, in a manner dependent on its prion-like domain. Deletion of the nuclear localisation sequence of FUS rescues toxicity, indicating that toxicity occurs primarily via a gain-of-function in the nucleus. We are currently testing whether the association of FUS with chromatin depends on an interaction with poly(ADP-ribose) or the C-terminal domain of RNA polymerase II (Polr2A).

(d98) High-throughput drug screening to identify modifiers of TDP-43 protein aggregation

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Despite decades of research, the only widely approved medicine for the treatment of Amyotrophic Lateral Sclerosis (ALS), riluzole, has limited efficacy. Novel medicines are therefore urgently needed. Accumulation of cytoplasmic aggregates of TAR DNA-binding protein 43 (TDP-43) is the pathological hallmark of ALS, observed in 97% of cases including both familial and sporadic cases. Multiple rodent in vivo studies have shown that these cytoplasmic TDP43 aggregates are deleterious to motor neurons leading to motor impairments and premature death and, crucially, clearance of these TDP43 aggregates (genetically) in these models results in functional recovery and extension of lifespan. Thus, the identification of therapeutic treatments which can reverse TDP43 aggregates formation and/or enhance its clearance are strong candidates for evaluation in clinical trials. We have established an in-vitro multiparametric high-throughput drug screening platform to identify drugs that alter TDP-43 aggregation upon oxidative stress in HEK293T cells, followed by secondary reconfirmation in human stem cell-derived spinal motor neurons. Using this platform, we performed the unbiased screen of a library of 6652 small molecules that includes FDA-approved drugs. This accelerated approach led us to the identification of several hit compounds that are now being further evaluated in secondary follow-up assays. Top candidate drugs will be reviewed and fast-tracked for the MND-SMART human clinical trial.

(d99) Characterisation of NMJ pathology in the Thy1-hTDP-43 ALS mouse model

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects both upper and lower motor neurons, presenting as progressive muscle weakness and atrophy. Several studies support the "dying back" hypothesis, where disease pathology initiates distally at nerve terminals at the neuromuscular junction (NMJ) before progressing back towards the motor neuron cell body. Here, we studied pathological changes occurring at the NMJ in the Thy1-hTDP-43 ALS mouse model at three different stages: pre-symptomatic (P8), early symptomatic (P15), and disease end-stage (P19,20). Using immunofluorescence, we labelled NMJ components in cranial muscles, forelimb lumbricals, transversus abdominis (TVA), and a range of hindlimb muscles (lumbricals, FDB, TA, EDL, PL, PB, GC, soleus, and plantaris). We also assessed muscle fibre type and area in hindlimb muscle cross sections. We found that denervation was already present at early symptomatic stages (P15) in muscles from the hindlimbs. At end-stage, we found the most severe disease pathology was present in the PL, PB, plantaris and GC muscles (>60% denervation, with the plantaris having the largest percentage of fully denervated NMJs). In contrast, soleus was the least denervated hindlimb muscle (< 25% denervation, with only 4% of NMJs being fully denervated). Moreover, forelimb lumbricals, TVA, and most cranial muscles did not show significant levels of denervation, even by end stage. Terminal Schwann cells at the NMJ were severely affected in the hindlimb muscles but not in cranial muscles; there was either a partial or a complete loss of S100 staining over the endplate of hindlimb muscles at end stage. Morphometric analysis of NMJs revealed that pathology was largely affecting presynaptic elements of the NMJ (axon diameter and nerve terminal area), whereas postsynaptic variables were largely preserved (e.g. AChR area, endplate compactness, and fragmentation). We did not observe any overt signs of muscle pathology, such as muscle fibre type switching, atrophy, or centrally positioned myonuclei. These results reveal a consistent and heterogeneous pattern of NMJ pathology occurring prior to motor neuron cell death in the Thy1-hTDP-43 mouse model of ALS, with hindlimb muscles being most susceptible.

(d100) Crosstalk of TDP-43 and Optineurin in myeloid cells

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Mutations in optineurin (Optn), a ubiquitin-binding adaptor protein linked to inflammatory signaling, protein trafficking, and autophagy, have been found in a subset of amyotrophic lateral sclerosis (ALS) patients. ALS is a neurodegenerative disease marked with chronic inflammation and protein aggregation. More than 95% of ALS patients have aggregated TAR DNA binding protein 43 kDa (TDP-43) in neurons and glia, whereby TDP-43 gets ubiquitinated, hyperphosphorylated, and mislocalized to the cytoplasm. Mutations in optineurin also cause aggregation and mislocalization of TDP-43, but the putative mechanistic link between optineurin and TDP-43 pathology is still elusive. To address the putative direct crosstalk of TDP and optineurin, we are using: 1) optineurin knockout (KO) neuronal and microglial cell lines made by CRISPR/Cas9 technology, and 2) primary microglia from optineurin insufficiency mouse model (Optn470T) mimicking the loss-of-function mutations found in ALS patients. We also analyzed a new two-hit ALS model made by crossing Optn470T to transgenic TDP-43G348C mice. We found elevated basal TDP-43 protein levels in Optn KO BV2 microglial cell line and the primary Optn470T bone marrow-derived macrophages (BMDM) and microglia. We did not detect differences in TDP-43 mRNA levels at the basal state, arguing that TDP-43 was post-translationally regulated. Subsequently, we analyzed TDP-43 levels upon blockage of two main protein degradation pathways: ubiquitin-proteasomal system and autophagy. TDP-43 was degraded by both pathways in WT, but not in Optn KO BV2 and Optn470T microglia. To test the role of inflammation on TDP-43 levels, we stimulated BV2, BMDMs, and microglia with lipopolysaccharide (LPS) to mimic bacterial infection. LPS did not affect TDP-43 transcription in neither WT nor Optn470T cells. Notably, on the protein level, we observed a significant increase in TDP-43 in WT cells upon LPS stimulation, which was absent in both Optn KO and Optn470T cells. In the latter, TDP-43 remained at the same elevated state as in the basal conditions. We saw a similar TDP-43 accumulation in the basal state and upon LPS treatment in Optn470T x TDP-43G348C BMDMs. We hypothesize that lack of functional optineurin leads to a state of chronic activation in the myeloid cells but further experiments are necessary to elucidate the mechanism of crosstalk of these proteins in the ALS pathogenesis.

(d101) Rescuing locomotor deficits in an ALS mouse model by Extended Synaptotagmin 1 (ESYT1) overexpression

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Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by a progressive inability to execute movement. Although the disease is characterized by the loss of spinal motor neurons (MNs), novel evidence from our laboratory has pointed towards the implication of interneurons (INs) in the progression of the disease. Specifically, V1 spinal inhibitory INs (positive for Engrailed 1 – En1) lose their connections to MNs at early pre-symptomatic stages, which might lead to a MN hyperexcitability and cell death. The present study aimed to investigate whether forced overexpression of the presynaptic protein Extended Synagtotagmin 1 (ESYT1) in INs could stabilize MN-IN connectivity and ameliorate associated behavioral deficits. ESYT1, which is downregulated in spinal neurons early in disease, has been shown to enhance neurotransmission and modulate membrane trafficking. Here, intraspinal lumbar injections of the cre-dependent AAV8-hSyn-DIO-hESYT1-W3SL viral construct were performed in SOD1G93A mice crossed with En1cre mice. Four genotypes were investigated: SOD1, SOD1;En1cre, En1cre and wild-type. Locomotor behavior assessment between P49 and P112 showed that viral injections attenuated the deficits in SOD1;En1cre compared to SOD1 animals, in parameters such as speed, peak acceleration, step frequency, and stride length. MN quantification showed that SOD1;En1cre exhibited a significantly higher number of MN compared to SOD1 littermates and wildtype mice. Together, our results suggest that interneurons can be a potential therapeutic target to delay the development of ALS motor symptoms and point to a relevant role of ESYT1 in promoting MN survival.

(d102) Dissecting the role of microglia in C9ORF72 ALS using brain organoids.

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease characterized by progressive loss of upper and lower motor neurons, for which no curative treatment is available. Recent studies show that motor neuron death is multifactorial and suggest that the immune system, especially microglia, are involved in disease pathogenesis and progression. Activated microglia are a hallmark of ALS pathology, however, their exact role in the pathogenic process underlying ALS remains elusive. Current models used are limited in their ability to recapitulate the functional heterogeneity of human microglia during ALS pathogenesis. To fill this gap, we have previously developed a model of 3D brain organoids where microglia develop innately. This allows the study of interactions between microglia and surrounding cells, including neurons and astrocytes. By developing organoids carrying the C9ORF72 hexanucleotide expansion, the most common cause of ALS, we study the contribution of this gene defect to altered immunity and neurodegeneration. Our goal is to characterize C9ORF72 microglia at the functional level by assessing phagocytic capacity, cytokine secretion, lysosomal activity and morphological features. Here we generated organoid-grown microglia derived from healthy and C9ORF72-ALS iPSC lines. Using live imaging and fluorescently labeled pHrodo bacterial particles, a decrease in the phagocytic capacity of C9ORF72-ALS organoid-derived microglia (oMG) was found. This observation is supported by transcriptomic profiling of oMGs using RNA sequencing, which shows downregulation of phagocytosis-associated genes. To further validate these findings, oMG morphology and phagocytosis within organoids will be assessed, as well as cytokine release. Our studies will further dissect the role of microglia in C9ORF72-ALS and in the longer term contribute to the development of more effective therapeutic strategies.

(d103) Characterisation of a Rat Model of ALS8

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VAPB is a ubiquitously expressed membrane-bound protein implicated in a number of cellular roles vital for cell survival and function. Mutations in this gene have been linked to ALS with patients presenting with typical rapid-progressive ALS, late-onset spinal muscular atrophy, or atypical slow-progressive ALS with postural tremor (ALS8). The first identified and most common mutation in VAPB is an autosomal dominant mis-sense mutation substituting proline with serine at position 56 (P56S) at the centre of the highly conserved major sperm protein (MSP) domain. To complement existing murine models, VAPB knock-out and VAPBP56S knock-in alleles were produced in Fischer 344 rats using the CRISPR/cas9-based "CLICK" system. A cohort of rats were matured to 18 months. Catwalk performance was measured at 6, 12, and 18 months. A progressive gait abnormality was detected in homo- and heterozygous VAPBP56S and knockout animals. Histological analysis revealed a reduction in the number and size of motor neurons in VAPBP56S/+ compared to wild-type. Proteomic analysis of brain synaptosomes indicated that expression of the VAPBP56S mutation is associated with biochemical changes also found in other forms of neurodegenerative disease. These results support a role for VAPBP56S in degenerative motor neuron disease and provide a new experimental model system with which to analyse mechanistic details of the pathology.

(d104) Sigma-1 receptor is a pharmacological target to promote neuroprotection in the SOD1G93A ALS mice

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There is no available cure for amyotrophic Lateral Sclerosis (ALS), thus, novel therapeutic targets are urgently needed. Sigma-1 receptor (Sig-1R) has been reported as a target to treat experimental models of degenerative diseases and, importantly, mutations in the Sig-1R gene cause several types of motoneuron disease (MND). In this study we compared the potential therapeutic effect of three Sig-1R ligands, the agonists PRE-084 and SA4503 and the antagonist BD1063, in the SOD1G93A mouse model of ALS. Pharmacological administration was from 8 to 16 weeks of age, and the neuromuscular function and disease progression were evaluated using nerve conduction and rotarod tests. At the end of follow up (16 weeks), samples were harvested for histological and molecular analyses. The results showed that PRE-084, as well as BD1063 treatment was able to preserve neuromuscular function of the hindlimbs and increased the number of surviving MNs in the treated female SOD1G93A mice. SA4503 tended to improve motor function and preserved neuromuscular junctions (NMJ), but did not improve MN survival. Western blot analyses revealed that the autophagic flux and the endoplasmic reticulum stress, two pathways implicated in the physiopathology of ALS, were not modified with Sig-1R treatments in SOD1G93A mice. In conclusion, Sig-1R ligands are promising tools for ALS treatment, although more research is needed to ascertain their mechanisms of action.

(d105) Modulation of VDAC promotes motoneuron survival after brachial plexus injury

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Traumatic lesions of the spinal roots cause severe loss of neural functions, leading to dysfunctions that result in social and labor impairment. The preservation of the motoneurons after axotomy is essential to allow successful axonal regeneration and recovery. Here we set up a model of brachial plexus injury in adult mice that represents a traumatic lesion often observed after road accidents. We focused on strategies to preserve motoneurons after brachial plexus injury by modulating components of the complex located at the mitochondria associated membranes (MAMs), in which Sigma-1 receptor (Sig1R), voltage dependent anion channel 1 (VDAC1) and inositol 1,4,5- trisphosphate receptor type 3 (IP3R3) are involved. Brachial plexus injury induced motoneuron loss to 28% after 3 weeks, increased reactive gliosis and accumulation of VDAC1 in the motoneuron. Treatment with DIDS, a pan-inhibitor of VDAC oligomerization, promoted motoneuron survival to 86% and reduced microglial reactivity to 56% and astrocytic activity to 40% of untreated mice values. VDAC1 seems to induce survival of motoneurons through the downregulation of pro-apoptotic proteins, such Cytochrome c (Cyt C) and Apoptotic inducing factor (AIF), showing a distinct decrease in cytosolic levels of both proteins after treatment. Therefore, our data suggest that, in a spinal nerve injury, the modulation of VDAC induces a neuroprotective effect.

(d106) Metabolic switch as a tool to unmask mitochondrial failure in MEFs carrying TARDBP mutations

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The involvement of mitochondrial failure in ALS pathology has been well described in the literature, and it is considered one of the earliest pathophysiological events. Acute metabolic switch assay has been traditionally performed, replacing glucose with galactose in the culture medium to unmask the mitochondrial liability of chemical compounds in cells. We use a modified glucose-galactose assay on murine embryonic fibroblasts (MEFs) carrying three different Tarbp mutations, namely Q331K, M323K, and F201I, to evaluate the effect of Tarbp genetic background on mitochondrial function. A method is described here, maintaining mutant MEFs in pyruvate-deprived culture medium containing galactose as the only energy source to sustain cellular growth. Several indicators of mitochondrial fitness are evaluated in these stressful conditions in comparison with optimal glucose and pyruvate availability, such as ATP production rate, membrane-potential preservation, and changes in the mitochondrial mass. Our results show an evident variability in response to metabolic stress, depending on TARBP mutation. Such heterogeneity underlines the unique effect of different Tarbp mutations on mitochondrial health, providing a valuable tool to relate mutations located in other regions of TDP-43 protein with a spectrum of mitochondrial damage involvement in ALS.

(d107) Amyotrophic lateral sclerosis at single cell resolution.

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In vitro-derived neurons that originate from induced pluripotent stem cells (iPSC) can model disease phenotypes and intracellular dysfunction but the cross-comparison of donor-derived lines is confounded by different genetic backgrounds. In order to create isogenic models for amyotrophic lateral sclerosis (ALS) across causative mutations in FUS and to unravel its cellular function, we have genome-edited several point mutations and a knockout of FUS into an iPSC control line. Their differentiation into spinal motor neurons permits us to study a primary disease target from an in-vitro source with the complication of heterogenous cultures that need single cell resolution to dissect the transcriptomes faithfully. The benefits of this approach are that we can not only assess dysfunction in different subpopulations of neurons but also uncover the disease-associated changes specific to motor neurons in contrast to other neurons.
We have therefore used two differentiation protocols yielding cultures that are either highly enriched in spinal motor neurons (approx. 80%) or that contain a mixture of spinal interneurons (approx. 50%) and few motor neurons (approx. 5%). We also show that FUS R495X and P525L motor neurons recapitulate FUS mislocalisation, a hallmark of FUS pathology. Following isolation of single live cells and Smart-Seq2 single cell mRNA sequencing, we have classified the different spinal neurons across isogenic cell lines based on their molecular identity, including motor neurons expressing cholinergic markers (CHAT, SLC18A3, SLC5A7) along specific transcription factors (ISL1, ISL2, MNX1) and V2a interneurons expressing VSX2 and SOX14. We find a total of 950 differentially expressed genes (DEG) between the mutant motor neurons and control, of which 197 are shared among FUS R495X and P525L lines. Using the FUS knockout line, we can separate out 31 DEG that are potentially associated with nuclear loss of function (LOF) in FUS-ALS in contrast to 166 DEG that are not. The genes that are putatively regulated in response to FUS LOF act in RNA metabolic processes and nucleocytoplasmic transport. In contrast, the DEGs not associated with FUS LOF likely represent cytoplasmic gain of function and are involved in mitochondrial processes and energy metabolic functions among others. Altogether our data furthers the understanding of the motor neuron-intrinsic roles of FUS in health and ALS and we identify targets that could be modulated in the future.

(d108) Retrograde tracing of monosynaptic inputs to MCH+ cells by mutant rabies virus in SOD1G93A ALS murine model

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Weight loss and hypermetabolism are the major non-motor symptoms observed in almost all ALS patients, correlated with worse prognosis and related to hypothalamic impairment. Hypothalamus, a key brain region that controls energy balance, is atrophied in ALS patients and pre-symptomatic gene carriers. Most recently, disruption of hypothalamic network was described in ALS patients, while projections to lateral hypothalamic (LHA) were found rearranged in murine models. However, which LHA circuits may drive hypermetabolism remains unknown. Hypothalamic melanin-concentrating hormone (MCH) and orexin neurons that maintain energy homeostasis, could be dysfunctional either intrinsically or by extrinsic detrimental inputs and thus contribute to ALS metabolic impairment. To address these questions, we first assessed these neuronal populations and we showed that while MCH neurons are preserved in pre-symptomatic SOD1G93A mice (P45) they degenerate in the late symptomatic stage of the disease (P110). In contrast, orexin neurons were maintained during the life span in SOD1G93A mice. Together, data indicate intrinsic-hypothalamic dysfunction and point to MCH neurons as a clinically relevant population. Consequently, to investigate the role of extrinsic pathways controlling MCH cells, we mapped the brain-wide, monosynaptic inputs selectively to MCH neurons. To do so, we employed retrograde tracing based on combining the modified rabies virus (RVΔG/ENV-mCherry) together with Cre-inducible viral vectors coding for TVA-eGFP receptor (enabling RVΔG selective infection) and rabies glycoprotein RVG (enabling RVΔG synaptic crossover), and targeted to MCH neurons by injecting into the LHA in Pmch-Cre;SOD1G93A transgenic mice and their Pmch-Cre;WT littermates. This strategy allowed the separation of MCH neurons (mCherry+/eGFP+) from distinct neurons that innervate them monosynaptically (mCherry+/eGFP-). We observed networks of input neurons in both hypothalamus and remote extra-hypothalamic regions, with the hypothalamic paraventricular and arcuate nuclei being the hotspot input areas. Results unraveled disease-related re-modelling of the MCH-specific connectivity map. Further in vivo targeted functional assessment of structurally affected MCH input networks by the means of multiplexed pharmacogenetics will define their contribution to ALS and therapeutic potential.

(d109) Longitudinal in vitro study for ALS-like phenotypes in hiPSC-derived motor neurons

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterised by the progressive denervation of the skeletal muscle, culminating in muscle paralysis and death. Axonal retraction at the neuromuscular junction (NMJ) level is one of the earliest out of a succession of events that occur prior to complete loss of muscle control, preceding the motor neuron soma degeneration in the spinal cord. The mechanisms underlying this dying back axonal degeneration are yet to be fully understood. However, it suggests that ALS-causing mutations affect axon stability, resulting in different early-stage phenotypes. The identification and characterization of such phenotypes in an in vitro model overcomes the challenges faced when using in vivo models and post mortem tissue, allowing a better understanding of ALS progression. With this longitudinal study we aim to characterise subtle ALS-like phenotypes in motor neurons derived from human induced pluripotent stem cells (hiPSC) harbouring ALS-causative mutations. This will allow us to identify the critical time point for axonal degeneration. In order to describe these ALS-related in vitro phenotypes, ALS mutant hiPSC and respective isogenic controls are differentiated into motor neurons and seeded individually in the form of motor neuron spheres. Live microscopy, immunocytochemistry and image analysis are carried out to evaluate axonal morphology and growth at different time points, by comparing both computer based and manual quantification method. Our findings suggest the existence of significant differences in the axonal length and morphology in ALS mutated lines when compared to isogenic controls, and implicate such analysis as tool for drug screening in ALS.

(d110) Modelling the human neuromuscular junction in vitro

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Neuromuscular junctions (NMJ) are affected early in amyotrophic lateral sclerosis (ALS). An in vitro model that replicates the NMJ and its destruction in ALS would further our understanding of this early event in the disease and may aid in finding disease modifiers. The aim of this study is to generate a robust and reproducible in vitro model of the NMJ using human primary myoblast-derived myotubes and spinal MNs derived from human pluripotent stem cells (PSCs). We also want to model the early destruction of NMJs in ALS and towards this purpose we use PSC lines harbouring ALS-causative mutations. In this project, we differentiate spinal MN from Hb9::GFP human embryonic stem cells, as well as human induced PSCs from a healthy donor and isogenic lines with genome engineered ALS-causing mutations. Human skeletal muscle cells were specified from human primary myoblast expressing the fluorescent calcium indicator MHCK7::GCaMP6f. Skeletal myogenesis was assessed by qRT-PCR for myogenic markers at different time points of the differentiation protocol. For generation of neuromuscular co-culture, spinal MN clusters were transferred on top of human derived skeletal muscle cultures. The functional connectivity between motor endplates and myofibers was studied with calcium imaging in live cells. With immunohistochemistry we evaluated NMJ formation through co-localization of TUBB3 and SV2A with BTX on MF20-positive myotubes. We found an increase in the gene expression of MYOG, and decrease in the expression of MYOD and PAX7 during myotube formation of the human primary myoblast and subsequently, mature myofibers expressing the myosin heavy chain marker MF20 were detected. AChR clusters were identified by BTX in the third week of co-culture. Interactions between MN processes and AChR clusters on myotubes were detected by immunostaining for TUBB3, SV2A and BTX, indicating formation of NMJ-like structure. Functional NMJ connectivity was detected by an increase in fluorescence intensity produced by the fluorescent calcium indicator of the myotubes after glutamate stimulation of MNs. Further experiments will include RNA-sequencing to study the transcriptomic changes derived from MN-myofiber interaction and analysis of NMJ disruption in ALS. This study shows an in vitro model that generates functional NMJ connections and allows us to study the influence of MNs with ALS-causing mutations on motor neuron-muscle connectivity.

(d111) Resistant and vulnerable motor neurons show unique temporal gene regulation in SOD1G93A ALS

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We have investigated the longitudinal transcriptional response of resistant and vulnerable motor neuron populations in the SOD1G93A mouse model. Specifically, we have conducted RNA sequencing of resilient oculomotor and trochlear (CN3/4) motor neurons, as well as visceral motor neurons of the dorsal motor nucleus of the vagus nerve (CN10) and compared these to vulnerable hypoglossal (CN12) and spinal motor neurons (sMNs) isolated from the SOD1G93A mouse model at presymptomatic (P56) and symptomatic (P112) stages. Differential gene expression analysis showed that spinal motor neurons display the largest number of dysregulated genes among the isolated neuron populations. Each disease stage showed distinct gene regulation with only a minority of genes being regulated across ages. However, deregulated pathways related to autophosphorylation, PERK-mediated unfolded protein response, ER stress and metabolic processes were regulated across time points in vulnerable sMNs. An in depth bioinformatics comparison with other published transcriptomics data sets on sMNs across SOD1 mutations to identify robust vulnerability signatures revealed that PERK-mediated unfolded protein response and positive response to ER stress pathways were common to SOD1G93A and SOD1G37R mutations. Resilient CN3/4 motor neurons showed a very minor transcriptional adaptation to the SOD1G93A mutation, while visceral CN10 motor neurons had a large number of uniquely regulated genes at the presymptomatic stage with subsequent normalization to control littermates at the symptomatic stage. We are currently investigating this initial gene regulatory response of CN10 motor neurons to ALS to identify presumably protective responses in these unaffected neurons. In conclusion our analysis shows that each motor neuron subpopulation responds uniquely to the SOD1G93A mutation, and that different disease stages give rise to distinct transcriptional responses. Our data also indicates that visceral motor neurons need to adapt their transcriptome to cope with disease, and within this response may lie clues to their resilience to ALS.

(d112) Investigating the upregulation of adenosine deaminase in ALS astrocytes

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Astrocytes are key neuronal support cells and are vital in understanding motor neuron (MN) degeneration in ALS. Data in ALS patient derived astrocytes (iAstrocytes) demonstrated adenosine metabolism dysfunction, caused by adenosine deaminase (ADA) loss which catalyses breakdown of adenosine into inosine. Loss of this purine metabolism enzyme led to several negative consequences for ALS iAstrocytes including increased sensitivity to adenosine-mediated toxicity, whilst higher endogenous ADA expression was able to protect against this. Inosine supplementation to bypass the defect was beneficial bioenergetically in iAstrocytes and decreased iAstrocyte-mediated MN toxicity in co-culture. Our data led us to the hypothesis that restoring ADA levels will be beneficial for ALS iAstrocytes and decrease iAstrocyte-mediated MN toxicity. Our objectives are to investigate the effect of increasing ADA expression in iAstrocytes by lentiviral gene therapy on adenosine-specific toxicity, bioenergetics, purine metabolism, DNA damage and iAstrocyte support for MNs in co-culture. Lentiviral gene therapy was used to increase ADA levels in ALS iAstrocytes. ADA levels and a panel of cellular targets were used to characterise the effect of ADA increase. Adenosine mediated toxicity and ATP output were also measured in lentiviral treated cells. The effect of gene therapy on ADA activity, inosine levels, uric acid levels, DNA repair, other purine metabolism enzymes and iAstrocyte-mediated support for MNs is currently underway. ADA activity and inosine levels were reduced in ALS iAstrocytes, confirming our previously published data. ADA gene therapy ameliorated these disease affects and restored ADA expression to levels comparable to endogenous control iAstrocytes. Markers of ALS pathogenesis (P62 and NQO-1) and levels of cytoskeletal markers (actin and tubulin) were unaffected by ADA gene therapy. ADA gene therapy overall did not affect adenosine-mediated toxicity or increase ATP levels unlike inosine supplementation. The effect of gene therapy on uric acid levels, DNA damage markers and MN survival is in progress. Further study is required to allow more robust characterisation of the effect of ADA gene therapy, including its effect on purine metabolism, antioxidant capacity, DNA repair and MN survival in co-cultures.

(d113) Understanding Disease Trajectory in Amyotrophic Lateral Sclerosis

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Background and methods: My research attempts to explain ALS phenotype through genotype, focussed on structural variation and disease progression, using bioinformatics as a means of bringing these elements together.

Results and contribution: I have shown that structural variation is a frequent event in ALS, and that an insertion in ERBB4 greatly predisposes to respiratory onset ALS, an otherwise rare phenotype (Al Khleifat, A. et al. npj Genom. Med. 2022). My findings suggest that respiratory onset ALS may be a specific genetic subtype, and potentially explains why progression is slower than expected in those with respiratory onset. I showed that longer telomeres are associated with ALS (Al Khleifat, et al. ALS & FTD 2019), replicating this across international cohorts, and that the same pattern of telomere elongation is seen in ALS brain tissue, despite the lack of mitosis in neurons. I investigated plasma NfL in 2311 individuals across 15 neurodegenerative diseases. Using the single-molecule array (SIMOA), I showed that NfL was significantly increased in all cortical neurodegenerative disorders, and particularly ALS and FTD. I further demonstrated that plasma NfL is clinically useful in detecting FTD in people with psychiatric disorders such as moderate and severe depression, and in identifying frontotemporal dementia in people with cognitive impairment (Ashton, N.J., Janelidze, S., Al Khleifat, A. et al.Nat Commun. I organised NEUROHACK; a 4-day competitive international hackathon in January 2022 at hubs in London and Los Angeles. Over 160 participants from six continents were selected to attend the event. As part of the hackathon, three pilot grants of £10,000 were awarded to NEUROHACK teams to continue to develop the most promising ideas, two of which were awarded to MND teams. I created the Trans-Ancestral Genetics working group that is part of the Project MinE consortium, with a focus on diversifying genetic research in ALS to include multi-ethnic populations and accurately represent ALS genetics-related disease risks in all populations.

Conclusions: I have explored the relationship between ALS phenotypes, disease progression, survival and genetics; developing and using various tools for measurement and assay of these parameters. My work has led to the discovery of a number of genetic factors that contribute to the risk of ALS with some being investigated further given their potential as drug targets for the development of new therapies.

(d114) Reduction of oxidized phospholipids or misfolded protein aggregates by AAV-VectAbs in ALS pre-clinical models

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It is accepted in the field that protein misfolding underlies multiple neurodegenerative diseases. As the pathogenic mechanisms usually include both a loss of function (of the wild-type protein) and a gain of toxicity (through the misfolded or aggregated protein), strategies are required which specifically target the toxic (misfolded) protein species, and not the wild-type protein. Misfolded TAR DNA-binding protein-43 (TDP-43) has been associated with the pathology of 97% of sporadic as well as genetic forms of Amyotrophic lateral sclerosis (ALS). Here, misfolded TDP-43 interferes with the translation of mitochondrial proteins at the neuromuscular junction (NMJ) leading to mitochondrial dysfunction. A library of AAV-delivered scFv's (VectAbs) were developed which are expressed as an intrabody, and specifically recognize misfolded TDP-43 species. In addition, these identified candidates show to effectively interfere with TDP43 aggregate formation. This library allows selection of a lead candidate for further development of a Gene Therapy for the treatment of ALS. In addition, this concept forms the basis of similar approaches in other neurodegenerative or neuromuscular diseases with the formation of misfolded protein aggregates that cause cellular toxicity. ALS motor neurons are selectively sensitive to axonal/NMJ mitochondrial dysfunction because of the axon length (up to 60 cm) and the high energy requirement. Mitochondrial dysfunction in ALS motor neurons leads to the formation of oxidized phosphocholine (OxPC) species that are extremely toxic. We have developed a library of secreted OxPC VectAbs that protect motor neurons from OxPC-induced toxicity and cell death. In addition, it was demonstrated that expression levels of AAV-delivered scFv's are at therapeutic levels in ALS-relevant areas within the CNS, including the spinal cord and brainstem. We are currently investigating the efficacy of these approaches in preclinical models.

(d115) MicroRNA dysregulation as a driver of WNT activation and motor neuron degeneration in ALS

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Disruption to RNA processing events has emerged as a common theme between sporadic and familial ALS (sALS/fALS). It is yet unclear how such defects drive motor neuron (MN) degeneration.

In this study, we deploy patient-derived iPSC disease modelling to investigate the mechanisms driving ALS-associated MN degeneration. iPSCs harbouring mutations in the FUS gene were differentiated into motor neurons alongside isogenic and independent healthy control cells. We performed detailed phenotypic and transcriptomic analyses of iPSC-derived MNs, alongside computational analyses of existing sALS datasets, to discover RNA perturbations and key signalling pathways that drive neurodegeneration.

As a common feature of fALS and sALS, we explored disruption to microRNA networks in our model. We identify downregulation of a MN-specific microRNA, and identify this microRNA as a regulator of a key signalling pathway called WNT. Suppression of WNT improves FUS-MN survival, whereas stimulation of WNT reduces healthy-MN survival, highlighting its role as a driver of MN degeneration. Importantly, we uncover that these molecular defects are also observed in sALS MNs, highlighting the shared dysregulation of RNA metabolism in familial and sporadic ALS.

These findings expose WNT signalling as a driver of MN degeneration in ALS. In FUS-ALS MNs this is, at least in part, driven by FUS-mediated disruption to non-coding RNAs.

Session 2 – Thursday

e) Neuroinflammation

(e116) Increased cleaved GSDMD expression in white matter microglia is associated with neuronal loss in the ALS motor cortex

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Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of motor neurons in the motor cortex, brainstem and spinal cord. Although ALS is considered a motor neuron disorder, neuroinflammation also plays an important role. Recent evidence in ALS disease models indicates activation of the inflammasome and subsequent initiation of pyroptosis, an inflammatory type of cell death, due to the presence of pathological proteins. In this study, we determined the expression and distribution of the inflammasome and pyroptosis effector proteins in post-mortem brain and spinal cord from ALS patients (n = 25) and controls (n = 19), as well as in symptomatic and asymptomatic TDP-43 A315T transgenic mice and wild-type mice, and evaluated its correlation with the presence of TDP-43 pathological proteins and neuronal loss. Expression of the NLRP3 inflammasome and pyroptosis effector proteins cleaved Gasdermin D (GSDMD) and IL-18 was detected in microglia in the human ALS motor cortex and spinal cord. The number of cleaved GSDMD-positive precentral white matter microglia was increased compared to controls and was associated with a decreased neuronal density in human ALS motor cortex. Neither of this was observed in the spinal cord. Similar results were obtained in TDP-43 A315T mice, where microglial pyroptosis activation was significantly increased in the motor cortex upon symptom onset and correlated with neuron loss. There was no significant correlation with the presence of TDP-43 pathological proteins both in human and mouse tissue. Our findings emphasize the importance of microglial NLRP3 inflammasome-mediated pyroptosis activation in ALS, and opens the way for new therapeutic strategies countering motor neuron degeneration in ALS by inhibiting microglial inflammasome/pyroptosis activation.

Acknowledgements: EVS is funded by an SB PhD Fellowship of the FWO (1S46219N). Additional funding for this project was provided through C-1 internal funds from KU Leuven (C14-17-107), FWO Odysseus grant G0F8516N and the ALS Liga Belgium.

(e117) Therapeutic potential of Naringenin on primary microglia derived from mutant G93A-SOD1 mice

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Dysregulation of microglial properties and function appears to be involved in the pathogenesis of ALS. It has been shown that mutant SOD1 microglia are more neurotoxic than wild-type microglia [1]. Activated microglia, the immune cells of the nervous system, can assume either a pro-inflammatory M1 phenotype or a neuroprotective M2 phenotype, which can be manipulated by different substances [2]. Modulation of the glial phenotype and function could represent an interesting therapeutic approach in ALS. The naturally occurring plant-derived compound Naringenin seems to be a promising neuroprotective drug candidate capable of interfering with microglial phenotype and properties [3]. Beneficial effects were shown in animal models of neurodegenerative diseases like Alzheimer's and Parkinson's disease [3]. We now intend to investigate effects of Naringenin in primary microglia derived from mutant G93A-SOD1 mice. Treatment effects of Naringenin we assessed via measurement of protein and mRNA levels of microglia phenotype markers in primary microglia derived from mutant G93A-SOD1 mice. In addition, we currently analyse the impact of treatment-induced modulation of microglial phenotype on motor neurons in co-cultures of primary motor neurons and microglia. Arginase-1, one of the best characterized markers of M2 phenotype [4], was significantly enhanced at the mRNA level and a tendency could be detected also at the protein level in mutant SOD1 transgenic cells treated by Naringenin. Arginase-1 can effectively outcompete iNOS and downregulate production of nitric oxide via the same ligand arginine [4]. In accordance, mRNA expression of the M1 marker iNOS [4] was significantly reduced in transgenic cells after treatment with Naringenin. However no significant Naringenin-induced changes for TGFß mRNA expression have been detectable so far, and for IL-1ß we only observed a slight reduction. As there is no cure for ALS so far, it is important to study drugs with potential impact on known disease mechanisms. Naringenin seems to have effects on factors and pathways involved in ALS progression. Our preliminary work in primary microglia derived from mutant SOD1 mice shows promising results regarding a shift of pro-inflammatory M1 microglia to a protective M2 phenotype.1. PMID: 175555562. PMID: 255983543. PMID: 316841424. PMID: 24889886

(e118) A novel proposed panel of inflammatory and redox genes as biomarkers in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease causing the progressive loss of motor neurons. There is a pressing need for biomarkers of disease progression and target engagement to enhance the efficacy of clinical trials in ALS. AMBRoSIA (A Multicentre Biomarker Resource Strategy in ALS) has allowed systematic longitudinal biosampling of ALS patients attending 3 MND centres in Sheffield, Oxford and University College London. A targeted transcriptomic study evaluating the expression of 145 inflammatory and 84 redox genes in the white blood cells of 49 newly-diagnosed AMBRoSIA ALS patients and 31 matched-controls was performed with the aim of identifying possible biomarker candidates. We found a total of 21 significantly differentially expressed genes in patients compared to controls. Significant differences were also observed between fast (ALSFRS-R-score decline/month >1) and slow progressors with a selection of genes being characteristically expressed in each group. Our results are consistent with a mixed and ALS-specific transcriptional phenotype at early disease stages when both up- and down-regulation of protective and harmful genes simultaneously coexist. Lastly, a logistic regression model, which can predict, with high sensitivity (0.94), specificity (0.89) and accuracy (0.92), the rate of ALS disease progression, was generated based on the expression of four key genes (CCL4, TNFSF4, IL5RA and KRT1) and on the patient age at diagnosis. These changes require validation, but if confirmed could serve as biomarkers to aid prediction of disease prognosis and could potentially be used to monitor ALS disease state when therapeutic interventions are administered.

(e119) TDP-43 Immunotherapy decreases neuropathology and confers neuroprotection through microglial engagement in mouse models of ALS/FTD

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TDP-43 mislocalization and pathologic aggregation in neurons and glia is a hallmark of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) and limbic-predominant age-related TDP-43 encephalopathy (LATE). Staging of TDP-43 pathology in patients demonstrates propagation of pathology within the central nervous system, suggestive of extracellular spreading. Therefore, targeting aggregation and spreading of pathological TDP-43 in the extracellular space has the potential to mitigate disease progression. Currently, no treatments in clinical development directly target the clearance of pathological TDP-43. By developing an immunotherapy against different domains of TDP-43, we investigated whether it would prevent templated aggregation and promote removal of pathological species. For this, multiple monoclonal antibodies (mAb) were generated using AC Immune's proprietary SupraAntigen® platform and characterized in vitro, from which two pan-TDP-43 mAbs (ACI-5891 and ACI-5886) were selected for evaluation in animal models of ALS/FTD. In an aggregation assay using recombinant TDP-43, ACI-5891, a high-affinity mAb binding in the C-terminal region of the target, showed significant inhibition while, ACI-5886, binding in the RNA-recognition motif (RRM), had no effect. Moreover, ACI-5891, but not ACI-5886, demonstrated target engagement to pathological TDP-43 derived from patient brain in an immuno-depletion experiment. These in vitro findings translated in vivo as ACI-5891 significantly reduced TDP-43 pathology as compared to ACI-5886 in a transgenic TDP-43 mouse model. Moreover, ACI-5891 showed neuroprotection in a mouse model with TDP-43 pathology induced by FTLD-TDP patient brain derived seeds. Importantly, neuroprotection was demonstrated only for a mAb with full-effector function, illustrating the contribution of Fc-receptor-mediated clearance. The relevance of this mechanism was further highlighted using ALS patient derived microglia in which phagocytic function was restored when treated with the pathological protein in the form of immune complexes. This study demonstrates for the first time the importance of targeting the C-terminal region of TDP-43 to achieve a reduction of pathology and neurotoxicity in vivo as well as the benefit of immunotherapy to restore protective mechanisms of the patient's immune cells, reinforcing the rational for targeting TDP-43 with a mAb.

(e120) Investigating the microglial role of ALS/FTD-associated gene TANK-binding kinase 1

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Introduction: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two devastating and interlinked neurodegenerative disorders that form a continuous ALS/FTD disease spectrum. Whilst neuronal dysfunction and degeneration is central to both diseases, chronic neuroinflammation is a hallmark feature of ALS/FTD and it is increasingly being recognised that non-neuronal cells contribute to disease pathogenesis in a non-cell autonomous manner. Importantly, a subset of recently identified ALS/FTD risk genes have immune roles, highlighting immune dysfunction as a key early pathogenic mechanism. One such gene is TANK-binding kinase 1 (TBK1), which encodes a serine/threonine protein kinase central to numerous cellular signalling pathways. We aim to investigate the molecular function of TBK1 in microglia and understand how ALS/FTD-associated mutations may intrinsically impair microglial function to drive disease pathogenesis.

Methods: We have used pharmacological TBK1 inhibitors and immortalised microglial cell lines to establish an in vitro model of TBK1 loss-of-function in microglia. We have investigated how TBK1 haploinsufficiency affects canonical microglial functions, such as immune signalling and phagocytosis, and have conducted TMT-based LC-MS/MS global proteomics to provide an insight into other dysregulated pathways. In parallel, we are generating a human pluripotent stem cell-derived microglial model of TBK1-associated ALS/FTD.

Results: We have demonstrated that TBK1 loss-of-function attenuates microglial immune signalling, specifically IRF3 activation, and disrupts phagocytosis of pHrodo-conjugated E. Coli bioparticles in a dose-dependent manner. Additionally, TBK1 kinase inhibition results in dysregulation of the global proteome, with 143 proteins becoming differentially expressed. Gene ontology and protein-protein interaction analysis of this dataset supports deficits in immune signalling and phagocytosis, and highlights potential impairments in antigen presentation, DNA damage repair and transcriptional regulation upon TBK1 loss-of-function.

Conclusion: We have shown that TBK1 loss-of-function results in microglial dysregulation in a cell autonomous manner and have shed light on novel dysregulated pathways that may present novel therapeutic avenues in TBK1-associated ALS/FTD. Future work will seek to further characterise findings in human pluripotent stem cell-derived microglial models of TBK1-associated ALS/FTD.

(e121) A multicentric approach to monocyte alterations in ALS

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Neuroinflammation is a major disease hallmark of ALS. Besides the involvement of the immune system in the central nervous system, increasing evidence also indicates a contribution of peripheral immune cells such as monocytes to the disease. Monocytes can be categorized into 'classical' (CD14++CD16-) and 'non-classical' (CD14+CD16++) monocytes according to their expression of surface markers and molecular function. While classical monocytes are involved in tissue maintenance, pathogen clearance and induction of adaptive immune responses, non-classical monocytes mainly play a role in tissue homeostasis and local regeneration. In a previous study, we showed that this monocyte subtype composition is dysregulated in ALS patients and pre-symptomatic mutation carriers. Furthermore, we observed an impaired phagocytic activity of ALS-associated CD14+ monocytes. The aim of this follow up study was to validate alterations of monocyte subtype compositions and phagocytic activity in familial and sporadic ALS patients as well as in pre-symptomatic mutation carriers in a multi-centric approach. PBMCs were isolated from ALS patients and pre-symptomatic mutation carriers of three independent cohorts. As controls, we chose individuals without known neurodegeneration or inflammatory manifestation and additional disease-mimicking controls. Surface markers of isolated PBMCs were immunostained and different monocyte subpopulations were examined using flow cytometry. We were able to replicate the previously observed increase in CD14/CD16 ratio in freshly isolated PBMCs. However, this effect was not visible in frozen PBMCs. The same experiment was carried out with PBMCs of pre-manifest mutation carriers. Again, a tendency towards a shift in the ratio of monocyte subpopulations was detected in fresh PBMCs although it was not statistically significant. Additionally, CD14+ monocytes were isolated, incubated with fluorescently-labeled zymosan bioparticles and also analyzed by flow cytometry to determine phagocytic activity. The previously observed reduced phagocytosis of CD14+ monocytes could not be replicated in this cohort. Taken together, this study demonstrates the significance of multi-centric validation of experimental results analogous to therapeutic clinical studies.

(e122) The role of C3 inhibition in an iPSC NMJ model of neuroinflammation

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Introduction: The complement cascade is a critical component of the immune system, and dysfunction of complement is implicated in ALS. Components of complement, including C3, are reported to be deposited on neuromuscular junctions (NMJs) of muscle biopsies in people with ALS. While the therapeutic potential of complement system modulation has been explored using embryonic knockout animals of ALS, its effects in clinically relevant human cell-based models are unknown.

Objectives: Evaluate the effect of complement C3 inhibition on NMJ function in response to inflammatory stimulations in a human iPSC-derived NMJ model.

Methods: NMJ systems were established by plating human iPSC-derived motoneurons, skeletal myoblasts, Schwann cells, microglia, and inactivated THP monocytes or activated M1 macrophages in a compartmentalized co-culture system. Activated and inactivated monocytes were plated at ratios of 4:1-1:50 (vs. skeletal myoblasts). ALS model systems were created using SOD-1 (E100G) or TDP-43 iPSC-derived cells compared to control NMJs established from wild-type iPSC-derived cells. To determine the role of C3 on NMJ function, the C3 inhibitor pegcetacoplan (50 µg/mL) and human complement serum (0.05%) were acutely dosed for 3 hours. C3 expression was assessed by immunocytochemistry, and NMJ number and fidelity were calculated by assessing the number of functional myotubes under indirect stimulation and the ratio of number of successful contractions to number of pulses at a given frequency, respectively. All experiments were replicated twice in triplicate.

Results: Functional NMJ systems were assessed with the addition of various ratios of activated or inactivated monocytes to skeletal muscle-side of the culture chamber. Addition of activated monocytes resulted in reduced NMJ number and function. In addition, while C3 expression was observed with THP-monocytes, activation of THP monocytes to M1 macrophages increased C3 activity. Human complement serum potentiated the effects of M1 macrophages, further decreasing NMJ numbers and reducing NMJ fidelity. Acute treatment with pegcetacoplan attenuated these effects. SOD-1 and TDP-43 NMJ systems reduced NMJ number and fidelity versus wild-type. The reduction in SOD-1 NMJ number and fidelity was greater than that of TDP-43 NMJ system.

Conclusions: These data demonstrate that modulating C3 with pegcetacoplan in the presence of an inflammatory NMJ environment could improve overall function.

(e123) The interferon signalling pathway as potential therapeutic target in amyotrophic lateral sclerosis and frontotemporal dementia – a systematic review and meta-analysis

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The immune system plays a prominent role in the pathogenesis of many chronic diseases, including amyotrophic lateral sclerosis frontotemporal spectrum disorders (ALS-FTSD). These disorders exhibit, albeit heterogeneously, features of immune system dysfunction such as excessive inflammation, inefficient immune response, and autoimmunity, and recent studies have identified interferon signalling as a key player in this dysfunction. Given that interferon dysregulation may contribute to disease heterogeneity and pathogenesis, we set out to perform a systematic review and meta-analysis to enhance our understanding of the role of interferon signalling pathways in ALS-FTSD.

Here we perform a comprehensive review of the ALS-FTSD preclinical literature to compile a list of (i) interventions affecting interferon signalling pathways and (ii) specific interferon signalling pathway targets that may be manipulated for therapeutic benefit in patients with ALS-FTSD. We assess the literature for the effects of interferon signalling manipulation in studies comparing models of ALS-FTSD to controls, with the primary outcome measure being survival, and secondary outcome measures including histological, biochemical and behavioural metrics. We also carry out a structured quality assessment of the literature to provide recommendations for future studies. Taken together, these data better our understanding of the contribution of inflammation to ALS-FTSD pathogenesis, as well as highlight targets for further preclinical and clinical studies that aim to improve personalised therapies for people with ALS-FTSD.

(e124) Microglial engulfment of ALS synapses

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Synapse loss is an early feature of neurodegeneration in ALS, which happens both in the CNS and in the periphery at the neuromuscular junction. Neurons have long been the focus of neurodegeneration research, but growing evidence suggests that non-neuronal cells such as microglia has a pivotal role in pathogenesis. In other neurodegenerative diseases, such as Alzheimer's disease, it has been already shown that microglia physically remove synapses by phagocytosis. As microgliosis is a prominent and early feature in ALS, moreover, it is correlated with diseases progression we hypothesized that microglial engulfment of synapses could be a potential driving mechanism of ALS pathology in the brain

To investigate the potential effect of microglia on synapse loss, we generated synaptoneurosome fractions by serial filtration from human fresh frozen tissue of (primary motor cortex) BA4 and (occipital cortex) BA17 brain areas from 5 ALS patients and 5 healthy gender- and age-matched controls. We used BA4 as this area is the primary site of the disease and BA17 serves as internal control, as the visual cortex is thought to be little affected in ALS. After sample validation by Western blot and fluorescent tagging with a pH-sensitive dye (pHRodo-Red), we performed in vitro real-time phagocytosis assays using the BV2 microglia cell line and primary rodent microglia. To further investigate ALS-associated microglial phagocytosis in postmortem human tissue, we analysed Cd68+ (a marker of microglial lysosomes and therefore phagocytosis) burden in BA4 and BA17 areas in a collection of ALS cases and gender/age matched controls.

We found that microglia preferably phagocytose ALS synapses, from different brain areas, than controls. This was surprising as we did not expect to see a difference in BA17, due to the general belief that this region is little/late affected in ALS. This interesting data highlights that ALS synapses may contain attractive "eat me" signals for microglial engulfment which could drive and worsen the pathogenesis of ALS.

f) Neuropsychology

(f125) Factors affecting anticipatory grief: Do severity of the disease and behavioural changes predict anticipatory grief in family carers of MND?

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Background: Family carers of people living with MND (PwMND) experience multiple losses during the disease which occur before death leading to the phenomenon of anticipatory grief (AG). There are few studies investigating AG in MND carers. Studies in dementia revealed that disease progression and changes in patients' behaviour negatively impact in carers' AG but their association with MND carers' AG has not been explored.

Objective: To explore if disease severity and behavioural changes in the PwMND are significantly related to MND family carers' AG.

Methods: Thirty-three family carers of PwMND recruited in the UK completed the Marwit-Meuser Caregiver Grief Inventory-Short Form, which measures AG; the MND Behavioural Instrument, which assesses behavioural changes in the PwMND and the ALS-Functional Rating Scale-R, which evaluates disease severity. To understand the effects of disease severity and behavioural changes to AG, firstly, two separate regression analyses were conducted with these factors as independent variables and AG as dependent variable. Secondly, a multiple regression analysis was conducted including both factors simultaneously as independent variables to investigate the adjusted effect of each variable and understand which of the two predicted AG better.Results: Mean age of carers was 64.0 (SD=10.6) and 72.7% were female. Almost all carers lived with the PwMND (97%); were spouses/partners (97%); had been caring for the PwMND for almost 2 years (mean=22.8 months, SD=22.9) and 27.3% provided 100 or more hours of care per week. Mean age of the PwMND was 67.2 (SD=9.9) and 30.3% were male.

Results indicated that AG was predicted by disease severity (β=-0.68, 95% CI: -1.15 to -0.21 p=0.006) and behavioural changes (β=1.15, 95% CI: 0.16 to 2.14 p=0.025) in separate simple regression models and explained 22.5% and 15.7 % of the variance of AG respectively. When modelled simultaneously, the adjusted effect of disease severity remained significant (β=-0.55, 95% CI: -1.04 to -0.05 p=0.031), but the effect of behavioural change became statistically insignificant (β=0.75, 95% CI: -0.24 to 1.75, p=0.132). Both factors together explained 28.4% of the variance of AG.

Conclusions: These preliminary findings suggest that progression of MND, such as motor problems, may be experienced as losses by the family carer. Although behaviour changes showed some effect in AG, this was not statistically significant. This may be due to lack of statistical power.

(f126) Loneliness is associated with behavioural changes and fronto-parietal networks in ALS

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Introduction: Loneliness, also termed "perceived social isolation", impacts on neurobiological architecture, ensuing significant consequences on mental and physical health. In elderly people, it represents a risk factor for dementia. The possible backlash of loneliness on frontotemporal dysfunctions of ALS has not been investigated.

Objectives: To explore the association of loneliness with behavioural and cognitive symptoms of ALS, verifying the underpinning cortical signatures.

Methods: Loneliness was measured using the 3-item UCLA Loneliness Scale in 200 consecutive ALS patients who performed a comprehensive assessment for the evaluation of the cognitive efficiency, including social cognition, behaviour, mood, alexithymia, emotional regulation and quality of life (QoL). Seventy-seven ALS patients performed also 3T MRI scans for the measurement of cortical thickness. Spearman rho and Jonckheere-Terpstra tests examined neuropsychological profiles and cortical signatures of loneliness.

Results: One-hundred twenty-five patients reported no loneliness, 65 were classified as low/moderately lonely; 10 felt highly lonely. UCLA scores were associated with behavioural change, mood, emotional dysregulation and QoL ($p< 0.001$). Cognitive and motor disabilities were not related to loneliness. A significant cross-sectional effect of cortical thinning was observed in bilateral rostral-middle frontal cortex, left inferior parietal cortex, right superior parietal cortex and right precuneus ($p < 0.01$). Correlation analyses showed that the thickening of left inferior parietal cortex was also related to depression and emotional dysregulation ($p < 0.01$).

Discussion: The satisfaction of social environment is associated with a sense of life well-being that is not limited to the functional motor status. Loneliness was strongly related to neurobehavioral functioning and not with cognitive abilities. Altered structure in extra-motor brain regions within the default mode network underpin loneliness levels. This suggests that loneliness may act as a risk factor or may exacerbate behavioural symptoms in ALS patients.

Conclusion: Paying attention to social isolation in patients with ALS will help clinicians to intervene in neurobehavioral and psychiatric symptoms at an early stage.

(f127) The Sustained Attention to Response Task evokes sensorimotor beta ERD/ERS and enables quantification of motor and cognitive pathophysiology

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Objective: To identify sources generating cortical oscillations during performance of the sustained attention to response task (SART) and identify sources of their abnormalities in amyotrophic lateral sclerosis (ALS).

Methods: A randomised SART was undertaken by 30 ALS patients and 40 controls during 128-channel electroencephalography. Linearly constrained minimum variance (LCMV) beamforming was applied to localise sources of event-related (de)synchronisation (ERD/ERS) associated with performing the SART. We investigated relationships between these oscillations at specific cortical sources and measures of task performance and motor, cognitive and behavioural change in ALS.

Results: During correct Go responses, beta ERD and subsequent beta ERS were deemed to primarily originate from the sensorimotor (SM1) and posterior parietal cortices (PPC), more so in the left hemisphere (contralateral to the hand used to perform the task). During correct response withholding (NoGo), beta ERD demonstrated similar origins, but beta ERS was less potent in the left SM1 and PPC. Both beta ERD and beta ERS in the left SM1 and PPC showed significant correlations to SART response time and accuracy measures. ALS patients showed greater anticipation and poorer Go trial response accuracy compared to controls. ALS patients also demonstrated lesser beta ERD in the left PPC during Go trials and the left SM1, PPC and fusiform cortex during NoGo trials. Lower beta ERS in ALS originated from these and additional sources, including the left temporal pole and medial and lateral frontoparietal regions. Theta ERS and alpha ERD were predominantly localised to sensation and cognition-associated regions and also showed significant correlations to SART performance measures, with theta ERS uniquely relating to task performance in ALS patients.

Conclusions: The SART evokes beta ERD/ERS characteristic of established sensorimotor activation and inhibition measures, alongside theta and alpha ERD/ERS in non-motor domains, enabling dissection and quantification of normal, compensatory and pathological motor and cognitive cortical physiology in ALS.

(f128) Caregiving burden in Amyotrophic Lateral Sclerosis (ALS): Association with cognitive changes and emotional distress

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Background: Caregiver burden is a multidimensional construct that impact directly on the quality of life of ALS patients and their relatives. Factors of the care provider and others associated with the disease itself may contribute to the onset of caregiver overload.

Objectives: Our aim is to explore the impact of ALS patient's cognitive changes and caregiver's emotional distress on caregiver burden.

Method: The study sample consisted of 21 patients with ALS according with El Escorial criteria and their primary caregivers. Zarit Burden Interview (ZBI) was used to measure caregiver burden and their emotional distress through Emotional Distress Detection (EDD), a Spanish validated scale. To determine cognitive function in patients The Edinburgh Cognitive-Behavioural ALS Screen (ECAS) was used, and Wisconsin Card Sorting Test (WCST) was administered as a complex executive task of cognitive flexibility. Revised ALS functional rating scale (ALSFRS-R) was used for determining disease progression. T-tests were used to compare the differences between groups and partial correlations coefficients were calculated to determine the relationship between continuous variables.

R**esults:** Mean age of ALS patients was 64.0 ±11.8, 38. 1% were women and 90.5% had limb onset. Significant higher ZBI scores were found in impaired ECAS- ALS and Total scores group (according to Spanish cutoffs) compared to non-impaired group (t= -0.4, p = 0.006). Negative partial correlations (adjusted by ALSFRS-R) were found between ZBI and ECAS - ALS (r=-0.526, p = 0.017) and ECAS - Total (r= -0.532, p =0.016). Positive correlation, although almost significant were found between ZBI with WCST- perseverative responses (r= 0.430, p = 0.054) and perseverative errors (r = 0.435, p=0.055). EDD scale was positively correlated with ZBI (r = 0.580, p =0.007).

Conclusion: In our cohort, caregiver burden is associated with ALS patients' cognitive symptoms without the influence of functional status. Also, an increased burden of caregivers is associated with their emotional distress. This study suggests that assessments of cognitive and behavioral symptoms could enable to identify those caregivers with a greater burden, and thus initiate a more targeted intervention program. Further studies are needed to study the association between cognition and caregiver burden in ALS.

(f129) Altered gaze control during emotional face exploration in Amyotrophic lateral sclerosis

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Background: Amyotrophic lateral sclerosis (ALS) primarily affects motor function, leading to progressive immobility and impaired verbal communication. In up to 50%, cognitive function and behaviour may worsen, including emotion recognition in faces. Abnormal recognition of facial emotions in ALS may be also explained by altered visual exploration as measured from scan path, an observation that appears to be independent from cognitive impairment.

Methods: ALS patients (n=45) and healthy controls (n=37) underwent neuropsychological assessment and video-based eye tracking. All subjects were cognitively unimpaired according to ECAS (above age- and education-adjusted cut-offs). Eye movement trajectories were recorded while viewing human faces expressing different emotional content as compared to non-facial stimuli consisting of houses mimicking faces. Main area of interest (AOI) were emotional-relevant regions as the area around the eyes (provides key information of the emotional state). The remaining areas (i.e. the cheeks) were defined as non-emotional-relevant regions.

Results: As compared to controls, analyses of eye movements revealed that ALS patients fixated significantly longer to non-emotional-relevant regions when faces expressed fear [p = 0.007] and disgust [p = 0.006]. Moreover, patients (vs. controls) fixated significantly more frequently on non-emotional-relevant regions when faces expressed fear [p = 0.035]. However, there were no significant differences in the number of fixations [p = 0.082] or fixation time [p = 0.100] to the eye region or for other informative face areas. Fixations were not significantly correlated with markers of cognitive, physical and affective state or disease duration.

Discussion: Scan paths for exploring emotional faces in cognitively unimpaired ALS patients were altered as non-salient features retrieved more attention. A possible explanation is the onset of impaired top-down processing with subliminal frontotemporal area involvement despite preserved cognitive profile. This may account for indistinctness in emotion recognition regarding facial expressions for fear and disgust in previous studies, as non-relevant areas retrieve more attention at the expense of relevant face areas as a unique feature of ALS pathology but unrelated to general frontotemporal dysfunction.

(f130) Reliability and validity of the remote administration of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)

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Introduction: The COVID-19 pandemic has created unprecedented challenges to ALS clinical care and research, leading to a reduction in face-to-face visits. Assessments had to take place remotely. A fundamental component in the provision of appropriate care for people with ALS (pwALS) and that is often used as an outcome measure in clinical trials is the assessment of cognition and behaviour. A well-established screening instrument for detecting cognitive impairment in ALS is the Edinburgh Cognitive and Behavioural ALS Screen (ECAS; https://ecas.psy.ed.ac.uk). However, the ECAS has been developed and validated for face-to-face administration only.

Objectives: To determine the reliability and validity of the remote administration method of the ECAS, and explore the experiences of pwALS, clinicians and researchers who have completed or administered the ECAS remotely.

Methods: The validation process consists of three components. (1) Administering two randomised versions of the ECAS (A and B) to healthy controls (HC), completing one-in-person and the other remotely. (2) Administering the ECAS remotely to 30 pwALS, with a second rater involved to independently score the ECAS. (3) Inviting pwALS, clinicians and researchers to take part in an online survey to explore their experiences of completing/administering the ECAS remotely.

Results: The study is still in progress, but preliminary data will be available for presentation. Proposed analyses will include: (1) Exploring the equivalence of in-person and remote administration. This will involve repeated measures t-tests (or Wilcoxon signed-rank test) to compare the means and medians, Kolmogorov-Smirnov test to compare the shape and spread of distribution, and Bayesian statistics to directly test the null hypothesis and examine the probability that the two administration methods are the same. (2) Intra-class correlation to determine inter-rater reliability. (3) Descriptive statistics of quantitative survey data and thematic analysis of qualitative data to explore the experiences of those who have completed/administrated the ECAS remotely.

Conclusions: We hope to demonstrate that the remote administration of the ECAS is a reliable and valid assessment method for pwALS. We hope that clinicians, researchers and patients view it as a good alternative to face-to-face administration.

(f131) Quality of life in adult patients with spinal muscular atrophy

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Introduction: Spinal muscular atrophy (SMA) is an autosomal recessive disease, caused by loss of the SMN1 gene, leading to alpha motoneuron damage. Based on the number of SMN2 genes and the clinical presentation, 5 types of SMA have been described. The aim of our study was to analyze the quality of life of adult patients with SMA (type 2 and type 3) in Serbia. Material and methods: Our study included 21 patients with SMA, seven patients had SMA2, while fourteen had SMA3. The diagnosis was based on clinical presentation and genetic analysis. Following scales were used: Fatigue Severity Scale (FSS), ALS Functional Rating Scale (ALS-FRS-r), Beck's depression inventory (BDI) and SF-36. Results: The average age of the patients was 38.04 ± 11.67 years. The ratio of SMA2: SMA3 was 1: 2. The average score of FSS was 4.9 ± 1.4, and 15 patients (71.4%) had clinically significant fatigue. Significant correlation between both worse FSS and fatigue with BDI score (6 (0.5-11,5)) was noted. Six patients (28.6 %) had depression with significant association with worse FSS. The mean ALS FRS-r was 34.6 ± 7.2 and was associated with higher FSS. The scores of both physical component (PCS) and total SF-36 (46.16 ± 20.93; 54.04 ± 23.21; respectively), were highly affected, with significant correlation with worse ALS FRS-r, FSS and BDI score. Mental component of SF-36 (MCS) (61.53 ± 26.34) was also affected and was associated with worse FSS and BDI score. Conclusion: Fatigue is the most debilitating symptom of adult patients with SMA and is associated with depression and functional disability. Combined influence of fatigue, depression and functional disability, caused lower quality of life, affecting both physical and mental aspects in our group of patients.

(f132) Assessing mental capacity in people with MND: What healthcare professionals think, know and do

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Background: People with MND (pwMND) are required to make important and difficult decisions throughout the course of the disease. Up to 50% show changes in cognition/behaviour which could affect decision-making. Healthcare professionals (HCPs) may have to judge patients' capacity to make specific decisions, however what HCPs think, know and do with regards to mental capacity assessments in pwMND is unknown. Objectives: Explore HCPs knowledge of mental capacity and current practices of assessment in the clinical care of pwMND in the UK.Methods: HCPs working in UK MND clinics were invited to participate in an online survey. Questions were related to: knowledge and involvement in mental capacity assessments, context and frequency, features of the assessment process and cognition. Results: Participants (n=86) and were mainly allied HCPs (36.1%), doctors in neurology/palliative medicine (26.7%), and psychologists (11.7%). 59.3% of the total sample had received training in mental capacity. When tested on true/false statements relating to mental capacity, only 57% got every statement correct. Over half of participants had been involved in assessing mental capacity in pwMND, of which 9/10 had encountered pwMND who lacked capacity to make decisions. Assessments often took place in the patient's home and the time taken to administer them varied. The majority of respondents completed ≤10 assessments per year, with the most common assessment method being a general discussion with the patient/carer (68.9%) rather than a formal standardised interview. Only 46.7% said they always made patients aware that their mental capacity was being assessed. 55.6% said that having a cognitive impairment would prompt a capacity assessment for a specific decision. When questioned if life-prolonging interventions would still be offered to those with cognitive impairment, only a third said yes, with two thirds saying that it would depend (often on the nature/degree of impairment). Finally, 88.7% of participants felt confident in their ability to assess mental capacity in pwMND. Conclusions: Many participants had encountered pwMND who lacked capacity to make decisions. The majority felt confident undertaking capacity assessments, although only 59% had undergone training and 43% made errors in their understanding of mental capacity. HCPs are aware of mental capacity, many are involved in assessments, but more thorough training is needed when a capacity assessment is required.

(f133) Treatment management and quality of life in ALS patients

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Background: Up to date, Riluzole is the only disease-modifying therapy available in ALS. However, the frequent swallowing problems of ALS patients increase the treatment burden. Our aim was to evaluate the role of treatment burden in ALS patients' quality of life (QoL), and its primary causes and consequences in order to improve the management of therapies.

Methods: ALS patients were enrolled between November 2020 and February 2022 and evaluated at T0 and after three months (T3) at NEMO center (Milano) and IRCCS Fondazione Mondino (Pavia). The Multimorbidity Treatment Burden Questionnaire (MTBQ) was used to evaluate patients' treatment burden, and was associated to the QoL related to the perceived treatment effects (INQoL's specific subscore), the change formulation needs, and the treatment adherence (Morisky Medication Adherence Scale).

Results: Up to now, 55 ALS patients were recruited (mean age at recruitment: 61.51 years ± 13.46, male/female ratio: 2.44). At T0, patients with a low burden (MTBQ < 10) reported a higher QoL related to the perceived treatment effects than patients with a medium to high burden (25.00 [8.33 – 41.67] vs 16.67 [-16.67 – 16.67]), although not statistically significant. Moreover, patients with swallowing problems who had to change the drug formulation reported a significantly higher treatment burden than patients who did not have to change the drug formulation (27.50 [15.00 – 42.50] vs 5.00 [0.00 – 8.75], p=0.0005), and treatment burden resulted to be significantly related to adherence, i.e. the higher the treatment burden the lower the treatment adherence (rho= -0.40, p=0.0028). Finally, patients who changed their burden level from low to medium/high burden after the 3-month follow-up period, reported a significantly decrease in QoL related to the perceived treatment effects than patients who remained in the low burden classification (-25.00 [-33.33 – 0.00] vs 12.51 [-8.33 – 25.00], p=0.0369).

Discussion: Swallowing problems due to tablet formulation seem to be the cause of a high level of treatment burden, which can reduce patients' adherence to treatment. Furthermore, the increase in treatment burden seems to be related to a significant reduction in the QoL related to the perceived treatment effects. In light of these findings, oral solutions could represent an improvement in the management of therapies in patients. However, a larger sample size and a longer follow-up are necessary to confirm these findings.

(f134) Holistic Assessment of Non-Motor Symptoms for People with Motor Neuron Disease (NMS-MND).

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Background: People with motor neuron disease (pwMND) often experience non-motor symptoms (NMS) in addition to physical symptoms. NMS that may affect pwMND include pain, fatigue, gastrointestinal issues, poor sleep, low mood, anxiety, problematic saliva, apathy, emotional lability, cognitive impairment and sexual dysfunction. These occur secondary to, or distinct from, motor degeneration, and can significantly reduce quality of life.

Aim: This study explored the frequency, severity and impact of NMS. We also investigated pwMND's perspectives on how frequently these NMS occur, and whether they should be incorporated into clinical care and clinical trial design. In addition, we investigated pwMND's preferences for how symptoms are evaluated.

Methods: People registered on CARE-MND (the Scottish MND register) were invited to complete a questionnaire exploring NMS available in online or paper format. Participants also completed a self-reported ALS-FRS(R) (Amyotrophic Lateral Sclerosis Functional Rating Scale) on functional ability. This was supplemented by clinical data from CARE-MND.

Results: 120 people participated (91% opted for paper format), a response rate of 39%. 34% were on riluzole, 20% used non-invasive ventilation, and 18% had a gastrostomy. ALS-FRS(R) ranged from 3 to 47, with an average score of 30 (SD = 9). All but one person experienced at least one NMS, with 72% reporting five or more NMS. Pain and fatigue were the most commonly reported symptoms (76% for both), with pain and problematic saliva reported as the most impactful on pwMND (51% respondents selected 'Yes' to "Is this a significant issue?", for both symptoms). 73% (n = 109) were content with how often NMS were assessed in clinical care. 80% indicated they believe NMS are important to include in clinical trials. The preferred method of symptom assessment was remote/community based questionnaire evaluation.

Conclusions: NMS are common and impactful in pwMND. The majority of people experience multiple NMS. Pain and fatigue are particularly prevalent. PwMND in this study reported they were content with evaluation of NMS in clinical practice and an overwhelming majority indicated a preference for NMS to be assessed in clinical trials. Ethical approval was provided for this study on 19th October 2021 (Research Ethics Committee number: 21/YH/0226).

g) Neuropathology

(g135) TDP-43 aggregates accumulate in the gastrointestinal tract prior to symptom onset in amyotrophic lateral sclerosis

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Objective: Neurodegenerative conditions/diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) are traditionally considered strictly neurological disorders. However, clinical presentation is not restricted to neurological systems, and non-central nervous system (CNS) manifestations, particularly gastrointestinal (GI) symptoms, are common. Our objective was to understand the systemic distribution of pathology in archived non-CNS tissues, taken as part of routine clinical practice during life from people with ALS.

Design: We requested all surgical specimens of non-CNS tissue taken during life from 48 people with ALS, for whom CNS evidence of the characteristic proteinopathy associated with ALS had been identified after death (i.e. the pathological cytoplasmic accumulation of phosphorylated TDP-43 (pTDP-43) aggregates). Of the 48 patients, 13 had sufficient tissue for evaluation; 12 patients with sporadic ALS and 1 patient with a C9orf72 hexanucleotide repeat expansion. The final cohort consisted of 68 formalin-fixed paraffin embedded tissue samples from 21 surgical cases (some patients having more than one case over their lifetimes), from 8 organ systems, which we examined for evidence of pTDP-43 pathology. The median age of tissue removal was 62.4 years old and median tissue removal to death was 6.3 years.

Results: We identified pTDP-43 aggregates in multiple cell types of the GI tract (colon and gallbladder), including macrophages and dendritic cells within the lamina propria; as well as ganglion/neuronal and glial cells of the myenteric plexus. Aggregates were also noted within lymph node parenchyma; blood vessel endothelial cells; and chondrocytes. We note that in all cases with non-CNS pTDP-43 pathology, aggregates were present prior to ALS diagnosis (median=3years) and in some instances, preceded neurological symptom onset by more than 10 years.

Conclusion: These data imply that patients with microscopically unexplained non-CNS symptoms could have occult protein aggregation that could be detected many years prior to neurological involvement.

(g136) Alternative counterstains improve detection and digital analysis of chromogenic BaseScopeTM in situ hybridisation signal in human post-mortem tissue

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Background: BaseScopeTM in situ hybridisation (ISH) is designed to amplify ISH signal, with high sensitivity and specificity, to allow for the visualisation of single mRNA molecules, which can be quantified with single cell resolution in a tissue section. Many choose to use this technique with chromogen detection kits to allow for cell-type information to be retained through haematoxylin counterstaining and to limit the substantial and highly variable autofluorescent artefact seen in (formalin fixed paraffin embedded) FFPE brain tissue. However, digital analysis of haematoxylin counterstained images is often difficult, especially with red chromogen, due to overlapping signal in the red/magenta channel. As such, digital detection and cell segmentation algorithms can be less accurate than manual counting, especially when analyzing a neuropil predominant chromogen signal.

Methods: We therefore set out to test two alternative counterstains (methylene blue and light green) and to compare (i) the visualization of probe binding events in the neuropil, white matter and grey matter (ii) adequacy of digital cell segmentation/superpixel analysis; (iii) the performance of concomitant fluorescent imaging and (iv) the resolution accuracy of cell-type histological features. These two alternative counterstains are used in routine histological practice as optimal counterstains for detecting small organisms (e.g. tuberculosis; red tinctorial staining with methylene blue counterstaining) and fungal organisms (e.g. aspergillus; using a silver treatment to highlight organisms and light green as a cellular counterstain).

Results: We found that these two alternative counterstains allow for optimal contrast in colour to facilitate improved detection of the red chromogen used in BaseScope ISH. Specifically, we demonstrate improved detection of white matter and neuropil ISH signal using light green and improved digital detection of ISH signal in all regions using methylene blue.

Conclusion: We present two alternative counterstaining methods to improve digital analysis of human tissue stained with chromogenic BaseScope ISH.

(g137) Cytoplasmic mislocalization and aggregation of TDP-43 in brains of ALS and FTD patients

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Introduction: TDP-43 has been identified as a major component protein of ubiquitin-positive inclusions in brain cortex from ALS and FTD patients. This protein is involved in transcriptional repression and alternative splicing (1). It is expressed in nucleus in the majority of healthy neurons, but in pathological neurons, it phosphorylates and translocates into the cytoplasm generating neuronal cytoplasmic inclusions (2,3). The aim of this study is to determinate the nuclear and cytoplasmic levels of TDP-43, as well as the presence of aggregates in pyramidal neurons.

Method: Cytoplasmic mislocalization and aggregation of TDP-43 was evaluated by immunofluorescence in post-mortem brain and spinal cord tissue of ALS patients, FTD patients and controls. Images are obtained with Zen 2009 Software (Carl Zeiss) and analyzed with Volocity Software. Statistical analysis was performed using SPSS Statistics version 22 (IBM, Spain).

Results: TDP-43-positive inclusions were found in brains from patients with ALS and FTD. TDP-43 appears in the nucleus of neurons but, in degenerating regions of ALS patients, the nucleus is almost empty, and TDP-43 appears in the cytoplasm of the pyramidal neurons. Fungal-like structures such as yeast and hyphae were found in the motor cortex, the medulla and the spinal cord, in ALS patients.

Conclusions: The pathological TDP-43 that appears in the cytoplasm of affected areas could be phosphorylated, ubiquitinated and cleaved to generate carboxy-terminal fragments. Fungal infection may play a part in the etiology of the ALS or may constitute a risk factor for patients.

Acknowledgements: This work was supported by grants PI17/00491 from the Instituto de Salud Carlos III (ISCIII), the support of Comunidad de Madrid (B2017/BMD-3813 "ELA-Madrid"), CM-Fundación H12O agreement (BOCM 142 – 17/09/2019 pg. 105), and Plataforma de afectados de ELA.
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(g138) High-resolution imaging of synapse density in ALS brain and its association with clinical presentation

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Approximately 50% of ALS patients display cognitive impairment and have a worse prognosis due to a faster disease progression. Previously, synapse loss in the dorsolateral prefrontal cortex (Brodmann area BA9) has been associated with cognitive decline, but the regional specificity of synaptic degeneration and its correlation with the presence of symptoms remains to be assessed.

Therefore, we aim to build on our previous work and generate a database of comprehensive patient information that ranges from clinical cognitive profiling to post-mortem high-resolution synaptic anatomy. Here, we present data on synaptic density in Brodmann area BA44/45 (Broca's area), associated with language function, and Brodmann area BA17/19, part of the visual cortex and thought to be spared in ALS. This analysis has been performed in samples from the same donors as our previous work, which means we have synapse density data from BA9, BA4 (motor cortex), BA44/45 and BA17/19 from each donor, collected using both electron microscopy and array tomography.

The ALS patients were stratified based on their cognitive profile derived from their Edinburgh Cognitive and Behavioural ALS Screen (ECAS) scores, which can be further broken down into cognitive tasks. This allows us to compare region-specific synapse density with particular aspects of cognitive change such as executive dysfunction and language fluency. Lastly, we have combined our synaptic-level analyses with cortical thickness measures as well as regional neuropathology analysis (presence of TDP-43, Tau or Amyloid) and we have access to full demographic information on each individual donor.

Surprisingly, we observed no difference in synaptic density in BA44/45 regardless of cognitive status, contrary to what we found in nearby area BA9. However, we found a decrease in cortical thickness in BA44/45, suggesting more advanced pathology and neuron loss. Interestingly, the thinning in BA44/45 seems to be exclusive to patients with cognitive decline. Remarkably, we found cortical thinning in the visual cortex, which was independent of cognitive impairment. We are currently assessing synapse density in BA17. Finally, we are in the process of aligning these data with regional neuropathology and demographic data.

In summary, we have unique human dataset combining detailed cognitive assessment, regional neuropathology, and single synapse analysis to try and uncover the underlying pathology associated with cognitive decline in ALS.

(g139) Rapamycin reverts TDP-43 splicing defects and oxidative stress-induced alterations in a human in vitro model of TDP-43 proteinopathy

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Aggregates of phosphorylated and ubiquitinated TDP-43 protein in the cytoplasm of neurons are an ALS neuropathological hallmark. Response to stress and formation of stress granules (SG) have been proposed as possible initiators of TDP-43 pathological aggregation. We recently showed that chronic oxidative stress by arsenite induces SG formation in fibroblasts and iPSC-motor neurons from TARDBP and C9orf72 patients. This insult also leads to the formation of phospho-TDP-43 aggregates, which are more abundant in C9orf72 cells and resemble those seen in ALS autoptic brains.

Aim of our study was to generate a cell model of TDP-43 pathology for screening drugs able to prevent or reduce TDP-43 pathological inclusions. Given the high variability in the response to stress observed in ALS patients' cells, we reproduced a chronic oxidative insult in human neuroblastoma SK-N-BE cells by exposure to low doses of arsenite for a time frame ranging from 9 to 24 hours. Our data showed TDP-43 mislocalization from the nucleus to the cytoplasm in a dose- and time-dependent manner and a block in the autophagic flux. Of interest, in this condition we also observed a defective splicing activity of TDP-43 towards selected RNA targets, including UNC13A, STMN2 and POLDIP3. Chronic arsenite treatment is therefore able to reproduce both TDP-43 nuclear loss-of-function and its cytoplasmic mislocalization and aggregation, the two neuropathological hallmarks of TDP-43 proteinopathy. Since autophagy impairment favors TDP-43 pathological aggregation, we tested two autophagy enhancers, Rapamycin and Lithium carbonate. We found that Rapamycin, but not Lithium, was capable of rescuing arsenite-induced loss of TDP-43 splicing activity on RNA targets, of reducing insoluble TDP-43 content and of re-establishing the autophagic flux. We already confirmed the efficacy of Rapamycin on TDP-43 splicing activity in C9orf72 patient-derived fibroblasts exposed to chronic oxidative insult and further studies are now in progress to validate findings also in C9orf72 iPSC-motor neurons.

In conclusion, we have set up an experimental in vitro model of TDP-43 pathology in which Rapamycin proved to be beneficial, thus supporting the rationale for targeting autophagy in clinical trials. Moreover, this in vitro model can be exploited as a valuable platform for future drug screening approaches.

h) Clinical

(h140) Faculty Development in a Multidisciplinary ALS Clinic

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Background: The impact of multidisciplinary clinics on the care and outcome of patients with amyotrophic lateral sclerosis (ALS) is well established. Providers from multiple disciplines, each with expertise in their own area, collaborate in a shared setting to optimize care, prolong survival and enhance quality of life. Information on the approach to faculty development in these settings, however, is lacking.

Method: The Multidisciplinary ALS Clinic at Rush University Medical Center initiated a monthly pre-clinic Lunch and Learn Session in August 2018 for team members. Knowledge relevant to the care of patients with ALS was assessed before and after the session, along with an evaluation of the presentation. On a rotating basis, a provider from each discipline presented a 20-30 minute topic discussion with objectives relevant to the care of ALS patients. Immediately prior to the discussion, a pre-session quiz was completed by the attendees. After the discussion, the same questions were presented in a post-session quiz. Quizzes and session evaluations were numerically coded to allow for individual comparison before and after the session.

Results: Data for six monthly sessions were analyzed. Comparing the percentage of correct answers, there was an overall significant difference ($p<0.0001$) between the pre- and post-sessions. The topics associated with the most significant difference were "The Role of Nutrition in ALS Management", "The Role of the Speech Pathologist as a Member of the Interdisciplinary ALS Clinic", and "Orthotic Considerations for ALS Patients". For each of these topics, the correct response rate was 67% before the session and 100% after the session. Evaluation forms were available for five of the six sessions. For all five sessions, 100% of the attendees reported that the objectives were met, the presenter exhibited content expertise, and based on the content presented, they would be better able to collaborate with multidisciplinary team members. 87-100% reported that the content presented was applicable to their care of patients. All attendees rated the sessions as either "Excellent" or "Good" on a four point Likert scale.

Conclusions: Due to the varied backgrounds in training, faculty development to promote an understanding of the role of each multidisciplinary provider and advances in the respective fields can enhance the knowledge and collaborative approach to optimize the care of ALS patients.

(h141) UPDATED: The United States National Amyotrophic Lateral Sclerosis (ALS) Registry Advances Research Domestically and Internationally

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Objective: Describe how the National ALS Registry supports and advances research in the United States and abroad.

Background: The National ALS Registry is the largest database of persons with ALS in the United States. One of the purposes of the Registry, as defined by Congress, "is to facilitate research." In addition to registering patients with ALS and collecting epidemiological data, a National ALS Biorepository was added in 2015 after the completion of a pilot project. The purpose of the Biorepository is expand ALS research in areas such as genetics, biomarker identification, environmental exposures, and disease progression. Because recruiting interested PALS can be difficult and time- consuming for researchers, the Registry can assist with recruiting persons with ALS into clinical trials and research studies. The Registry can also provide de-identified self-reported epidemiological data and/or biological samples for a subset of persons in the Registry.

Design/Methods: Developed mechanisms that allow eligible persons with ALS to be informed about clinical trials and research studies, provide specimens to the Biorepository, and self-report epidemiologic data securely. Researchers, both domestically and internationally, can apply to receive data from the Registry and/or samples from the Biorepository as well as have recruitment emails sent for their studies. Researchers who want to use one of these mechanisms submit an application to CDC/ATSDR with information about the study and provide documentation that an Institutional Review Board has approved the study. CDC/ATSDR maintains a scientific review committee.

Results: To date, over 65 institutions (pharmaceutical companies and academia) have used the Registry to recruit for their clinical trials and studies. These include notable clinical trials for Amylyx, Inc., MT Pharma., and others. Analyses have been completed for the Biorepository specimens (blood/urine/serum/saliva) for the following areas: heavy metals, persistent organic pollutants, and genotyping. Almost 50 patients have completed a post-mortem component, which covers whole brains, CSF, spinal cord, and tissue and over 1500 patients have completed the in-home collection (blood, urine, saliva). Data are available for researchers from 18 risk factor modules.

Conclusions: The National ALS Registry provide researchers access to a large national group of ALS cases and data for their research national and internationally.

(h142) Robotic assessment with artificial intelligence in the evaluation of ALS disease progression

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Background: ALS is a rapid and progressive neurodegenerative disease; thus, the evaluation of patients' clinical status and prognosis may be challenging. Up to date, the main validated tool to assess patients' clinical condition is the ALS Functional Rating Scale-Revised (ALSFRS-R). In light of this, the aim of our study was to evaluate the ability of a robotic device with artificial intelligence to administer the ALSFRS-R.

Methods: ALS patients referred to the NEMO clinical centre between April and January 2022 were enrolled. The ALSFRS-R was assessed by both a human operator and the robotic device. Moreover, the STAI-Y 1-2 were provided to evaluated the levels of patients' anxiety before and after the robotic device's administration.

Results: Twenty-two ALS patients were recruited for the study (median age at evaluation: 62.37 years [53.81 – 71.00], male/female ratio of 2.14). A good to excellent agreement resulted in the ALSFRS-R total score (ICC=0.95 [0.89 – 0.98]) between human and robotic operators' administrations, reporting a bias (mean difference) of 0.36 points with -3.44 and 4.17 points as 95% limit of agreement. Among subscores, a moderate to good agreement resulted in the bulbar subscore (ICC=0.76 [0.52 – 0.89]), a good to excellent agreement resulted in the motor subscore (ICC=0.91 [0.80 – 0.96]), and an excellent agreement resulted in the respiratory subscore (ICC=0.96 [0.91 – 0.98]). Moreover, no significant clinically differences in patients' anxiety levels emerged between pre and post robotic device's administration.

Discussion: The ALSFRS-R administered by the robotic device appears to be in moderate to excellent agreement with those administered by the human operator, without increasing the patients' anxiety. These findings suggest the potential benefit of a robotic device with artificial intelligence in an hospital context, reducing also the operators' workload.

(h143) Assessment of the upper motor neuron in ALS using Combined Patellar Tendon Reflex-MEP to Lower Limb: a monocentric cohort

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Objective: To evaluate whether Combined Patellar Tendon Reflex-Motor Evoked Potentials to Lower Limb (T-MEP-LL) is relevant to assess corticospinal function in amyotrophic lateral sclerosis (ALS), in a monocentric retrospective study.

Methods: T-MEP-LL was performed on 100 patients with motor neuron disease (MND) and 35 patients with other neurological pathologies, during routine diagnosis explorations. Clinical evaluation of the patients included neurological examination, the revised ALS Functional Rating Scale (ALSFRS-R) and Medical Research Council (MRC) score. Awaji and Gold Coast criteria were determined for each patient. Survival was calculated if death occurred.

Results: Three parameters of T-MEP-LL are of particular interest to detect corticospinal abnormalities. Depending on the T-MEP-LL parameters chosen to detect MND, sensitivity raises up to 80% and specificity to 74%. Among MND patients without sign of upper motor neuron, T-MEP-LL shows a greater specificity. T-MEP-LL can help to improve diagnosis criteria, by correcting 50% of Gold Coast diagnosis error. T-MEP-LL results are not correlated with ALSFRS-R and MRC score but it could be a prognosis factor knowing that patients with normal T-MEP-LL have delayed survival.

Conclusions: T-MEP-LL is an easy and painless technique applicable in daily clinical practice, and it has a good sensitivity and specificity to detect motor neurone disease. T-MEP-LL might be an interesting prognosis factor that needs to be confirmed by other studies.

(h144) Validation of the DYALS (dysphagia in amyotrophic lateral sclerosis) questionnaire for the evaluation of dysphagia in ALS patients

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Background: Dysphagia is a common symptom during the trajectory of ALS, and it can significantly impact on the quality of life and prognosis of patients. Nowadays, no specific tool for the screening of dysphagia in ALS is validated, and the approach is heterogeneous across the Italian centres.

Objective: To validate the DYALS (dysphagia in amyotrophic lateral sclerosis) questionnaire, adapting the DYMUS (dysphagia in multiple sclerosis) questionnaire, for the assessment of dysphagia in ALS patients, in order to uniform the evaluations across the Italian ALS network.

Methods: We included 197 patients diagnosed with ALS following the El Escorial criteria, in sixteen Italian ALS centres between 1st December 2019 and 1st July 2020. For each patient, we collected clinical and demographic data and obtained ALSFRS-r score, ALSAQ-5 score, DYMUS score, and EAT-10 score.

Results: Across the 197 patients, the ratio M/F was 113/84, and the median age was 64 years (IQR 56–72.5). Bulbar patients were 20%, and spinal patients 80%. The median ALSFRSr total score of patients was 35 (IQR 28–39). DYALS score was statistically higher in bulbar ALS than in spinal ALS (median = 6, IQR 4.5–9 vs median = 1, IQR 0–5, $z = 6.253$, $p < 0.0001$). DYALS questionnaire showed a high internal consistency (Cronbach's alpha = 0.88). There was a statistically significant correlation between DYALS and EAT-10 ($rho = 0.90$, $p < 0.0001$).

Conclusions: DYALS scale is reliable, manageable, and easily usable for the screening of dysphagia in ALS. It can be shared with all the Italian ALS centres in order to collect uniform data for therapeutic strategies and clinical trials.

(h145) Impact of ALS subtypes on disease progression: A continuous temporal multivariate approach

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Objectives: Amyotrophic lateral sclerosis (ALS) is a rare disease with heterogenous progression rates ranging from slow to rapid progression. Studies aiming to characterize the factors associated with the progression rate have focused on survival but few concerned the functional decline trajectory [1].

Methods: Using PROACT database (8,569 patients), we select spinal and bulbar patients with at least a baseline and a second follow-up visit. We randomly select spinal patients to get two balanced groups of 1,380 patients.The following steps were performed: 1) We built a multimodal ALS course map that grasped long-term disease progression in a mixed-effects fashion [2] with Leaspy. We used 6 features: the four subscores of ALSFRS-R, forced vital capacity (FVC) and BMI. 2) We extracted the progression rate and onset age, and the relative progression of each feature of each patient from the parameters of the model. 3) We also computed from the disease course, the conversion age to a progression threshold for each feature: 8 points for ALSFRS-R subscores in reference to FT9 score, 18.5 for the BMI and 2.43 litres for FVC.Finally, we compared the distributions of the extracted parameters and conversion ages using independent t-test.

Results: We found that ALS starts 2.84 years later for bulbar patients, but progresses 1.36 times faster. We observed, for bulbar patients, that ALSFRS-R fine and gross motor progress 3.7 and 4.4 months later than the spinal patients but ALSFRS-R bulbar progression starts almost 8 months before. Bulbar patients reached endpoint thresholds define above, in average after spinal patients for: ALSFRS-R fine motor (8 points, 49.7 months), gross motor (44 months), bulbar (7.5 months) and respiratory (23.3 months), FVC (24 months).

Conclusions: We build a modelling framework for describing ALS subtypes effect on functional endpoints from multimodal screening assessments. This model also allows describing and predicting individual progression which can pave the way to discriminate fast and slow progressor for stratification of clinical trial. This methodology could also be applied in clinical trial to compare treated and placebo arms.

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(h146) The value of the El Escorial Criteria (EEC)

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Introduction: In the first Edaravone trial (Abe K et al, 2014) and later in the PRO-ACT dataset (Braun N et al, 2019) we could show that the EEC 'probable laboratory supported' category induces a bias towards slow progressors in ALS clinical trial populations. In addition patients in this category exhibited a significantly longer diagnostic delay (13.5 months versus 11.7 months, $p<0.001$). It is unclear whether this bias is only present in a clinical trial population (e.g. PROACT dataset) or in the general ALS population.

Methods: We therefore analysed prospectively entered data from ALS Progeny database from Belgium (n=411), Ireland (n=79), Italy (n=252), the Netherlands (n=623) and Switzerland (n=298) with at least two ALSFRS-R and vital capacity (VC) measurements. We used a linear mixed effect model with a random slope and intercept per patient and country to analyse ALSFRS-R-Score and VC changes.

Results: Diagnostic delay between EEC categories possible, probable lab supported, probable, definite (means 8.7; 9.5; 10.8; 11.5) were significantly different ($p=0.005$). The mean rate of decline was significantly faster with a worse EL Escorial category for the ALS FRS-R score ($p<0.001$) and VC ($p<0.048$). Considering the change from baseline (cumulative difference from baseline values for individual patients) the slopes for ALSFRS-R and VC of the categories lab supported and probable behaved similarly.

Conclusion: The conclusion that the EEC "probable lab supported" category creates a bias toward slow progressors does not hold true in an unselected, incident ALS population. Patients belonging to this EEC category come earlier to diagnosis, which was one of the goals of the EEC revision, and should not be excluded from clinical trials. Moreover, the significantly different progression rates between EEC categories suggest they should be considered as a stratification tool for ALS clinical trials.

(h147) Serum Chloride is a low cost marker of respiratory failure in Amyotrophic Lateral Sclerosis

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Introduction: Serum chloride is a metabolic indicator of the degree of respiratory acidosis easily obtainable by routine blood analysis. We investigated the role of serum chloride analysed at diagnosis as a prognostic factor in a population-based series of ALS patients.

Methods: We collected all the serum chloride in patients followed up in Turin ALS Centre as part of the Piemonte and Valle d'Aosta Register for ALS from January 1st, 2007 to December 31st, 2019. We also collected clinical data such as age at diagnosis, sex, date of onset, site of onset, date of death/tracheostomy, ALSFRS-r score at diagnosis, weight loss and FVC at diagnosis.

Results: One thousand four hundred and eighty-four ALS patients were included in the analysis. Serum chloride showed a significant but small correlation with FVC ($R=0.149$, $p<0.001$) and respiratory symptoms at diagnosis ($R=0.179$, $p<0.001$), measured using ALSFRS-R. Survival analysis performed using both Cox proportional hazard models, adjusted for many different prognostic factors, and Kaplan-Meier curves (two groups according median value) demonstrated a significantly lower risk for death/tracheostomy for patients with high serum chloride (HR 0.982 95%CI 0.969-0.995, $p=0.007$; log rank test $p<0.001$). Stratifying patients according to NIV usage, baseline low serum chloride was associated with a shorter time-to-ventilation (median 11.5 months, 4.0-19.0 vs. 14.0 months, 8.0-26.0).

Conclusions: Serum chloride can be used as a low cost screening test at diagnosis and during follow-up to monitor respiratory dysfunction in ALS patients.

(h148) Simulating motor neuron degeneration and reinnervation in motor neuron disease based on surface-electromyography recorded single motor unit potentials

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Introduction: Surface-electromyography (EMG) methods provide relevant insights on the disease pathology of motor neuron diseases (MND), which has been suggested to underlie two prominent mechanisms with motor neuron death as the primary process. The secondary process involves reinnervation, where denervated muscle fibers become reinnervated by still functioning nerve fibers. As a result, muscle strength may for a certain time be relatively maintained. The interaction between the two mechanisms may vary greatly between patients. By simulating their interaction, we aim to provide insights into their impact on the sensitivity of surface-EMG methods to monitor disease progression in MND.

Objectives: To develop a muscle model to simulate progressive motor neuron loss and enlarged motor units (MUs) due to collateral reinnervation.

Methods: The basic building block for the model consisted of a large dataset of high-density surface-EMG recorded MU potentials (MUPs) from which normal sized MU pools were randomly selected. Progressive MU loss was simulated by removing the MUPs one-by-one at random. The removed MUPs underwent partial to complete reinnervation by neighbouring MUPs depending on the overlap in their topographical fingerprints. The model was subsequently tailored to generate compound muscle action potential (CMAP) scans, which is a promising neurophysiological method to quantify disease progression in muscles affected by MND. This allowed us to compare simulated and recorded CMAP scans in healthy controls and patients with MND.

Results: Simulated baseline maximum CMAP showed values up to 12 mV. During progressive MU loss and reinnervation, the CMAP scan pattern showed a clear transition from a smooth sigmoidal towards a more discrete stepwise pattern, which matched well with experimental observations. Reinnervation was successfully reflected by increases in MU size resulting in enlarged MUs up to 2 mV when only a few MUs are left, which are sizes also occasionally observed during experiments.

Conclusions: The muscle model was able to capture on average the pathological MU characteristics observed in patients with MND and healthy controls. Further refinements are possible towards more patient-specific patterns over time. The model may potentially be used as surrogate reference to compare motor unit number estimate (MUNE) methods, which may also aid in designing more sensitive biomarkers for monitoring disease progression of MND.

(h149) Characterizing Hospitalization as an Outcome Measure in ALS Clinical Trials

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Background: Composite endpoints that include time to 1st hospitalization and other clinical outcomes have been described in recent ALS trials to evaluate potential long-term delays in disease progression.

Objective: To establish a method to characterize and analyze risk of hospitalizations in COURAGE-ALS, a phase 3 trial of reldesemtiv in ALS.

Methods: Non-fatal serious adverse event narratives associated with hospitalizations in a 56-week phase 3 trial of tirasemtiv (VITALITY-ALS [CY 4031]) and a 16-week phase 2 trial of reldesemtiv (FORTITUDE-ALS [CY 5022]) were reviewed to identify reasons leading to hospitalization. They were classified as related to underlying ALS (RU-ALS), related to ALS progression (RP-ALS), unrelated to ALS (U-ALS) or indeterminate (IN). A survey of 13 ALS experts was conducted to determine the degree of agreement

in defining terminology and classifying events. The survey described clinical scenarios of gastrostomy tube placement, traumatic injuries, respiratory, thromboembolic, urologic, and neuropsychiatric events; respondents classified the hospitalization as RU-ALS, RP-ALS, U-ALS or IN.

Results: In CY 5022, 1st hospitalization occurred in 27/457 (6%) patients and in 67/566 (12%) in CY 4031; the difference is likely due to study duration. The most common reason for the hospitalization was U-ALS in CY 5022 (10/27, 38%) and RP-ALS in CY 4031 (26/67, 39%). Respiratory events were the most common cause of hospitalization in both trials. While there was 77-100% agreement with proposed classification definitions, no clinical scenarios were classified in the same manner by all. Agreement in classification ranged from 92.3% (example: non-ambulant patient with DVT and PE) to 46.5% (example: patient with dyspnea). After review of diverse results classifying clinical scenarios, the group concluded that simply distinguishing related from unrelated was preferred. Challenges to appropriate identification included variable resources at home, advance care planning, and geographic differences in criteria for hospitalizations.

Conclusions: A fair number of hospitalizations in our analyses were U-ALS, and classification of clinical scenarios by ALS experts was not always consistent despite high agreement for defining RU-ALS, RP-ALS, and U-ALS. Given these results, a consistent and simplified approach may lead to more clinically meaningful and rigorous analyses for patients, clinicians, and payers.

(h150) Utility of the spirometry, arterial blood gas analysis and nocturnal oximetry in the respiratory outcome of motor neuron disease

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Motor neuron disease (MND) encompasses a heterogeneous group of neurologic disorders affecting the upper and lower motor neurons. Since respiratory failure represents the main cause of death in MND patients and to assess the need for non-invasive mechanical ventilation adaption, a careful monitoring of respiratory function with spirometry test, arterial blood gas (ABG) analysis and nocturnal oximetry (NO) is highly suggested. The aim of the present study is to evaluate the prognostic role of NO and ABG measurements in addition to the forced vital capacity (FVC) in a large cohort of MND patients.

We retrospectively analyzed respiratory data of 472 MND patients referred to our department (2001-2019), collecting for each patient, when available, an FVC and ABG and NO measurements performed during the same evaluation. We applied two-tailed unpaired Mann–Whitney U test and Kruskal–Wallis test with Bonferroni post hoc comparison to verify differences among two or more groups, respectively. Survival curves were estimated by the Kaplan-Meier analysis and compared using the log rank test, while univariate Cox-regression was carried out to derive unadjusted hazard ratios (HRs) for death/tracheostomy of each measurement. Cox multivariate analysis was subsequently carried out to evaluate the role of each measurement as independent factors on survival.

The overall study population included 450 MND patients. Tests comparing median values between FVC and ABG and NO measurements showed a significant drop in FVC when an increase of pCO2 , HCO3-, standard base excess (SBE), percentage of recording time spent with oxygen saturation at <90% (T-90%) or a reduction in pO2, mean percentage saturation (MPS) and average minimum saturations (AMS) occurred. Tests comparing median values between ABG and NO showed that pCO2, HCO3- and SBE were significantly higher when an increase in T90% or a reduction in MPS and AMS occurred. Kaplan-Meier survival analysis with Cox univariate analysis, demonstrated significant risk stratification for patients showing a reduction in FVC for each interquartile range, and when pCO2, HCO3-, SBE increased over a specific cut-off as when pO2 and MPS decreased below specific cut-off. Cox multivariate analysis demonstrated an independent effect on survival of FVC%, MPS and ABG parameters.

These results might help the neurologist to predict prognosis, stratify patients in clinical trials and might facilitate the respiratory management of these patients.

(h151) Repeated training improves administration of the ALSFRS-R

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Background: The most frequently used primary outcome measures in current ALS trials is the ALSFRS-R. Reliable and accurate performance of this instrument is thus obviously critical. The Barrow Neurological Institute Clinical Research Organization (BNI-CR) has been performing evaluator training and certification for the ALSFRS-R since 2011. We wished to determine whether we could observe an effect of training on evaluator performance using standard patient vignettes.

Methods: We reviewed training records and identified evaluators who had been trained and certified by the BNI-CRO at least twice since 2011. Certification of successful completion of training is documented by an evaluator scoring the ALSFRS-R on patient vignettes, in which adequate performance is determined by a total score of no more than 2 points different than an expert panel rating, with a difference of no more than 1 point on any individual item also required. Successful performance on 2 vignettes is required for certification. To determine whether prior certification impacted subsequent performance, we assessed the number of vignettes required to be scored in order to generate 2 successful attempts. The extent to which increasing time intervals between successive trainings impacted successful performance was determined; we also assessed whether mode of training impacted successful vignetted scoring.

Results: 208 evaluators completed training either via an interactive in person training, an interactive remote training, or by completing a self training module. 117 evaluators completed at least 2 training and certification sessions. The interval between trainings impacted the number of vignettes required for certification during the second training, with poorer performance noted when the interval between trainings was 2 years or greater. Mode of training also impacted performance; interactive in-person and remote sessions were associated with better performance than use of self training modules.

Conclusions: Standard training of evaluators has an impact on performance of the ALSFRS-R, with shorter intervals between training positively impacting performance. Interactive training sessions allowing for real time questions also are associated with better performance. Continued training is important to maintain high quality of ALSFRS-R assessment.

(h152) Baseline Speech Assessment and Vital Capacity Measured Remotely and in Clinic in the HEALEY ALS Platform Trial

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Background: The HEALEY ALS Platform Trial is an innovative effort to streamline ALS clinical trials by reducing placebo treatment assignment and maintaining an active network of sites studying continually enrolling participants into treatment regimens. Participation includes 24 weeks of placebo-controlled treatment. The first 4 regimens have completed enrollment. Standard ALS outcomes are assessed as well as exploratory outcomes, including coached home spirometry (ZephyRx app) and quantitative voice characterization using a mobile app (Aural Analytics). Here we describe baseline characteristics of these exploratory measures of vital capacity and their ability to predict in-clinic vital capacity assessments.

Results: As of 31 Dec 2021, 870 participants with ALS were consented and screened; 653 were randomized within the first 4 regimens. Mean age at baseline was 59 years; 241 were female, 111 had bulbar onset, and mean ALSFRS-R was 35. Mean baseline vital capacity (VC) as measured at the study sites and by home testing was 77%-predicted; the measures were well correlated (Pearson r=0.86 for volume and r=0.73 for %-predicted). Home VC ≥65%-predicted was a good predictor of in-clinic VC meeting the same threshold (positive predictive value = 88%, negative predictive value = 71). An automated feature of the home VC device determined whether testing characteristics met ATS standards for acceptability; this was the case in only 71% of coached sessions. Home VC was on average lower than the in-clinic value when not meeting ATS acceptability standards among participants with bulbar onset (by 4.9%-predicted). The automated speech analysis produced estimates of 9 speech attributes: maximum phonation time, pause rate, breathy vocal quality, pitch instability, regulation of voicing, articulatory precision, speaking rate, articulation rate, and monotonicity. Duration of sustained phonation was combined with demographic variables to predict VC.

Discussion: The HEALEY ALS Platform study is an ongoing effort to improve the care of ALS patients through drug discovery. A related goal is to discover new outcome measures that can increase the efficiency of ALS trials. Baseline data presented here show that home vital capacity assessment is accurate; use of a sustained phonation task will be presented.

Acknowledgements: The HEALEY ALS Platform Trial is supported by The Sean M. Healey and AMG Center for ALS and partially supported by Tackle ALS, The ALS Association

(h153) Autonomy and self-determination. Are we respecting them in our patients?

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Introduction: The importance of respecting the dignity and autonomy of patients have been described in literature. Self-determination refers to the possibility of making decisions, about ourselves and our future and respecting our values. Medical advance directives are increasingly recognized as instruments for increasing patient self-determination and dignity.

Objective: To describe advance directives fulfilled documents in a cohort of ALS patients

Methods: We reviewed the advanced directives of patients visited by the psychologist of the ALS Unit of Hospital la Fe between 2017 and 2022.

Results: 105 ALS patients fulfilled the advanced directives document during this period and 38 patients were currently working on them. From January 2018 until March 2022, 68 patients, who had a routine follow-up in the ALS Unit of Hospital la Fe, died. 52 of them (76.5%) had fulfilled the advanced directives document. Most of the decisions made in the document were maintained throughout the disease course. The most frequent reasons for not completing the advanced directives were dementia and rapid or sudden death.

Conclusions: In this descriptive study, 76.5% of patients with a routine follow-up in an ALS Unit fulfilled the advanced directives document. The advanced directives helps the multidisciplinary team to plan a better care, while reinforces patient's autonomy, dignity and self-determination.

(h154) Prognostic Modelling of Motor Neuron Disease in Scotland

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Background: Predicting outcomes in MND is limited by phenotypic and prognostic heterogeneity. Population-wide recruitment using multiple sources of data, and accounting for missing data, is considered the optimal approach. Clinical Audit Research and Evaluation of MND (CARE-MND) is a platform for prospective data collection of demographics and health-related variables of people with MND (pwMND) in Scotland. We aimed to use these data to identify predictors of disease prognosis.

Methods: A hypothesis-free data-led approach was taken, whereby a comprehensive breadth of variables were included to identify predictors. Variables with zero variance and highly correlated variables were excluded. Missing data were imputed using Multiple Imputation by Chained Equations (MICE). Sensitivity analyses were performed to select optimum imputation level. Cox regression modelling was used to assess the effect of multiple variables on survival.

Results: Of 619 pwMND diagnosed in Scotland in 2015-2017, 437 (70.6%) consented to share their data. Mean follow-up time was 23 months. 24 predictors were included in the model. Imputation of all missing data resulted in the best model with a pseudo-R2 of 0.538 (95% CI 0.531-0.545). Increasing Age of Onset (p=0.012), Family History of MND (p=0.002) and ALSFRS-R Preslope (p=0.001) predicted death. Increasing Time to Diagnosis (p<0.0001), Classification: Other (including PLS, PMA or PBP) (p=0.002) and a history of Ever Smoking (p=0.001) predicted survival. Exposure to Heavy Metals or Pesticides was associated with poorer survival (p=0.042). Interventions such as riluzole, gastrostomy and NIV did not influence survival when controlled for other variables.

Conclusions: Collectively, these observations suggest that rapid, widespread manifestation of upper and lower motor neurone degeneration in older adults results in faster time to death. This has been observed previously but our study shows that these factors predict survival independent of other medical and environmental influences. The influence of smoking requires replication. Exposure to Heavy Metals or Pesticides was an amalgamation of two different observations of toxin exposure and it is not possible to draw conclusions about its impact on survival. Further study of these variables in Scotland using case-control methods is merited.

(h155) Phenotypic Characterisation and Genetic Epidemiology of Motor Neuron Disease in Scotland

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Background: Scotland benefits from a culture of prospective, longitudinal monitoring of MND. The Clinical Audit Research and Evaluation of MND (CARE-MND) database captures 99% of people with MND (pwMND) in Scotland. Using this platform and the established Scottish Regenerative Neurology Tissue Bank, we aimed to described the phenotypic characteristics and genetic epidemiology of an incident cohort diagnosed 2015-2017.

Methods: Key phenotypic markers were identified from the CARE-MND platform. Sequence analysis of a panel of 48 genes causally associated with MND was carried out using a custom-designed QIAseq assay. Variants were classified using the American College of Medical Genetics and Association for Molecular Pathology (ACMG-AMP) framework, adhering to Association for Clinical Genomic Science (ACGS) UK guidelines. Samples were also tested for C9orf72 hexanucleotide expansions using repeat prime PCR methodology.

Results: Of 619 pwMND diagnosed 2015-2017, 437 (71%) consented to share their data. Male-to-female ratio was 1.7:1. Mean age of onset was 64 years and median time to diagnosis 12 months. 9% had a family history of MND; 4% a family of early-onset or frontotemporal dementia. Lower limb was the most common site of disease onset (33%) and ALS the most typical manifestation (77%). Cognitive impairment was present in 39% of patients. 338 (55%) donated a DNA sample. Applying strict ACMG-AMP criteria, 46 (14%) had a pathogenic or likely pathogenic mutation. Of these, 29 (9%) of individuals had a C9orf72 repeat expansion. Having a C9orf72 expansion was associated with a lower Edinburgh Cognitive and Behavioural ALS Screen ALS-Specific score (p=0.0005). 9 (3%) of pwMND had the Scottish p.Ile114Thr mutation with a predominant limb-onset ALS phenotype. Rare gene mutations included those in FUS and NEK1. 1 individual carried both a C9orf72 expansion and the SOD1 p.Ile114Thr variant.

Conclusions: The CARE-MND database provides an accurate summary of MND demographics which guides service provision. Structured variant classification using available guidelines gives a realistic estimate of the proportion of gene carriers in the MND population, with an expected number of C9orf72 and SOD1 carriers and a low prevalence of rare variants. C9orf72 status correlates with ALS-Specific ECAS score. Scotland is enriched for the SOD1 p.Ile114Thr mutation and further modelling of this variant would be of benefit to our population.

(h156) Coexistence of CASQ1-related myopathy and Amyotrophic Lateral Sclerosis in an Italian patient: a case description

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder involving upper and lower motor neurons. It may start with isolated cramps and fasciculation, leading sometimes to misdiagnosis with myopathies. CASQ1-related myopathies are a spectrum of vacuolar or tubular aggregate myopathies related to mutation in the CASQ1 gene, coding for the main buffer calcium Calsequestrin, and clinically characterized by neuromuscular hyperexcitability. According to dying back hypothesis, muscular affections may lead to motor neuron diseases. CASQ1 mutations seem not to affect motor neurons, though the common occurrence of brisk reflexes in these myopathies have raised the hypothesis of a possible involvement of pyramidal tracts. Still, coexistence of ALS and myopathies is extremely rare; here, we describe a unique case of ALS in a patient with CASQ1 mutation. A 55-year-old female came to our attention at Neuromuscular Unit in Santa Chiara Hospital, Pisa, Italy for a history of severe cramps and fasciculations started two years prior. Her father and her aunt in paternal line also reported a long history of cramps and fasciculation, without any movement impairment. Her first neurological examination revealed only diffuse and severe limbs' fasciculation and cramps. An electromyogram showed myopathic pattern in all examined muscles. CK were slightly increased. Due to signs of neuromuscular hyperexcitability and her family history, a NGS panel for channelopathies was performed and revealed a heterozygous mutation in CASQ1 predicted to be pathogenetic for tubular aggregate myopathy. Three months after the first evaluation, she developed a progressive hyposthenia in right foot dorsiflexion. The neurological examination revealed subtle hypotrophy of first interosseus of the right hand, hyposthenia in right tibialis anterior and positivity to Hoffmann's sign. The electromyogram this time showed neurogenic pattern with acute denervation and a brain MRI showed bilateral hyperintensity of the corticospinal tract, in a picture consistent for ALS. We hypothesize that, since deregulation of Ca2+ homeostasis due to CASQ1 mutation may cause an increased amount of calcium in mitochondria leading to muscle damage, a possible backward spread of damage to motor neuron may have happened. Alternatively, a coexistence of CASQ1 myopathy and ALS may be assumed. These observations support the need of further studies to profile genetic mutations and their pathogenic role

(h157) Capillaroscopic alterations in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that predominantly affects upper and lower motor neurons. We have sufficient evidence of vascular involvement in ALS. The capillaroscopy is a non-invasive technique that visualizes the capillaries of the nail bed using a light source and a magnifying optical system.T he aim of this study was to evaluate the possible capillary abnormalities in ALS patients.

Methodology: we include 34 ALS patients according to the El Escorial ALS criteria and 27 healthy controls. Those patients with rheumatic diseases that could alter the result of capillaroscopy were excluded. Additionally, We exclude two patients and one healthy control due to bad technique visualization and one patient due to a capillaroscopy Raynaud's pattern and two healthy controls due to an active sclerodermia pattern. We used videocapillaroscopy equipment. We save the images on the computer for the posterior analysis. The parameters of capillary density, avascular zones, capillary diameter, morphology (tortuosity and ramifications), hemorrhage, thrombosis and visibility of the venous plexus were evaluated.

Results: 31 patients (19 men and 12 women) and 24 healthy controls (8 men and 16 women) were analyzed. The mean age (years) of the patients was 60.55 (SD 10.01) and of the controls 55.21 (SD 6.27) (p=0.19). The median of the patient's evolution was 32.85 months [17.92; 60.05]. 25 patients had spinal phenotype and 6 bulbar. 6 patients and 3 controls had dilated capillaries (p>0.05). We did not observe avascular zones in the participants. 48% of patients and 58% of controls had mild capillary tortuosities, 19% of patients and 29% of controls had moderate capillary tortuosities, 32% of patients and 4% of controls had severe capillary tortuosities, 8% of controls had no tortuosities. 51% of patients and 12% of controls had capillary branches, p=0.02. Three patients presented significant hemorrhages that did not present any healthy control. No thrombosis was evident in any participant and the venous plexus was visible in almost all subjects. The parameters of capillary density and capillary diameter didn't show any significant differences between cases and controls.

Conclusion: Our data suggest that patients with ALS present more capillary tortuosities, more branches of these capillaries as well as significant hemorrhages. The presence of hemorrhages suggests endothelial damage and branches are indicative of angiogenesis.

(h158)Altered Angiopoietin like proteins correlate with lipid metabolism in ALS

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Dysregulated energy metabolism is an emerging phenotype of Amyotrophic lateral sclerosis (ALS). With more than half of ALS patients exhibiting hypermetabolism, identifying new players that can prolong survival is imminent. Angiopoietin-like proteins (Angptls) 3, 4 and 8 inhibit Lipoprotein lipase (LPL), the rate limiting enzyme catalysing the transfer of Triglyceride rich lipids into free fatty acids. Using enzyme linked immunosorbent assays, Angptl3, 4 and 8 were measured in the serum of ALS patients (sporadic, SOD1 and C9orf72 mutations) with corresponding age and gender matched clinical controls. Angptl3 and 4 was found decreased in patients with SOD1 and C9orf72 mutations, compared to sporadic cases and controls. Interestingly, sporadic females had higher Angptl3 than males, which is also independent of age or body mass index. Angptl8 remained fairly unchanged between the groups. Proprotein convertase subtilisin/kexin type 9 (PCSK9) that regulates cholesterol metabolism was additionally measured, with decreased levels in C9orf72 mutated patients. Measuring the activity of LPL in these samples, reaffirmed the inverse correlation between LPL and the Angptl3-4-8 triad. Our results provide the first insight into Angiopoietin-like proteins as mechanistically involved in lipid disturbances observed in ALS patients. With recent successes in pharmacologically targeting Angptl3 for cardiovascular disorders, our study is a point of entry to investigate Angptls in ALS metabolism.

(h159) Lived Experience of Persons with Amyotrophic Lateral Sclerosis Who Are Participating in a Clinical Trial

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With only two therapies currently approved for the treatment of ALS, clinical trials are crucial for the development of new drugs. Patient participation in clinical trials is vital to progress the development of potential therapies, and being sensitive to patient needs and preferences can allow for enhanced trial design and delivery. Patient-centred approaches to study designs can accelerate drug development processes by understanding and overcoming potential barriers, such as recruitment and retention. Despite the importance of patient-centred designs, there is a dearth of research assessing the lived experiences of clinical trial participation of persons with ALS (PwALS). As such, the objective of the present study is to evaluate PwALS' experience in clinical trials. Using a grounded theory approach, semi-structured interviews were conducted with PwALS participating in a clinical trial at a Canadian research unit. Preliminary analyses suggest the patient experience is encompassed by a variety of positive factors for their care, and motivations such as helping (oneself, future generations, research, etc.), however, barriers such as uncertainty and study procedures remain important factors. Based on these findings, we hope to build a framework to serve as a basis for a larger scale quantitative investigation on standardized measures of patient trial experience that provide insight in implementing patient-centred trial designs that will expand patient involvement.

(h160) Bringing health care closer to patients – two years of the home care ALS programme in Slovenia

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Ljubljana ALS Centre takes care of the majority of patients with ALS in Slovenia. Due to limited access to health care during COVID-19 pandemic, we have started a home care ALS programme to improve care of patients with ALS. We developed eligibility criteria to decide which patients could benefit from home visits. The criteria included problematic transport due to patient immobility, mechanical ventilation use, gastrostomy-related problems and need for advanced directives discussion. During March 2020 and March 2022, we performed 190 home visits in 67 patients. This represents 20% of all ALS out-patient visits and 30% of the patients treated at our centre during this period. The patients were visited on average every 3.3 months (range between visits 3 days – 15 months). Only 4% of the visits were done due to a sudden unexpected clinical deterioration. The neurologist that performed home visits was usually accompanied by one or two members of our multidisciplinary team (mostly by respiratory therapist and sometimes by nurse, social worker or team coordinator). 58% of the patients visited were using gastrostomy, 72% were using non-invasive ventilation (NIV) and 9% were using invasive ventilation (IV). The main procedures / tasks performed at home visits were: arterial blood gasses analysis (in 72% of all visits), assessment of NIV (in 60%), adjustment of symptomatic therapy (in 41%), advance directive regarding mechanical ventilation (in 28%), prescription of existing therapy (in 13%), discussion on possible gastrostomy (in 13%), gastrostomy assessment (in 10%), gastrostomy care (in 10%), replacement of gastric tube (in 5%), assessment of IV (in 5%), botulinum toxin application (in 5%), decision to withhold treatment (in 4%), introduction of NIV (in 2%), introduction of cough assist (in 2%), discussion on treatment withdrawal (in 1%).The home care ALS programme provides an improved health care for patients with ALS, especially for those in advance stages of the disease. Many of these patients would probably not be able to attend regular out-patient visits at the hospital. The multidisciplinary programme integrates different aspects of ALS care, the most important being home ventilation and palliative care. Based on our experience, it can be cost and time effective with appropriate planning. The programme has recently received long-term funding by the Health Insurance Institute of Slovenia as a part of mobile palliative teams initiative.

(h161) A systematic review of wearable technologies for evaluating disease progression in motor neuron disease.

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Background: The current gold-standard measure of disease progression is the ALS Functional Rating Scale – Revised (ALS-FRS(R)), a clinician-administered questionnaire providing a composite score on physical functioning. The sensitivity and objectivity of the ALS-FRS(R) is under scrutiny. Digital health technologies and wearable devices, such as accelerometers, gait sensors and smart phone apps, offer potential alternatives for assessing aspects of motor progression in both clinical practice and trials.

Objective: The objective of this project is to evaluate previous studies reporting use of wearable devices in people with MND (pwMND), the properties of these devices and their suitability to measure motor progression in research and clinical care.

Method: We investigated studies evaluating the utility and suitability of wearable sensors and accelerometers to evaluate disease progression in pwMND. We systematically searched Google Scholar, PubMed, and EMBASE applying no language or date restrictions. We extracted information on devices used and additional assessments and also assessed the quality of individual studies.

Results: 14 studies, including 462 pwMND, were included (median n=24) . Sensor type included accelerometers (n=9), activity monitors (n=5), smartphones (n=25), gait (n=3) or Microsoft Kinect sensors (n= 2). Length of follow-up varied from single time-point to 36 months, with a median of nine. 10 (71%) of studies used the ALS-FRS(R) to evaluate concurrent validity. Participant feedback on sensor utility was evaluated in six studies (43%) was generally positive. All studies showed initial feasibility, warranting larger longitudinal studies to establish suitability. Risk of bias in the included studies was high, likely due to a large amount of information to determine study quality being unclear.

Conclusion: Measurement of physical symptom progression using wearables technologies is an emerging, and promising, area of MND research which has shown to be beneficial in other neurological conditions. Prospective studies, with larger sample sizes, longer follow-up durations and user feedback are required to evaluate the utility of devices and establish which devices are most suitable in MND. The development of strategic guidelines would be beneficial to harmonise approaches and inform future study design and clinical care integration.

(h162) Genetic-environment interactions to discover novel risk factor in ALS: a proof-of-concept application in a population-based register

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Background: Amyotrophic Lateral Sclerosis (ALS) etiology cannot be entirely explained by our current knowledge of genetic or environmental factors alone. Indeed, most ALS cases are likely due to different combinations of environmental exposures and genetic susceptibility.

Objective: To apply a candidate gene-environment interaction methods to identify environmental exposures that may be associated with ALS etiology for future study.

Methods: We extracted residence at diagnosis of □1000 ALS patients from a large population-based registry, the Piemonte and Valle d'Aosta Register for ALS (PARALS), who underwent whole-genome sequencing. We geospatially estimated local residential exposure to several environmental factors based on region-level data from the Agenzia Regionale per la Protezione Ambientale (ARPA). Patients were divided based on the status of four SNPs in PON1 gene (rs662, rs705379, rs705381, rs854560, rs854571, rs854572 e rs854573) that modify paraoxonase function and individual susceptibility to toxic agents. Interaction analysis was performed to detect geographical cluster of neurotoxicity-prone ALS cases. The candidate environmental sources were then screened to identify the toxicants that could underlie those gene-environment interactions.

Results: Top results in our analysis provided suggestive evidence that ALS rs705379 carriers are more exposed to surface water (p=0.0030) and gardens (p=0.0207). Both rs854572 and rs622 carriers also seem to live in close proximity to surface water (p=0.0031 and p=0.0049 respectively). Conversely, ALS carriers of PON1 rs854560 are closer to paddy fields (p=0.0176). Our nominates diaxozon, an herbicide which is metabolized by the paraoxonase enzyme and is the most frequent pesticide detected in Piedmont's surface waters, as a candidate agent responsible for this gene-environment interactions.

Significance: First, our study is a proof of concept that analyzing genes and environment on the whole-genome scale is feasible, and this approach can identify important environmental risk factors that could otherwise be missed. Our geospatial analysis integrated with genomic data support potential neurotoxic pesticide exposures as risk factors for sporadic ALS and provide suggestive evidence of novel gene-environment interaction effects that have biological plausibility for ALS risk. Further investigation of the role of toxicants in ALS in large and carefully studied samples is warranted.

(h163) ALS incidence by geographical area in England

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Background: The incidence of ALS varies by area in regions of the UK, and has previously been correlated with population density. However it is not known whether these trends extend to other geographical areas in the UK. In this research we investigate incidence by region using data from the MND Register for England Wales and Northern Ireland.

Methods: We extracted incident cases from the MND Register between 2017 and 2020 that covered a population base of 13.5 million people located in 28 postcode areas (each with populations between 200,000 and 1.1mn people) in England. Crude age- and sex-specific incidence rates in 28 postcode areas were estimated using population census records for the relevant postcodes from Office of National Statistics census data. These rates were standardised to the UK population structure using direct standardisation. Incidence rates were correlated to population density of the base population.

Results: There were 504 incidence cases available for analysis. Incidence varied between 1.6/100,000 person years (95% CI 0.19-5.9) and 9.7/100,000 (95% CI 5.1-16.7) person years. Correlation analysis showed that this is not related to population density (R2 = 0.04).

Conclusion: ALS incidence varies by geographical location in an area in England. More work on characterising the areas and the services available in each area should be performed.

(h164) Identification of ALS slow progressors through the Emilia Romagna regional registry: a possible target population for biomarker studies

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Background: Biomarkers are advocated for the implementation of clinical search in ALS[1], however most biological studies are limited by scarce clinical correlations; slow and fast progressors are often identified through arbitrary cutoffs extrapolated from population studies outside the cohort of interest[2]. Objective: To identify a posteriori, through Emilia Romagna Region ALS registry, a cluster of patients who can be referred as slow progressors(SP) and could be object of investigation through biomarker studies; to describe SP and determine if key clinical differences exist with other groups of progressors.

Methods: From 1489 incident ALS cases (1/1/2009-29/12/2020),1394 were left for analysis. Cox regression was used to determine clinical factors affecting survival and time to reach important disease stages. Latent class analysis was performed until a final model was achieved including disease progression rate at 12 months, time to reach MiTos stage 1 and King's stage 3, and diagnostic latency as dependent variables; sex, genotype, phenotype, age, and site of onset were the independent variables. After postestimation of individuals membership to each latent class, categories of different disease progressors were analyzed through descriptive statistics and multinomial logistic regression(MLR). Logrank analysis was performed to validate model prediction of class membership[3].

Results: Only 8.38% of patients could be defined as true SP; they presented with UMN-predominant and classic phenotype more commonly than intermediate progressors(IP) [RRR:44.7(CI:12.74-156) and 3.48(CI:1.86-6.5),respectively]. Hypercholesterolemia and hypertension remained in the MLR model as explanatory variables of slow progressors membership. Importantly, baseline ALSFRS-r scores, BMI and FVC could not predict group membership. Time to reach each disease stage was significantly longer in this subgroup of patients, especially when looking at King's stage 3 [p-value<0.0001; median time interval in days: 1255 (IQR:992-1562),vs 352(IQR:233-480) for FP and 463(IQR:296-549) for IP].

Discussion: True SP in ALS are a minority, and baseline clinical features are not always good predictors of this class membership. Coupling the identification of this subgroup of patients in population registries with a network of high-standard biobanking systems will open new avenues for translational research in ALS. Extension of this model to other registries is necessary to validate these findings

(h165) A systematic review of digital technology to evaluate speech dysfunction in amyotrophic lateral sclerosis.

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Background: Up to 30% of people with ALS are affected by bulbar symptoms such as impaired speech and swallow, with voice changes often predating motor dysfunction. Communication difficulties significantly reduce quality of life. The current gold-standard measure of disease progression, the ALS Functional Rating Scale Revised (ALS-FRS(R)), only has a single item on speech intelligibility. Digital speech assessment offers the opportunity for detailed, objective, analysis of a number of voice parameters that may indicate disease progression.

Aim: To systematically review studies evaluating digitally-enabled speech assessment in people with ALS (pwALS).

Method: We studied articles evaluating the use of novel assessment devices to evaluate speech as a marker of disease progression in pwALS. We systematically searched Google Scholar, PubMed, Medline and EMBASE. No language or date restrictions were applied. We investigated the sensitivity, specificity and discriminatory capacity of technologies identified in a narrative synthesis.

Results: 31 studies were included in our study which included apps and smartphones (n = 10), sensors in the vocal system (n = 7), digital microphones (n = 9), and in-built computer recording systems (n = 4) to collect data on voice and the speech system. Evaluated features of speech included: jitter and shimmer (n = 14), fundamental frequency (n = 20) and pause durations (n = 9). The degree of vocal impairment was analysed in ALS sub-groups, compared to other neurological conditions (n = 5) and healthy controls (n = 17).

Conclusion: A range of technologies for measurement of speech and vocal systems have been tested in small numbers of pwALS. These have been reported to help identify and compare more nuanced changes associated with voice dysfunction and offer objective and sensitive evaluation compared to historical questionnaire-based assessments. Further longitudinal studies involving larger samples are required to validate use of these technologies as diagnostic tools or prognostic biomarkers.

(h166) Use and subjective experience of the impact of a motor-assisted movement exerciser in people with ALS: a multicenter observational study

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Background: Motor-assisted movement exercisers (MME) enable device-assisted physical therapy delivered in domestic settings for people living with ALS (PALS). This observational study captures the frequency of MME use, subjective experience of the therapy provided, and MME recommendation made by PALS.

Methods: The prospective cohort study was implemented at ten ALS centers (02/2019-10/2020) in Germany and coordinated by the APST case management and research network (www.ambulanzpartner.de). Participants assessed symptom severity, frequency of use and subjective benefit of MME-assisted therapy on a numerical rating scale (NRS, 0 to 10 points). The likelihood of recommending the MME by PALS was determined by the Net Promotor Score (NPS).

Results: Data for 144 participants were analyzed. Weekly MME applications ranged from 1 to 4 in 41%, 5 to 7 in 42% and >7 in 17% of participants. Particularly positive results were achieved in the following domains: amplification of a sense of achievement (67%), diminution of the feeling of having rigid limbs (63%), diminution of the feeling of being immobile (61%), improvement of general wellbeing (55%) and

reduction of muscle stiffness (52%). Participants with more pronounced self-rated muscle weakness were more likely to note a beneficial effect on preservation and improvement of muscle strength under MME treatment ($p<0.05$). The NPS for the MME was high (+61).

Conclusion: High-frequency MME-assisted treatment (defined as a minimum of five sessions a week) was administered in the majority of participants in addition to physical therapy. Most participants stated to have achieved their individual objectives of MME intervention, as evidenced by a high level of satisfaction. The results underline the justification for early and extended MME treatment as part of the ALS care concept.

(h167) User expectation and user experience of a robotic arm in people with ALS: a multicenter observational study

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Background: Robotic arms compensating for physical limitations, as experienced by people living with ALS (PALS), have recently been introduced in the field of medical assistive devices. These devices are designed to substitute for severely impaired hands and arms, particularly with regard to fine motor skills and grabbing. The provision of robotic arms is an essential part of managed care for PALS experiencing these limitations. The aim was to analyze user expectation and experience among participants who were expected to or did receive a robotic arm.

Methods: A prospective cohort study was conducted within the APST case management and research network, deployed in 13 ALS centers in Germany (www.ambulanzpartner.de). The investigation captured the user expectation of PALS with an indication for the robotic arm and subsequently the user experience following the provision with the device.

Results: Over a period of 24 months, 158 individuals with an indication for robotic arm provision were identified in the network. 54% (n=85) were included in the survey. In 38% (n=32), the robotic arm was delivered. Fourteen participants (44%) offered information on their experience with the device.

Assessment of user expectation revealed that all participants considered the possibility to use a robotic arm to be significant to them. The majority (>80%) intended to use the robotic arm for handling objects,

personal care, pressing buttons, handing drinks, and opening closets and doors. User experience indicated the application of the arm as follows: handling objects (79%), serving drinks (79%), personal care (71%), pressing buttons (71%), handling food (64%), and opening doors (64%). All 14 users evaluated the benefit, safety, and satisfaction favorably.

Conclusions: The need for a robotic arm was high among PALS. Participants' expectations of such device revealed potentially significant impact on daily activities. Experience with the robotic arm largely confirmed this perception and showed a high level of satisfaction with the device. Thus, increasing availability and awareness of robotic assistance can improve the quality of life and promotes independence of people living with ALS.

(h168) Socioeconomic status and ALS

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Purpose: Socioeconomic status (SES) is defined as a measure of one's shared economic and social status and tends to be positively associated with better health. Socioeconomic status is of importance. It is a determining factor that can influence and be influenced by different variables. Socioeconomic impact on various health conditions has been widely investigated and nowadays, it is an established criterion with impact on disease occurrence and progression. SES studies focus on three common measures: education, income, and occupation. If ALS risk or progression varies by SES, this would imply that ALS risk is affected by factors usually linked with SES.

Materials and methods: We investigated 300 participants, both cases and controls. People diagnosed with definite, probable, or possible ALS according to the El Escorial criteria between 2008 and 2013 were recruited from three tertiary centers in London, Sheffield, and Birmingham. Using comparative statistics, we examined the male-female ratio, site of onset, and age of onset. We analysed age of onset, diagnostic delay, and survival for association with SES.

Findings: There was an inverse relationship between age of onset and salary (B:0.35 P value: 0.595) but no effect of education or occupation. There was no effect of any SES variable on diagnostic delay or survival.

Limitations: The number of cases and controls limits statistical power.

Conclusions: Income influences age of onset and diagnostic delay in ALS independent of other factors.

(h169) Timing of NIV initiation in patients with ALS based on forced vital capacity: a retrospective propensity score matched analysis.

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Introduction: Non-invasive ventilation (NIV) is well-known to improve survival of patients with amyotrophic lateral sclerosis (ALS). Optimal timing of NIV initiation remains unclear. Research in this domain is difficult due to low prevalence of ALS combined with clinical heterogeneity within this population and the use of different measures to evaluate respiratory dysfunction, leaving guidelines with the heavy task of creating clarity on this matter. To determine optimal timing, we explored whether early NIV initiation influences the positive effect of NIV on survival.

Methods: This retrospective study used data from 1238 patients diagnosed at the referral ALS center from the Leuven University Hospitals. Patients who remained in follow-up throughout their disease course were included. We performed a Cox regression analysis using a 1:1 propensity score matching based on the linear predictor from the ENCALS prediction model to deal with survival heterogeneity. This linear predictor is based on age, diagnostic delay, FVC at diagnosis, ALS functional rating scale at diagnosis, C9orf72 mutation, frontotemporal dementia, site of symptom onset and El Escorial diagnostic criteria. We compared patients with NIV to patients without NIV, as well as patients with different FVC cut-offs to investigate the effect of timing on tracheostomy-free survival. We matched patients for each analysis separately.

Results: Patients without NIV were older ($p<0.001$) and more likely to have bulbar onset ($p=0.013$) (n=230 in each arm). During the study period 302 patients (83%) died. NIV was associated with improved survival (HR 0.81; $p=0.043$). Using the cut-off FVCpredicted 50% we matched 71 patients in each arm, 84 patients were matched using cut-off 60% and 45 patients with cut-off 70%. The different cut-offs of NIV initiation where not associated with an added benefit in survival.

Conclusion: We found a positive effect of NIV on survival, yet no added effect of early initiation based on FVC values. With fundamental differences between ALS patients who start NIV and those who chose not to or those where initiation fails, more research is needed to determine whether FVC or another marker can play a role in determining the optimal timing for NIV initiation, with a strong need for randomized controlled trials. Furthermore, using the linear predictor from the ENCALS model to match individuals seems to be a feasible method to deal with heterogeneity within the ALS population.

(h170) Withdrawal of life-sustaining treatment in ALS patients: a Multicenter Italian Survey.

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Objective: To investigate the current status in Italy of clinical management for life-sustaining treatment suspension in ALS patients and to evaluate the impact of Law 2017/219 on italian Neurologists involvement and Advanced Care Planning (ACP) discussion in ALS patients.

Background. In Italy, recent significant juridical and social developments led to the entry into force of Law 219/2017, providing possibility of life-sustaining treatment suspension. However, no practical guidelines about life-sustaining treatment suspension are available in Italy.

Methods: We conducted a multicenter survey addressed to Italian ALS experts Neurologists, concerning the frequency of life-sustaining treatment suspension and ACP discussion before and after the entry into force of Law no. 219/2017, procedures and decisional steps applied.

Results: 38 forms were completed, from 33 Italian ALS Centers. Results of the present Survey show that the entry into force of Law 219 was followed by an increase of vital treatment suspension, percentage of DAT discussion and Neurologists involvement in this procedure. However, we also noticed an extreme variability in some aspects of practical management of the procedure, particularly concerning timing, Health professionals involved and Palliative Care Service participation.

Conclusions: the present Survey shows that the entry into force of Law 219 resulted in a higher frequency of invasive ventilation treatment suspension, a more consistent involvement of Neurologists in the procedure and an increase in frequency of ACP discussion. However, decisional steps, Health professionals and PCS involvement were variable.

(h171) Evaluation of the nation-wide implementation of ALS Home monitoring & Coaching: an e-health innovation for personalized care for patients with ALS

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Introduction: Despite technological advances, widespread implementation of ehealth for people with ALS is not yet established. We developed and implemented the care concept ALS Home Monitoring & Coaching (ALS H&C) in the ALS Center Utrecht and further implemented this in 10 regional multidisciplinary ALS teams of the ALS Care Network in The Netherlands. The aim was to evaluate implementation outcomes and user experiences.

Methods: We used an action research approach that was theoretically grounded on the implementation process model of Grol & Wensing. In meetings with stakeholder project groups (incl. patients and caregivers) ALS H&C was introduced, expected barriers/facilitators were identified, and action plans to resolve each barrier were executed. After a 3-month pilot phase in which patients receive ALS care with ALS H&C, implementation success (continuation of ALS H&C after completion of the pilot) was assessed and user experiences of patients and their healthcare providers (HCP) with ALS H&C were evaluated through an online survey. Outcomes between successful implementation and failed implementation were compared.

Results: Nine teams completed the implementation project. In 7 teams the implementation of ALS H&C was successful. Adoption by patients was high (67%). User experiences of patients (71 patients in 9 teams) were positive: satisfaction with ALS H&C was high (median 8, IQR 1 on a 0-10 scale), and most patients found the (web)app useful (76%), insightful (84%) and perceived feedback from the healthcare coach informative (70%) and easy to understand (81%). Healthcare providers (N=76) were moderately positive on the ALS H&C care concept (median satisfaction score 7; IQR 1), but were less positive about feasibility and usability. Teams with failed implementation were not convinced of the added value of ALS H&C (on quality of care, safety and financial costs) and had more fidelity issues including limited integration of ALS H&C into the workflow and staff availability for monitoring.

Conclusion: A participatory action research approach grounded in implementation theory led to a sustainable implementation of ALS H&C in most ALS teams. For successful integration of ehealth into ALS Care attention is needed to attune technology to the daily care processes in the organization and to ensure staff time for new tasks. Future studies should strengthen the evidence base for the impact on the quality, safety and efficiency of ALS care.

(h172) Effect of age on interventions and survival in people with motor neuron disease in Scotland

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Introduction: Motor neuron disease (MND) is rapidly progressive and usually fatal. The National Institute for Health and Care Excellence guidelines (2016) outline best practice for symptomatic interventions including riluzole, gastrostomy, and non-invasive ventilation (NIV). We investigated the effect of age on clinical features, interventions and survival in MND using data from the Scottish MND register (CARE-MND), hypothesising these factors may be influenced by increasing age.

Methods: We interrogated the CARE-MND database for incident cases in Scotland diagnosed between 01-Jan-2015 to 31-Dec-2020. Functional impairment at the first clinic visit was assessed using the revised amyotrophic lateral sclerosis functional rating scale (ALS-FRS(R)). Diagnostic latency describes time from symptom onset to diagnosis. These variables were assessed using the Kruskall-Wallis test. Survival was compared in different age cohorts using Kaplan-Meier curves and log-rank test. Management was compared in different age cohorts using Chi-squared test. Statistical analysis was completed using R studio.

Results: 828 individuals were included (median age at diagnosis 68.7 years (interquartile range 61.4-76.1)). We investigated age cohorts of <40, 40-60, 60-80, and >80 years. Median survival from diagnosis in each cohort was 24 months, 18 months, 11 months, and 4.5 months respectively ($p<0.001$). Initial ALS-FRS(R) decreased with advancing age ($p<0.01$). Diagnostic latency was a median of 48.5 weeks and did not differ significantly between age groups ($p=0.46$). Riluzole, NIV and gastrostomy were implemented least in the oldest group ($p<0.05$).

Conclusions: Our data demonstrate reduced survival of MND with increasing age, together with lower rates of riluzole prescribing, NIV use, and gastrostomy insertion, and offer insights into how care may be optimised in line with national guidelines.

(h173) Epidemiology of MND in Czech Republic

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Motor Neurone Disease is a group of diseases, including Amyotrofic lateral sclerosis and others. Due to the lack of effective cure, MND is economically very demanding. Therefore, effective healthcare management setup should be a priority of every country, with a special attention in countries with no public registry, such as Czech Republic. Here we report epidemiologic data collected from the Institute of Health Information and Statistics of the Czech Republic available from the 7 years of follow-up. Our data describes prevalence and the incidence of the diseases, sex and age distribution of the disorder comorbidities, hospitalizations per year, and specialized health care consumption based on medical specialty. Finally, we compare our data with the literature data from European population.

Supported by PRIMUS/012/MED

(h174) Validation of ENCALS ALS survival model on US Population

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Background: ALS is a motor neuron disease with a heterogenous course of progression but a tragically consistent outcome. Length of survival is variable with no certain explanations. ENCALS developed a model to predict survival endpoints in European patients with ALS based on data from 14 ALS Centers. The Royston-Parmar model was used as a structure. This paper shows the findings of external validation of the ENCALS model with US subjects.

Methods: NeuroBANK® (NB), a patient-centric clinical research platform comprises longitudinal and cross-sectional data from 6,500+ research participants from 24 observational studies in ALS/MND, captured by 84 sites in 14 countries. For the model validation, 5 NB-based studies were selected. To test the model on US data, non-US subjects were excluded, as well as participants with other motor neuron diseases and controls. This left 2,970 subjects.

Survival was defined as the number of months between onset date and the first of the following three dates: death, tracheostomy, or last follow-up. Some subjects were still alive; some lost to follow-up. The latter cohort's exact survival status is unknown, but some useful data on the length of survival could still be derived.

Results: ENCALS used c-statistic as a proxy for predictive accuracy. In the meta-analysis, the c-statistic was .78 (.77-.80). For the NB dataset, c-statistic was .83 (.82-.84), showing that predictive accuracy is similar for US subjects as for European subjects. To calibrate the model, a linear predictor (LP), an intercept to anchor all data points, was used. ENCALS used -6.41, whereas US dataset used a coefficient of -7.22, reflecting longer survival in the NB data.

The ENCALS model grouped subjects into 5 equally sized categories based on predicted survival length: very long, long, middle, short, very short. Each cohort contained 20% of the subjects. In the model output using NB data, 25% of subjects were predicted 'very long' and 16% predicted 'very short'. The 'Calibration-in-the-large' also suggested underestimation of survival. The two findings of a lower LP and 'Calibration-in-the-large' were consistent with a similar project using data collected by Emory.

Discussion: The ENCALS model may be used to predict survival in European and US patients. European datasets may have better quality for survival endpoints due to nationalized, single-point insurance systems. In fragmented US healthcare, survival data may be underreported. This requires 'censorship', which may underestimate the survival. The finding of 'longer-survival' in US subjects should be monitored for accuracy. Some studies continue to capture survival data. In addition to the eight factors used in the model, additional biomarkers, gene expressions, and demographics will influence survival. This model provides a benchmark of predictive accuracy; as new metrics are discovered; this model can be rerun to determine if the inclusion gives better insights into survival and patient clustering.

(h175) Initial results of the REVEALS study: Registry of Endpoints and Validated Experiences in ALS

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Respiratory insufficiency is the primary cause of mortality in ALS. The relationships between measures of respiratory function, symptoms and morbidity are poorly understood. The REVEALS study aimed to examine the rates of decline of respiratory measures and their clinical correlates.

This was a prospective, observational study conducted in six European ALS centres from January 2018 to February 2021. Participants, with Kings Stage 2 or 3 ALS at baseline, were assessed three monthly. Measurements included Peak Cough Flow (PCF), Forced Vital Capacity (FVC (L)), Slow Vital Capacity (SVC (L)), Sniff Nasal Inspiratory Pressure (SNIP cmH2O), ALSFRS-R, fatigue, sleep and secretion clearance. Chest infection incidence was tracked fortnightly. Analysis was conducted using R and Stata. A Bayesian multiple outcomes random effects model was constructed to investigate rates of decline with time from baseline and site of onset as fixed effects, in interaction with time from baseline, gender and cohort.

280 participants (males n=187 (66.8%), spinal onset n=227 (81.1)) with a mean age of 61.6 (SD12.41) years were included. A median of 3 assessments (IQR 2,4) were completed over 195 days (IQR 50.5, 382). In bulbar patients FVC declined at a rate of -0.02(0.01,-0.05)L/day, SVC: -0.02 (0.01,-0.04)L/day, SNIP: -1.01 (0.4,-1.8)cmH2O/day and PCF: -4.06 (1.63,-7.29)L/min/day. In spinal patents FVC (-0.02 (0,-0.03)L/day) and SVC (-0.02 (0,-0.02)L/day) declined at a similar rate to bulbar patients, but SNIP (-0.58 (0.11,-0.81)cmH2O/day and PCF (-2.5 (0.46,-3.38)L/min) declined more slowly. 258 participants responded to 5,013 chest infection checks over 55±38 weeks. A mean of 1.69 RTIs (range 1-7) were reported by 75 (26.8%) participants. Difficulty clearing secretions was reported in 83%.

Respiratory measures declined over time, but differentially according to site of onset. SNIP and PCF showed the greatest ability to differentiate between bulbar- and spinal-onset ALS. The incidence of respiratory tract infections with prospective measurement was high.

(h176) A cross-sectional study to evaluate the determinants of Quality of Life in patients affected by Amyotrophic Lateral Sclerosis

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a multisystem neurodegenerative disease primarily involving motor neurons. Because no effective treatment has been available up to now, most medical interventions aim to manage symptoms and improve the quality of life (QoL) of patients. The understanding of factors related to QoL in ALS may have major implications on the patients' care and the clinical research setting. Thus, the aim of our study was to evaluate which variables play a key role in the determination of QoL in ALS patients.

Methods: Fifty-five consecutive patients with definite, probable, or laboratory supported ALS according to El Escorial revised criteria were retrospectively recruited at NEuroMuscular Omnicentre (NEMO) in Milan from January 2019 to December 2020. QoL was evaluated with the ALS Assessment Questionnaire (ALSAQ-40) and was related to demographic, clinical, psychological and neuropsychological features that were analyzed as predictors.

Results: Among the QoL areas investigated by the ALSAQ-40, the most relevant variable was the physical functioning, resulted strongly and independently associated with all the ALSAQ-40 subscales, except for the emotional functioning. Moreover, also depression played a major role in determining QoL in ALS patients, especially when considering communication and emotional functioning aspects. The third most important factor in determining QoL was the level of apathy, in specific subdimensions of emotional and executive apathy. Regarding coping strategies, only positive re-interpretation appears to be a significantly determinant in emotional functions QoL.

Conclusions: Our study highlighted the multidimensionality of QoL. In detail, the entwining of physical functioning, perceived depression and perceived apathy showed to play a key role in explaining QoL in ALS patients. Coping strategies, in terms of positive re-interpretation, seemed to play a key role only in determining emotional functions QoL.Overall, our study showed some limitation and further data should be collected and analysed to better explain our current results. However, it sheds light not only on the importance of considering variables such as QoL in diseases with no treatment available, but also on the importance of defining which factors may explain perceived QoL in order to intervene in an effective way on patients to manage and maintain good levels of QoL.

i) Clinical trials

(i177) Statistical Model of the Relationship of Neurofilament with Clinical Function in the VALOR Phase 3 Study of Tofersen in Adults with SOD1-ALS

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Objective: To describe a statistical model used to assess the relationship between reductions in plasma neurofilament light (NfL) with tofersen administration and slowing of decline in disease progression, as measured by clinical and functional endpoints.

Background: Tofersen is an investigational antisense oligonucleotide designed to reduce synthesis of SOD1 protein through degradation of SOD1 mRNA. Tofersen is currently being evaluated for the treatment of SOD1-ALS. VALOR was a Phase 3, placebo-controlled trial to evaluate the clinical efficacy and safety of tofersen in adults with SOD1-ALS. Though tofersen was associated with robust reduction in NfL (55% reduction (95% CI: 48%, 61%) in the intention to treat (ITT) population), the clinical relevance of such a reduction has not been established.

Design/Methods: A statistical model with a causal inference component informed by data from the VALOR study was developed to evaluate plasma NfL as a potential surrogate biomarker predicting clinical outcome measures including ALSFRS-R, SVC, HHD, and patient-reported outcome measures. The model accounts for the fact that higher baseline NfL is associated with faster disease progression and therefore provides greater opportunity to demonstrate a treatment effect over a 6-month trial.

Results: At the sample mean baseline NfL (96.78 pg/mL), the model shows that every 10 pg/mL reduction in plasma NfL with tofersen administration at Day 114 is associated with a reduction in worsening on the ALSFRS-R and SVC at Day 197 (Week 28) of 0.772 points (p=0.00381) and 1.451 percent-predicted (p=0.0706), respectively. In addition, the model projects that tofersen-treated participants in VALOR (n=72), had they been randomized to placebo, would have experienced faster disease progression with an average of 3.83 points more decline on ALSFRS-R, and 10.6 percent-predicted more decline on SVC over 28 weeks.

Conclusions: The model suggests that reductions in plasma NfL with tofersen administration are associated with less decline in clinical function.

Study Supported By: Biogen

(i178) RNS60 in amyotrophic lateral sclerosis: a phase II multicentre, randomised, double-blind, placebo-controlled trial

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Rationale: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. The only marketed drugs for treatment are riluzole and edaravone, both having modest effects on disease survival and progression. RNS60 is a novel anti-inflammatory and cytoprotective drug that has shown efficacy in animal models of neuroinflammation and neurodegeneration. Methods: Phase II, multicenter, randomized, double-blind, placebo-controlled, parallel group trial. Patients diagnosed with Definite or Probable or Probable lab-supported ALS were randomly assigned to receive either RNS60 or placebo. The treatment was administered intravenously (375ml) once a week and inhaled (4ml/day) in the remaining days for 24 weeks, followed by additional 24 weeks off-treatment. Primary objective: to measure the effect of RNS60 on selected pharmacodynamic biomarkers (Monocyte Chemoattractant Protein-1, cyclophilin A, tyrosine nitrated-actin, 3-nitrotyrosine, Interleukin-17, neurofilament light chain, regulatory T cells) in peripheral blood. Secondary objectives: the effect of RNS60 on functional impairment (ALSFRS-R scale), respiratory function (forced vital capacity (FVC)), quality of life (ALSAQ-40 scale), self-sufficiency, and survival. Tolerability and safety were also evaluated. The intention-to-treat (ITT), per-protocol (PP) and completer-and-complier (CC) populations were assessed separately. Results: Included were 147 patients, 99 women and 48 men, aged 30-77 years. Spinal onset ALS was documented in 85.7% of cases. 37 patients (25.2%) did not complete the 48-weeks follow-up (13 died). 70.3% of drop-outs occurred after treatment discontinuation. No significant effects of treatment on candidate biomarkers were detected. Significant findings were observed in two secondary outcomes in the ITT population. Mean FVC decreased from 102.6% (baseline) to 91.4% at 24 weeks (end of treatment) (-0.46 per week) in the treatment arm and from 102.6% to 81.6% in the placebo arm (-0.87 per week) (p=0.0101). This difference was paralleled by a significant difference in the rate of change of the eating and drinking domain of the ALSAQ-40 scale, with the RNS60 group showing a slower worsening. No other differences were found. Safety and tolerability of RNS60 and placebo were similar. Conclusions: RNS60 showed no effects on selected biomarkers of disease progression, but it showed positive effects on measures of respiratory and other bulbar functions, warranting further investigation.

(i179) Plasma biomarkers of microbial translocation are modulated in ALS patients clinically responsive to NP001

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Objective: The goal of the present study was to test whether ALS patients clinically responsive to NP001, a regulator of innate immune function, would show changes in microbial translocation (MT) associated plasma biomarkers.

Background: MT is driven by gut bacterial translocation into the circulation. This MT process leads to a persistent chronic inflammatory state influencing the pathogenesis of ALS. NP001 regulates innate immune activation, converting inflammatory macrophages to a phagocytic wound healing state. A recently published post hoc analysis of combined phase 2A and 2B clinical trials in ALS patients treated with NP001 defined a subset clinically responsive as measured by halting loss of ALSFRS-R score over 6 months. These patients had baseline plasma CRP levels > 1.13 mg/L and were 40-65 years old. Rates of ALSFRS-R and Vital capacity (VC) decline were significantly slowed as compared to placebo.

Design/Methods: ALS patients in the NP001 phase 2A study received 2mg/kg of NP001 or placebo iv for 5 days the first month, then 3 days per month thereafter. At initiation of trial their average age was 54 years, time since symptom onset of 17 months, ALSFRS-R score of 38 and forced vital capacity (VC) of 3.8L, without significant differences between the two arms. Plasma specimens at baseline and 6 months from NP001 treated and placebos were evaluated for biomarker levels related to MT. Results of MT marker quantitation were compared between treated and placebo.

Results: From the NP001 2A study, 15 NP001 treated and 17 placebos completing the trial had plasma CRP levels > 1.13 mg/L and were 40-65 years of age. Decline over 6 months in ALSFRS-R ($p = 0.03$) and VC ($p = 0.03$) was significantly slowed by NP001 vs. placebo. Baseline markers of MT (LPS, LBP, sCD163, IL-18) all decreased significantly as compared with placebos ($p = 0.04$, 0.006, 0.02, 0.02, respectively) whereas the level of wound healing epidermal growth factor (EGF) increased in patients treated with NP001 ($p = 0.04$).

Conclusions: Biomarkers related to inflammation and MT improve significantly in patients treated with NP001 as compared to placebo. The statistically significant clinical improvement in these treated ALS patients that effect both the ALSFRS-R score as well as VC are unprecedented and coupled with NP001's mechanism of action suggests that MT may actively contribute to ALS pathogenesis.

(i180) When estimating causal effects in amyotrophic lateral sclerosis (ALS) randomized clinical trials, start by defining causal estimands — not estimators

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Consider a study whose goal is to estimate causal effects, with measures of estimated uncertainty. In the standard statistical literature, whether applied or methodological, frequentist or Bayesian, parametric or nonparametric, the focus remains on estimators at the expense of the explicit consideration of causal estimands. We argue that valid causal estimands must first be precisely defined as functions of potential outcomes, and only subsequently the focus turns to estimators. We illustrate our point in the context of a randomized clinical trial (RCT) on amyotrophic lateral sclerosis (ALS). In ALS RCTs estimating the effect of an active treatment vs. a placebo on a non-survival outcome (e.g., amyotrophic lateral sclerosis functional rating scale revised), it is common to encounter the issue of censoring due to death, that is, some participants die before the end of the study. In this setting, whereas valid causal estimands (e.g., always-survivor causal effect at the end of a study) are evaluable and have been previously described, the ALS literature essentially focuses on estimators that lack a causal interpretation. We advocate for alternative estimators.

(i181) Efficacy and safety of RIPK1 inhibitor SAR443820 in adult participants with amyotrophic lateral sclerosis (ALS): The Himalaya phase 2 trial design

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Background: Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is an intracellular protein involved in regulating inflammation, cytokine release, and cell death. In ALS pathophysiology, RIPK1 critically mediates necroptosis and inflammatory pathways. SAR443820 is a selective, orally bioavailable, central nervous system penetrant, reversible inhibitor of RIPK1. By inhibiting necroptosis and inflammatory signalling through RIPK1, SAR443820 has the potential to modify the course of ALS. Himalaya trial (NCT05237284) is a phase 2 study that will determine the efficacy and safety of SAR443820 in people with ALS.

Objective: To present the study design of the Himalaya trial

Methods: This is a multicenter, randomized, double blind, placebo controlled study followed by an open-label long term extension period. The trial will include approximately 260 participants, aged 18 to 80 years with diagnosis of possible, clinically probable, clinically probable laboratory-supported, or definite ALS, with disease duration ≤ 2 years and slow vital capacity (SVC) ≥ 60% at screening. Himalaya's design comprises a placebo-controlled period (Part A) and an open-label period (Part B). Part A is a 24-week, double blind, placebo-controlled treatment period where trial participants will be randomized in a 2:1 ratio to receive oral SAR443820 and placebo on top of pre-trial standard-of-care treatments. The primary endpoint of Part A is the change from baseline at Week 24 in the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) total score. Part B is a 1.5-year open label, long term extension period that will begin at the end of Week 24 and proceed through Week 106, where eligible participants will receive SAR443820. The primary goal of Part B is to assess the long-term efficacy and safety of SAR443820 in people with ALS.

Discussion: The Himalaya trial is designed to assess the effect of SAR443820 compared to placebo in reducing ALS progression as measured by ALSFRS-R. The trial includes a randomized, placebo-controlled, double-blind Part A to generate well-controlled efficacy and safety data, as well as an open-label Part B to provide long-term efficacy and safety data.

(i182) Preclinical evaluation of WVE-004, an investigational stereopure antisense oligonucleotide for the treatment of C9orf72-associated ALS or FTD

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Background: A large G4C2 repeat expansion in the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), a single genetic disorder that manifests across a clinical spectrum. This repeat expansion reduces gene expression and produces pathogenic RNA foci and dipeptide repeat (DPR) proteins. Several mechanisms have been proposed to explain how the repeat expansion drives disease, and we hypothesize that a variant-selective approach, in which transcripts affected by the repeat expansion are preferentially decreased, has the potential to address most of them. We report a stereopure antisense oligonucleotide, WVE-004, that executes this variant-selective mechanism of action.

Methods: WVE-004 in vitro activity was assessed in patient-derived induced pluripotent stem cell (iPSC) motor neurons under gymnotic conditions. In vivo activity and distribution were assessed in BAC transgenic mice containing the full human C9orf72 gene with repeat expansion. Mice were administered intracerebroventricular (ICV) injections of WVE-004 (50 µg) or PBS at day 0 and day 7.

Results: WVE-004 potently and selectively reduced repeat-containing transcripts in iPSC motor neurons (IC50 201.7 nM). In transgenic mice, WVE-004 reduced repeat-containing transcripts by 60-80% ($p<0.0001$) in spinal cord and by 40-50% ($p<0.0001$) in cortex 6 months after dosing. WVE-004 reduced DPRs by 94.2% ($p=0.001$) and 87% ($p<0.0001$) in spinal cord and cortex, respectively, at 6 months. Total C9orf72 protein levels were unaffected. Immunohistochemistry revealed widespread and sustained WVE-004 distribution in the nuclei of neurons in spinal cord and cortex.

Conclusion: In transgenic mice, WVE-004 substantially reduced repeat-containing C9orf72 transcripts and DPR proteins, which were sustained for at least 6 months, without disrupting total C9orf72 protein expression. These data support the advancement of WVE-004 as an investigational stereopure antisense oligonucleotide targeting C9orf72 for the treatment of C9orf72-associated ALS or FTD. WVE-004 is currently undergoing clinical evaluation in FOCUS-C9 (NCT04931862), a global, multicenter phase 1b/2a trial with an adaptive design that may allow for rapid assessment of the safety, tolerability, and pharmacodynamic effects of WVE-004 in humans.

(i183) Phase 3, Open-Label, Multicenter Safety Study of Oral Edaravone in Patients With Amyotrophic Lateral Sclerosis (MT-1186-A01): 48-Week Results

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Introduction: An intravenous (IV) formulation of edaravone (Radicava®/Radicut) has been shown to slow the rate of physical functional decline in amyotrophic lateral sclerosis (ALS). There is interest in a non-IV formulation of edaravone.

Objective: To assess the safety and tolerability of an investigational oral formulation of edaravone (MT-1186) in patients with ALS.

Methods: Study MT-1186-A01 is a global, multicenter, open-label, phase 3 study that evaluated the long-term safety and tolerability of investigational oral edaravone in patients with ALS. The primary safety analysis was assessed at Weeks 24 and 48. Patients received a 105-mg dose of oral edaravone administered in treatment cycles that replicated the dosing of IV edaravone.

Results: The safety analysis of the study included 185 patients. Oral edaravone was generally safe and well tolerated. The most common treatment-emergent adverse events (TEAEs) reported by ≥5% of patients were fall (22.2%), muscular weakness (21.1%), constipation (17.8%), dyspnea (10.8%), dysphagia (10.3%), and back pain (10.3%). TEAEs considered to be related to the study drug were reported by 46/185 (24.9%) patients, with the most common being fatigue (3.2%), dizziness (2.7%), headache (2.2%), and constipation (2.2%). Serious TEAEs were reported by 48/185 (25.9%) of patients, with the most common being worsening of ALS symptoms (6.5%), dysphagia (3.2%), dyspnea (2.7%), and respiratory failure (2.7%). TEAEs leading to death were reported in 12/185 (6.5%) patients, including respiratory failure (2.2%, n = 4), worsening of ALS (1.1%, n = 2), pneumonia (1.1%, n = 2), acute respiratory failure (0.5%, n = 1), lung disorder (0.5%, n = 1), diabetic ketoacidosis (0.5%, n = 1), feeding disorder (0.5%, n = 1), and suicide (0.5%, n = 1). TEAEs leading to discontinuation were reported by 2/185 (1.1%) patients (tremor, n = 1; gait disturbance, n = 1) both of which were considered by the investigator to be related to the study drug. There were no serious TEAEs, or TEAEs leading to death, related to the study drug.

Summary/Conclusion: This study demonstrates that oral edaravone was generally safe and well tolerated during 48 weeks of treatment, with no new safety concerns identified.Sponsorship: Mitsubishi Tanabe Pharma Development America, Inc., and Mitsubishi Tanabe Pharma America, Inc. Acknowledgements: p-value communications provided editorial support.

(i184) Phase 3, Open-Label, Safety Extension Study of Investigational Oral Edaravone Administered Over 96 Weeks in Patients with ALS (MT-1186-A03)

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Introduction: Radicava® (edaravone injection) is a US FDA-approved treatment for amyotrophic lateral sclerosis (ALS), shown to slow the rate of physical functional decline. The ongoing, multicenter, phase 3 studies are currently assessing the safety and tolerability of an investigational oral formulation of edaravone (MT-1186) due to interest in a non-intravenous (IV) formulation.

OBJECTIVE: To assess the continued long-term safety and tolerability of investigational oral edaravone in patients with ALS.

Methods: Study MT-1186-A03 is an extension long-term safety and tolerability study for patients who complete MT-1186-A01. Patients who continue to meet the enrollment criteria will be eligible for enrollment into the MT-1186-A03 study and will continue to receive oral edaravone for an additional treatment period of 96 weeks. Patients will continue to receive a 105-mg dose of MT-1186 administered in treatment cycles that replicate the dosing of IV edaravone. In addition to the primary safety analysis, Study MT-1186-A03 also includes exploratory end points, such as change from baseline in the revised ALS Functional Rating Scale (ALSFRS-R) score and time to death, tracheostomy, or permanent assisted mechanical ventilation.It is anticipated that Study MT-1186-A03 will include approximately 130 patients. The primary objective of this extension study is to evaluate the continued long-term safety of MT-1186, and the study includes exploratory efficacy end points in common with Study MT-1186-A01.

Results: Ongoing.

Summary/Conclusion: This extension study will provide important information on the continued long-term safety and tolerability of this investigational oral formulation of edaravone in patients with ALS.Sponsorship: Mitsubishi Tanabe Pharma Development America, Inc., and Mitsubishi Tanabe Pharma America, Inc.

Acknowledgements: p-value communications provided editorial support.

(i185) Determining Individual Substantial Response in Amyotrophic Lateral Sclerosis: Utilizing a New Method on CENTAUR Trial Results

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Background: In amyotrophic lateral sclerosis (ALS), determining whether individuals have a substantial response to therapy is a challenge for the field. ALS naturally progresses at variable rates, and a personalized approach is required to determine if individuals have a substantial response. A new method to evaluate individual response is proposed and applied to data from the randomized controlled phase (NCT03127514) of the CENTAUR trial of sodium phenylbutyrate and ursodoxicoltaurine (PB and TURSO).

Methods: In a post hoc analysis, CENTAUR participants whose actual rate of change from baseline in the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised (ALSFRS-R) at week 18 was less than or equal to their own prebaseline progression rate (ΔFS) were defined as having a substantial individual response in slowing ALS progression.

Results: Substantial individual response was observed in a greater proportion of participants receiving PB and TURSO (41%, n=87) vs placebo (19%, n=48; P=0.0076).

Conclusions; Response versus ΔFS provides a personalized metric to determine substantial individual response in ALS. ΔFS has been shown to be highly correlated with, but to proportionally underestimate, ALSFRS-R decline in clinical trials. Consequently, those who outperform the ΔFS may be considered to have a substantial individual response. Application to CENTAUR data demonstrates a greater proportion of participants with a substantial individual response in the PB and TURSO arm. These methods may enable greater personalization and analysis of individual response in ALS.

(i186) Sudden Changes in the ALSFRS-R in Three ALS Trials

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Background: The ALSFRS-R is the most commonly used primary outcome measure in late phase ALS trials, shown to be sensitive in detecting modest clinical benefits. However, concern exists that variability in either measurement or patient function can reduce its effectiveness.

Objective: To determine the frequency and impact of large visit-to-visit variations in the ALSFRS-R in several large trials.

Methods: We evaluated ALSFRS-R total score placebo data from phase 2 and 3 studies of tirasemtiv in ALS (BENEFIT-ALS, VITALITY-ALS), and the phase 2 study of reldesemtiv in ALS (FORTITUDE-ALS). Data were evaluated over 16 weeks for the phase 2 studies and 24 weeks for the phase 3 study. We identified visit-to-visit pairs in which the score of a given visit was ≥ 5 points greater than at the preceding visit. An identical analysis was applied to increases of 4 points. We calculated the slope of decline for all participants and for those in which there had been a ≥ 4- or ≥ 5-point increase. We evaluated whether any patient characteristics predicted the increases in 4 or 5 points.

Results: Overall, 1.4% of patients had visit pairs with ≥ 5-point increases, and 6.3% of patients had pairs with ≥ 4-point increases. For both the ≥4 and ≥5-point increases, no differences in such occurrences were seen as a function of site of ALS onset, time to symptom onset, or baseline ALSFRS-R total score. Slopes of participants with and without these increases were very similar, as were slopes calculated with and without the aberrant data point included. Overall, the very small number of subjects with these sudden changes contributed very little to the overall estimate of slope in the study population. In contrast to a previous study, participants with 4- or 5-point increases were distributed across study sites, with sites having at most 1 participant with aberrant data points, and others with none.

Conclusions: Overall, the occurrence of large visit-to-visit increases in ALSFRS-R of 5 points or more was a rare event in three robust, double-blind ALS trials. Even when such aberrant data points occurred, their impact on overall slope was small. The ALSFRS-R can be ascertained in a reliable manner, and the variability associated with its assessment is small enough to identify modest therapeutic effects of experimental agents.

(i187) DAZALS: A Phase 2, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study of Dazucorilant in Patients with Amyotrophic Lateral Sclerosis

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Introduction: Hypothalamic-pituitary-adrenal (HPA) axis dysregulation, such as distortion of cortisol levels plays a role in amyotrophic lateral sclerosis (ALS). Despite the known immunosuppressive effects of glucocorticoids (GCs), prolonged GC exposure can increase myeloid activity and proinflammatory factors in the central nervous system (CNS). Dazucorilant (CORT113176) is a small-molecule, selective GC receptor (GR) modulator (SGRM) that binds to GR competitively, reversibly, and with high affinity. In Wobbler mice, a model with spontaneous mutant motor-neuron degeneration, dazucorilant reduced forepaw atrophy, improved performance in the rotarod test, and inhibited neurodegeneration and inflammation. Dazucorilant distribution to the cerebrospinal fluid was observed in a healthy-volunteer study. Here, we describe the study design of DAZALS, a phase 2 study to evaluate the safety and efficacy of dazucorilant in patients with ALS.

Methods: DAZALS (EudraCT 2021-005611-31) consists of a 24-week randomized, double-blind treatment period followed by an optional 24-week open-label extension where all eligible patients will receive dazucorilant. 198 adult patients with ALS in Europe and the US will be randomized 1:1:1 to receive dazucorilant (150 or 300 mg/day) or matched placebo orally once daily. Patients will be stratified by prior use of ALS medication and region of disease onset. An ENCALS risk profile score, a multivariable combination of 7 prognostic patient characteristics, will be used to characterize ALS progression for eligibility. Patients with ENCALS risk profile score ≥-6 and ≤-3 will be eligible, which excludes those with very slow or very fast progression.

Safety and efficacy (change from baseline to week 24 in ALS Functional Rating Scale-Revised total score) are the primary endpoints. Key secondary endpoints include changes in muscle strength, quality of life, and the time to reach the composite event hospitalization, respiratory support, tracheostomy or death. Exploratory endpoints include pharmacokinetics, biomarkers, and patient-reported outcomes.

Conclusions: Disruption of the HPA axis and the proinflammatory effects of GCs in the CNS provide a strong rationale for the role of SGRMs in the treatment of ALS. DAZALS will be the first study assessing whether GR modulation with dazucorilant can reduce the neurotoxic effects of cortisol activity and benefit patients with ALS by slowing functional loss. Enrollment is planned to begin mid-2022.

(i188) A composite endpoint for ALS clinical trials based on patient preference: Patient-Ranked Order of Function (PROOF)

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Background: Patients with amyotrophic lateral sclerosis (ALS) show considerable variation in symptoms. Treatments targeting an overall improvement in symptomatology may not address what the majority of patients consider to be most important. Here we propose a composite endpoint for ALS clinical trials that weighs the improvement in symptoms compared to what the patient population actually wants.

Methods: An online questionnaire was sent out to a population-based registry in the Netherlands. Patients with ALS were asked to score functional domains with a validated self-reported questionnaire, and rank the order of importance of each domain. This information was used to estimate variability in patient preferences and to develop the Patient-Ranked Order of Function (PROOF) endpoint.

Results: There was extensive variability in patient preferences among the 433 responders. The majority of the patients (62.1%) preferred to prioritize certain symptoms over others when evaluating treatments. The PROOF endpoint was established by comparing each patient in the treatment arm to each patient in the placebo arm, based on their preferred order of functional domains. PROOF averages all pairwise comparisons, and reflects the probability that a patient receiving treatment has a better outcome on domains that are most important to them, compared to a patient receiving placebo. By means of simulation we illustrate how incorporating patient preference may up- or downgrade trial results.

Conclusions: The PROOF endpoint provides a balanced patient-focused analysis of the improvement in function and may help to refine the risk-benefit assessment of new treatments for ALS.

(i189) Developing a systematic framework to identify, evaluate and report evidence for drug selection in motor neuron disease clinical trials.

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Despite decades of clinical trials, effective disease modifying treatment options for motor neuron disease (MND) remain limited. There is a pressing need to innovate how we identify and evaluate candidate drugs in clinical trials. Motor Neuron Disease - Systematic Multi-Arm Adaptive Randomised Trial (MND-SMART; ClinicalTrials.gov number: NCT04302870) is an adaptive platform trial aimed at testing a pipeline of candidate drugs in a timely and efficient manner. We aim to develop a systematic and structured framework to identify, evaluate and prioritise candidate drugs for evaluation in MND-SMART, taking into consideration emerging data in different domains and expert opinion. Currently, the domains incorporated in our workflow include (i) published literature through Repurposing Living Systematic Review-Motor Neuron Disease (ReLiSyR-MND), a machine-learning assisted systematic review evaluating clinical studies of MND and other neurodegenerative diseases which may share similar pathological pathways, animal in vivo MND studies and in vitro MND studies; (ii) unbiased in vitro high throughput drug screening; (iii) pathway and network analysis, and (iv) pharmacological, feasibility and clinical trial data by mining drug, chemical and clinical trial registry databases. We compile an integrated list of candidate drugs including drugs which have been described in at least one clinical publication, positive hits in any of our in-house MND in vitro assays, and drugs which target pathways and networks of interest. For each drug, we obtain predictions on blood brain barrier permeability from admetSAR (http://lmmd.ecust.edu.cn/admetsar2/). Next, we generate a list of prioritised drugs deemed suitable for imminent repurposing in MND-SMART, taking into consideration availability in oral formulation, prescription-only medicine status in the British National Formulary, and evaluation of safety profile by clinical triallists in the context of common comorbidities. We further identify, evaluate, and synthesise evidence across the different domains for prioritised drugs and report these using automated workflows as interactive, curated, living evidence summaries. These summaries can be used to inform expert panel discussions on drug selection for future arms of MND-SMART at trial adaptation epochs.

(i190) The Impact of Diverse Therapeutic Targets and Treatment Modalities on Clinical Trial Design, Operations and Participation.

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Objective: We discuss the evolving therapeutic approaches, the characteristics of different treatment modalities and the implications for clinical trial design, clinical site infrastructure and the consequent burden of participation for both the clinical research site and centrally, the trial participants and their caregivers.

Introduction: Several molecular mechanisms have been hypothesized to contribute to ALS including mitochondria dysfunction, glutamate transport and excitotoxicity, protein aggregation, dysregulated RNA processing, axonal transport, microglia activation, oxidative stress, apoptosis and phosphorylation. Advancements in basic research have coincided with innovations within the biotechnology industry giving rise to an increase in the diversity of novel innovator therapeutics that are in development for the treatment of ALS.

Methods: Worldwide Clinical Trials is a full-service Clinical Research Organization which has conducted 15 clinical trials in ALS since 2017. These trials have ranged across development phase, geographical territory and class of investigational medicinal product (IMP). One of these trials is a First in Human (FIH) clinical trial conducted in an early phase clinical research unit in a healthy volunteer population and was therefore excluded from this research.

Conclusion: Clinical trial participation allows access to investigation medicinal products in supplement to the multidisciplinary care offered to PALS. There is an increasing variety of drug targets and classes of agents each with physio-chemic properties which define their administration route, dosing frequency and treatment period. Despite the attempts to bring consensus to ALS trial design and to broaden access to IMPs, the broad variety of therapies have significant consequences on the complexity of clinical trials. Clinical trials with IMPs which permit self-administration have the greatest versatility and may be able to implement decentralized clinical trial solutions such as telemedicine and home health. Biopharmaceuticals (biologics) and ATMPs may affect trial design due to the necessary additional restrictions on entry criteria and the increased safety follow-up assessments needed. The type of therapy being studied affects site requirements and consequently access to IMP while reasons for screen failure may be increased. Increasing clinic visits and invasive procedures negatively affect the burden of participation.

(i191) Design and Implementation of the Tofersen Early Access Program

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An early access program (EAP) is a non-promoted, optional program that provides eligible patients with access to an investigational treatment, before regulatory approval and commercial availability. EAPs are most commonly offered for rare diseases for which treatment options are unavailable or not tolerated, and typically for severe or life-threatening diseases, once an appropriate dose has been established and there is sufficient evidence of efficacy and safety to justify the use of an investigational drug outside the trial context. Biogen launched an EAP in July 2021 for the investigational drug tofersen (BIIB067) in people with amyotrophic lateral sclerosis (ALS) associated with mutations in the superoxide dismutase 1 (SOD1) gene (SOD1-ALS).The tofersen EAP was first made available in July 2021 when the last participant randomized in the Phase 3 placebo-controlled VALOR trial (233AS101; NCT02623699) received their first open-label tofersen dose in the extension trial (233AS102; NCT03070119). This ensured that VALOR participants were not asked to remain randomized to placebo while others received open-label tofersen outside the trial. This timing meant that the EAP was offered before the benefit-risk profile of tofersen was known. The EAP was therefore initially limited to persons with the most rapidly progressive disease (average monthly decline in the ALSFRS-R of 2.0 points or more).In October 2021 results of the VALOR trial revealed that although the primary endpoint did not achieve statistical significance, consistent evidence suggested slowing of decline in faster-progressing participants and an apparent stabilization of clinical function in slower-progressing participants. Tofersen was generally well-tolerated. Serious neurologic events, including myelitis, have been reported in several participants. In light of the severe unmet medical need, the EAP was then expanded to the broader SOD1-ALS population regardless of disease progression rate.The design of the tofersen EAP was informed by consultation with medical experts, bioethicists, and patient advocates. This resulted in a plan to make access to tofersen more inclusive, consistent with principles of medical ethics and prudent medical practice, in countries where an EAP is permitted per local regulations and future access is anticipated.As of March 10, 2022, the tofersen EAP was available in 31 countries; sixty-five patients had been treated in 7 countries.

(i192) First experience of the treatment with antisense nucleotide tofersen in ALS patient due to SOD1 mutation

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Background.: Tofersen is an antisense oligonucleotide that mediates the degradation of superoxide dismutase 1 (SOD1) messenger RNA to reduce SOD1 protein synthesis with a potential of slowing disease progression. Case presentation. A 46-year old female patient with a positive familial history for ALS, presented multiple episodes of sudden stridor and dyspnoea since May 2019, attributed to allergic asthma. In June 2020, she was hospitalised in ICU because of the acute respiratory insufficiency and haemoptysis, needing intubation, and complicated by pulmonary embolism. In September 2020, the patient was diagnosed with bulbar-onset ALS. Her symptoms included fasciculations of the tongue with discrete atrophy, dysarthria, hypophonia, rhinolalia, facial diplegia, exaggerated masseter reflex without any abnormality in muscle force or pyramidal signs. Electrophysiological studies were normal in other segments than bulbar, and imaging studies were negative. Further, a genetic testing in February 2021 revealed a SOD1 mutation. The treatment with riluzole and edaravone was initiated and the patient was enrolled in the early access program for tofersen. Protocol of tofersen. The standard dosage (100 mg/15 ml) initially was administered in 3 loading doses (day 0, 14 and 28) intrathecally in November 2021 according, following the pre-treatment blood tests (liver, renal function, electrolytes, complete blood count, C-reactive protein, coagulation parameters), evaluation of vital signs, and ECG before the first injection. After, the treatment was continued every 4 weeks (28 days). Injections were well tolerated except for a post-lumbar puncture syndrome after the first administration, which was treated with non-steroid anti-inflammatory medication. After modification of the volume of cerebrospinal fluid withdrawn, (10 ml vs.15 ml), there were no more side effects. Outcome. Four months on tofersen treatment, the tolerance of injections remained satisfactory (6 in total). Neurological status was stable with ALSFRS-R score at 42/48 points. Conclusions. In the Phase 3 VALOR study, the primary endpoint as measured by the ALSFRS-R scale from baseline to week 28 did not reach statistical significance, however, signs of reduced disease progression or stabilization of clinical function was observed. Given the high unmet need, together with other neuroprotective treatments, tofersen remains a mainstay therapy in patients with ALS due to SOD1 mutation.

(i193) Detectable Effect Cluster Analysis: A Novel Machine-Learning Subgroup Analysis Method for Drug Rescue

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Introduction: ALS drug development is plagued by high clinical trial failure rates. Subgroup analysis is a key tool used to account for patient heterogeneity, but current methods fall short. DEC analysis systematically groups and analyzes patients based on predicted disease path, creating more homogeneous patient subgroups with reduced noise around the endpoint.

Methods: A multivariate machine-learning model trained using PRO-ACT was used to rank order trial participants by predicted disease progression. Fifty initial subgroups were expanded by adjusting prediction thresholds in 2% increments until the FAS was reached. A matrix was plotted in which each block had distinct upper and lower thresholds. A series of analyses were performed to assess variance (RMSE), treatment effect (TE), effect size, and P-value, thus developing a series of heat maps that revealed subgroups with favorable conditions for detecting a significant effect. The method was applied to the Ceftriaxone-ALS and Topiramate-ALS data sets available from the US NINDS.

Results: We used the 285 patients in the Ceftriaxone-ALS dataset who remained on study for one year, which included 190 treated and 95 placebo patients. We randomly separated the 190 treated patients into two groups, one for the exploratory analysis and a second for hypothesis testing. One-year predictions using our validated percent expected vital capacity model were made for all patients in the dataset. A broad central region, a hot-spot, where moderately progressing patients localized, was detected and a subgroup, representing predicted 15% to 25% one-year decline in percent expected vital capacity was selected to determine whether the subgroup could be detected in the test set. Examination of the test group confirmed the results of the exploratory analysis. The topiramate trial reported a negative TE for the primary endpoint. Similarly, DEC analysis showed broad zones of negative TEs. This experiment provides a strong negative control for DEC analysis.

Conclusions: DEC analysis organizes trial participants in an unbiased way into homogeneous subgroups: • Reveals "hot-spots" of detectable TEs that could form the basis for a subsequent successful trial. Numerous ways to implement this approach are envisioned: • "Rescue" of drugs that failed late-stage clinical development • All-comers trials to identify patients with detectable effects that can be seamlessly expanded into a fully powered trial

(i194) Factors Influencing Trial Participation in Motor Neuron Disease (FIT-Participation in MND).

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Background: The Motor Neuron Disease Systematic Multi-Arm Randomised Adaptive Trial (MND-SMART) is a multi-site United Kingdom clinical trial seeking to address the paucity in effective disease modifying drugs for people with MND (pwMND). Historically, MND trials have been affected by suboptimal recruitment and high rates of attrition. Failure to recruit and/or retain trial participants can result in insufficiently representative samples, terminated trials, or invalid conclusions.

Objective: This is the first study to prospectively explore pwMND's reasons for joining, and remaining, in a clinical trial.

Hypothesis: We hypothesise patient-specific factors (neuropsychiatric symptoms, cognitive impairment, behavioural change, disease phenotype, quality of life (QoL), physical functioning, intervention use and attitudes to trials) will have a significant impact upon pwMND's decision to participate, and remain in, MND-SMART.

Methods: Participants were recruited from the Scottish MND Register, Clinical Audit Research and Evaluation MND (CARE-MND). A comprehensive set of questionnaires were completed by participants or caregivers (in online or paper format) and supplemented by data from CARE-MND and MND-SMART. 12 months from the date of completion for all questionnaires, data on trial recruitment and retention for our cohort will be collated.

Preliminary Results: 120 pwMND participated; with an equal preference for online and paper questionnaire completion. 67% were male, mean age 66 (range 39-85), 58% amyotrophic lateral sclerosis and 23% had bulbar onset. 40% of participants had been in other research. Most significant barriers to trial participation identified were travel and time requirements, with a desire to help others the most important reason for participating. Participants rated overall health related QoL as 'Good' and ALS-specific QoL as 97 (SD = 29), on average. 73% of participants had a Brief Dimensional Apathy Scale completed by a caregiver, with a mean total score of 7.8 (SD = 5.1).

Discussion: Recruitment was completed May 2021, with a quarter of the Scottish MND population completing questionnaires. Data to establish how many of our 120 pwMND cohort participated, and remained, in MND-SMART will be available in May 2022; we will also explore the relationships between study covariates and trial-related outcomes. Ethical approval was provided by the West of Scotland Research Ethics Committee 3 (20/WS/0067) on 12th May.

(i195) Design of an international, phase 3, randomized, placebo-controlled trial with daily oral edaravone (FNP122) in ALS: the ADORE study

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Objective: To describe the design of a Phase 3 trial, assessing the safety and the efficacy on disease progression and survival of once-daily oral edaravone in patients with ALS.

Introduction: Effective treatment of ALS continues to be a significant unmet need. FNP122 is an oral formulation of edaravone to be administered daily. Edaravone is a known free radical scavenger, and as an antioxidant, it has been shown to decrease oxidative stress and reduce death of neuronal cells, which play an important role in ALS' pathogenesis and disease progression.

Methods: Several TRICALS Centers will participate in this trial. The ADORE study aims to randomize 300 ALS patients to daily oral 100mg of edaravone or matched placebo in a 2:1 ratio. Patients will receive double-blind treatment up to a maximum of 72-weeks. Riluzole (100mg/day or less) may be used as background (add-on) therapy. The main inclusion criteria are: definite, probable, probably laboratory supported or possible ALS (based on the El Escorial and the revised Airlie House diagnostic criteria, Slow Vital Capacity (SVC) ≥70%, onset of first symptoms ≤24 months prior to randomization, and change in Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) score between 0.35 and 1.5 points per month (both inclusive) in the period between first symptoms and the screening visit. The primary efficacy endpoint will be the change from baseline in ALSFRS-R total score after 48w.Secondary efficacy endpoints include: combined assessment of function and survival (CAFS) at 48 and 72w, survival

time over 72w, change from baseline in SVC and in the overall mega score for the hand-held dynamometer (HHD), and serial assessments of patient-reported outcomes (ALSAQ-40 and EQ-5D-5L), and ECAS. Exploratory endpoints include measures of plasma and urine ALS biomarkers. After a patient completes the study, the possibility to roll over in an open label extension (ADOREXT) in which all patients daily oral edaravone will be offered.

Results: This study is currently recruiting patients across Europe. Enrolment started in October 2021.

Conclusions: The ADORE trial is an ongoing phase 3 study designed to compare active treatment vs. placebo for a longer duration (up to 72w of treatment) in patients with ALS. The results will provide relevant safety and efficacy data on this new oral daily formulation of edaravone (FNP122).

(i196) ALS Phase 3 NurOwn Trial: Insight into the primary outcome through ENCALS modeled trajectories

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The development of ALS treatments is complicated by clinical heterogeneity. The NurOwn phase 3 trial enrolled a broad set of participants, including some with advanced ALS disease (ALSFRS-R<25), making this trial subject to the impact of floor effects and reduced ALSFRS-R sensitivity (Mandrioli 2015). Participants with advanced ALS began the trial with Fine and Gross Motor Subscale items of 0, limiting the ability to measure progression (Cudkowicz 2022).

The ENCALS model, a composite endpoint model developed and validated to reliably estimate prognosis trajectories using a set of predictors, provides an alternative method to baseline characteristics (e.g., baseline ALSFRS-R) to identify participants likely to reach the composite survival endpoint in a "very-short" and "short" period, and more likely to be impacted by measurement error compared to the other ENCALS prediction categories. Clinical efficacy was evaluated using the primary endpoint from the trial, a clinical response criterion based on a change in ALSFRS-R of ≥1.25 points/month between pre- and post-treatment.

Participants included in the trial spanned all 5 ENCALS model prediction categories with baseline characteristics balanced by treatment, illustrating randomization was effective. Furthermore, 9% of participants were predicted to reach the survival endpoint "very short", and 15% in "short", illustrating that advanced and rapidly declining participants were included in this trial.

In participants predicted to have "intermediate," "long" or "very long" survival, there was 36% NurOwn response versus 21% Placebo (p=0.056). In the participants who were predicted to have "very short" and "short" survival, there was a paradoxical finding with an increased rate of response with Placebo (52%), which may suggest a misclassification error of response due to a floor effect of ALSFRS-R. This finding appears to be driven by a few outliers (rates of decline > 3 points/month).

The ENCALS prediction model provides a unique view into this Phase 3 trial. The primary endpoint appears to have been impacted by participants likely to reach the composite survival endpoint in a very short and short period of time, a result of misclassification. Analyses that isolate ALS participants with the most rapidly advancing disease, who are more likely to reach the floor effect of the ALSFRS-R, demonstrate a potential treatment effect in participants with less severe disease.

(i197) Preliminary Biomarker Findings from the RESCUE-ALS Double-Blind, Placebo-Controlled Study of CNM-Au8 to Slow Disease Progression in ALS

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RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 as treatment for early sporadic ALS. CNM-Au8 is a suspension of clean-surfaced, catalytically active gold nanocrystals shown to enhance neuronal metabolic energy, reduce oxidative stress, and improve protein homeostasis.

Pharmacodynamic (PD) biomarker investigation in RESCUE-ALS included SIMOA assay for neurofilament light chain (NfL), tau protein (Tau), glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase-L1(UCHL1); ELISA for urinary p75ECD; and untargeted metabolomic and proteomic assays. Here, we report the data from the neurodegeneration markers including urinary p75ECD and the SIMOA assay over the 36-week blinded study duration.
45 participants (73% limb onset, 27% bulbar) were randomized 1:1 to receive 30mg CNM-Au8 (active) or placebo daily for 36 weeks. Baseline clinical characteristics of the intent-to-treat (ITT) population include [mean (SD)], age: 59.1 (12.3), months from symptom onset: 15.8 (9.3), FVC % predicted: 81.5 (16.7); ALSFRS-R: 38.7 (6.0); ENCALS risk score: −4.4 (1.8). Amongst all evaluable PD samples, baseline urinary p75ECD/creatine (mg) were [mean (SD)], active: 5.6 (2.5); placebo: 6.1 (1.7); baseline UCHL1 values (pg/mL) were active: 108 (56), placebo: 155 (114); baseline NfL values (pg/mL) were active: 63.6 (35.5), placebo: 72.5 (36.8); baseline GFAP levels (pg/mL) were active: 127 (65), placebo: 170 (118).

Placebo treated participants demonstrated increased p75ECD/creatine and UCHL1 levels, whereas CNM-Au8 treated participants showed a trend for decreased p75ECD/creatine levels and significantly decreased UCHL1 levels versus placebo participants. Mixed effects model, mean difference (SE): p75ECD/creatine: -1.5 (0.9), p=0.10; UCHL1: -51 (24.8), p<0.05. NfL and GFAP changes did not significantly differ between active and placebo treated participants.
CNM-Au8 treated participants demonstrated decreased levels of neurodegeneration markers including UCHL1 and p75ECD/creatine levels relative to placebo treated controls over 36-weeks of treatment.

(i198) RESCUE-ALS Trial Results: A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study of CNM-Au8 to Slow Disease Progression in ALS

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RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 as treatment for early sporadic ALS. CNM-Au8 is a suspension of clean-surfaced, catalytically active gold nanocrystals shown to enhance neuronal metabolic energy, reduce oxidative stress, and improve protein homeostasis.

Participants were randomized 1:1 to receive 30mg CNM-Au8 or placebo daily for 36 weeks. The primary endpoint was the percent change in the summated motor unit index (MUNIX) scores for the abductor digit minimi, abductor pollicis brevis, tibialis anterior, and biceps brachii. Secondary and exploratory endpoints included respiratory function, ALS disease progression, and quality of life. 45 participants (73% limb onset, 27% bulbar) were enrolled. Baseline characteristics of the intent-to-treat (ITT) population include [mean (SD)], age: 59.1 (12.3), months from symptom onset: 15.8 (9.3), FVC % predicted: 81.5 (16.7); ALSFRS-R: 38.7 (6.0); ENCALS risk score: – 4.4 (1.8). The primary endpoint was not significant (least-squares [LS] mean difference: 7.7% (95% CI: –11.9% to 27.3%; p=0.430), which was driven by limited decline in bulbar-onset placebo participants (8% decrease in LS mean at week 36). In prespecified analyses of limbonset participants, there was a strong trend for reducing MUNIX decline (LS mean difference 20.9%, 95% CI: 2.2% to 44.0%; p=0.074). Notably, a significant reduction in ALS disease progression (defined as occurrence of death, tracheostomy, non-invasive ventilatory support, or gastrostomy tube placement) was evident in CNM-Au8 treated participants at week 36 (55% absolute risk reduction, p=0.013), and the proportion experiencing ≥ 6-point decline in ALSFRSR was significantly reduced in the CNM-Au8 treated patients (p=0.035, chi-square test). CNMAu8 treated participants demonstrated quality of life improvement (ALSSQOL-SF LS meandifference: 0.9; 95% CI: 0.2, 1.6; p=0.018). There were no significant adverse effects. RESCUE-ALS has established safety and suggested efficacy of CNM-Au8 in ALS. A larger study is underway to confirm efficacy of CNM-Au8 in ALS.

(i199) Evidence for a Potential Survival Benefit in ALS with CNM-Au8 Treatment: Interim Results from the RESCUE-ALS Trial Long-Term Open Label Extension

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RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 in early sporadic ALS, with an ongoing open-label extension (OLE) to evaluate the long-term safety and efficacy of CNM-Au8. CNM-Au8 is a suspension of clean-surfaced, catalytically active gold nanocrystals shown to enhance neuronal metabolic energy, reduce oxidative stress, and improve protein homeostasis.

Study participants were randomized 1:1 to receive 30mg CNM-Au8 or placebo daily for 36- weeks during the double-blind portion of the study, followed by an OLE with CNM-Au8 (30mg/day). The trial enrolled 45 participants [n=23 active (CNM-Au8), n= 22 matched placebo)].

During the 36-week double-blind period, 96% of CNM-Au8 treated participants completed, with one mortality event in the active-treatment arm (4%). Amongst placebo-treated participants, 86% completed with two mortality events and one withdrawal due to ALS disease worsening (14% death or withdrawal). Of the participants who completed the double-blind portion, one subject (active) was ineligible for the OLE due to relocation from Australia, and four elected not to continue in the OLE (1 active, 3 placebo) resulting in 90% of eligible participants entering the OLE.

Long-term observed survival across the entire study population from randomization through the latest OLE observation was compared to predicted median survival derived from the published ENCALS model based on each participant's baseline study characteristics. Data were censored at either last study contact or as 1-February-2022. Kaplan-Meier analyses showed a significant survival benefit with long-term open label CNM-Au8 treatment, resulting in approximately a 70% decreased risk of death versus predicted, log-rank hazard ratio = 0.314 (95% CI: 0.133 to 0.744, p=0.01). CNM-Au8 treatment was well-tolerated and there were no significant safety findings reported during the OLE. These results demonstrate improved survival with CNM-Au8 treatment compared to estimated median survival derived from the ENCALS prediction model.

(i200) Interim ALS Specific Quality of Life Results from the Long-Term Open Label Extension of RESCUE-ALS, a Double-Blind, Placebo-Controlled Study of CNM-Au8 to Slow Disease Progression in ALS

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RESCUE-ALS was a Phase 2 randomized, double-blind, placebo-controlled study of CNM-Au8 as treatment for early sporadic ALS. CNM-Au8 is a suspension of clean-surfaced, catalytically active gold nanocrystals shown to enhance neuronal metabolic energy, reduce oxidative stress, and improve protein homeostasis. Study participants were randomized 1:1 to receive 30mg CNM-Au8 or placebo daily for 36-weeks during the double-blind portion of the study, followed by a long-term open label extension (OLE) with CNM-Au8 (30mg/day) with subsequent visits occurring every 12-weeks. The trial enrolled 45 participants [n=23 active (CNM-Au8), n= 22 matched placebo)]. Baseline clinical characteristics of the intent-to-treat (ITT) population include [mean (SD)], age: 59.1 (12.3), months from symptom onset: 15.8 (9.3), FVC % predicted: 81.5 (16.7); ALSFRS-R: 38.7 (6.0); ENCALS risk score: −4.4 (1.8). During the 36-week double-blind period, CNM-Au8 treated participants demonstrated quality of life improvement (ALSSQOL-SF LS mean change, active: -0.3, placebo: -1.2; LS mean difference: 0.9; 95% CI: 0.2, 1.6; p=0.018). During the OLE, originally randomized CNM-Au8 participants continued to maintain ALSSQOL-SF scores through 84-weeks or more of treatment with a mean (SD) slope change from week 36 – 84 post-randomization (average point change/week = -0.008 (0.03). Ex-placebo participants who transitioned to the long-term OLE demonstrated similar stability following treatment with CNM-Au8 (average point change/week = -0.004 (0.02). CNM-Au8 treatment resulted in improved quality of life assessed by the ALS Specific QOL scale during the 36-week double-blind period, which was maintained during the OLE.

(i201) Adapting an adaptive randomised control trial for motor neuron disease to covid-19 pressures

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Motor Neuron Disease (MND) is a rapidly progressive disease involving the loss of motor neurons responsible for functions such a breathing, speech, swallowing, cognition, and movement, and proving fatal within a couple of years of diagnosis. In the last 10 years, over 125 trials have failed to identify any effective treatments1. There remains only one globally licensed drug approved in 1995 to prolong survival by 2-3 months and historically only 5% of people with(pw) MND have taken part in clinical trials. MND-SMART is a UK multi-centre adaptive platform trial for pwMND that will enable multiple potential investigational medicinal products (IMPs) to be definitively and efficiently tested. MND-SMART randomised its first participant on 27th February 2020, just prior to the Covid-19 pandemic. To reduce the impact of the new constraints on research, potential transmission to vulnerable people and ensure pwMND could still join MND-SMART several updates to the trial protocol and operations were made.

To reduce time required on site for research visits, the MND-SMART team introduced the use of routine clinical blood and respiratory results collected within specified timeframes. To further minimise participant burden, most assessments can be completed remotely or in the community and questionnaires emailed to participants to complete wherever best suits them. In particular, remote consent via an electronic system has been useful in participant follow-up to avoid unnecessary travel to sites. This is primarily used if re-consent to new trial information is required. With appropriate permissions, delegated staff from other sites have been able to support other sites with completing remote follow-up appointments. Partnering with courier services across the UK has also further enabled equity of access for participants to receive IMPs at home. PwMND have been at the heart of these developments and a sub-study was also introduced to allow further feedback on participant and carer experiences.

Despite starting recruitment in February 2020 and unlike many trials, MND-SMART continued to recruit during the pandemic and open trial sites, reaching 290 participants recruited and 17 trial sites (as of 11th March 2022).

Conclusion: There is an urgent need for delivering trials in MND, in particular with regards to innovation in drug delivery and patient assessments to ensure more people with MND have opportunities to take part. It is critical that we continue to incorporate flexibility in trial designs, highlighted by the advent of covid-19, to ensure ongoing recruitment.

1 Wong et al Clinical trials in amyotrophic lateral sclerosis: a systematic review and perspective, Brain Communications, Volume 3, Issue 4, 2021, fcab242, https://doi.org/10.1093/braincomms/fcab242

(i201a) Generation of synthetic placebo arms for amyotrophic lateral sclerosis clinical trials

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Background: Randomised clinical trials, where people receive either an active treatment or a placebo are the gold standard for validating ALS therapeutics. ALS is a rapidly progressive disease and withholding of treatment from the placebo group presents an ethical concern, however generation of synthetic placebo arms as an alternative to a real placebo arm has not been explored in ALS. In this work we test two approaches to modelling synthetic placebo arms for ALS trials, comparing the synthetic placebo arms to control arms in previous ALS clinical trials.

Methods: We extracted sample subsets from the UK MND register (n = 308) using an evolutionary algorithm such that the subset baseline variables (age of onset, age of diagnosis, site of onset and sex) matched a target trials group, either people enrolled in LiCALS (n = 106) or people included in the PRO-ACT resource (n = 171). We also applied trial specific exclusion criteria where possible or alternatively preferentially selected long term survivors using a 'time simulation algorithm'. Additionally, survival was predicted for LiCALS and PRO-ACT participants using the ENCALS Prognostic model. Generated survival probabilities from each method were compared to real placebo participants using Kaplan-Meier analysis and the log rank test, where a p-value of >0.05 represents no statistical difference between groups and therefore good matching.

Results: We found that the synthetic placebo groups derived from the MND register matched the target trials outcomes very well. Log rank tests showed a significant difference between the MND register and Proact (P = 0.02) but a non-significant difference for our extracted placebo groups (P = 0.89). The ENCALS model produces synthetic placebo groups that are significantly different to the real placebo groups, if overall survival probabilities are compared. However, when participants are censored at 6 month intervals, the synthetic group matches the target group very well with a non significant difference log rank p-value > 0.05 between 24 and 48 months indicating a possible timeframe that this method could be utilised.

Discussion: Both approaches generated synthetic placebo groups that matched placebo groups from historical trials. These methods need to be validated in prospective trials.

j) Cell Biology

(j202) Mislocalization of FUS in adult projection neurons impairs social memory and executive functions

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FUS (Fused in Sarcoma) is an RNA-binding protein involved in multiple steps of RNA metabolism. FUS is mostly a nuclear protein in physiological, yet is accumulating and aggregating in the cytoplasm of a subset of patients with amyotrophic lateral sclerosis (ALS) or fronto-temporal dementia (FTD). To circumvent the perinatal letality of constitutive homozygous truncation of FUS, we created a new conditional Fus mouse model (called Fus cKI) allowing the CRE-dependent truncation of the PY-NLS domain responsible for FUS nuclear import. Induction of recombination through adenoviral delivery of CRE led to the expected recombination and truncation of the FUS protein in cultured primary neurons. We then crossed Fus cKI mice to Thy1-CRE-ERT2 transgenic mice, expressing an inducible CRE in most projection neurons, to generate mice with inducible FUS truncation in neurons (FusΔNeuron). Tamoxifen induction in homozygous FusΔNeuron mice induced a robust recombination in the cortex and hippocampus at the DNA, RNA and protein level. FUS mislocalization was observed in most cortical and hippocampal neurons, as well as in spinal motor neurons. Two months after recombination, neither homozygous or heterozygous FusΔNeuron mice showed altered motor function, as judged using grip test and inverted grid. However, FusΔNeuron mice displayed severe abnormalities in social interaction and social memory in the 3-chamber test, as well as altered executive functions (nest building test). These defects appeared selective as FusΔNeuron mice showed normal performance in object recognition. RNAseq performed two months after recombination revealed widespread changes in expression of genes related to synaptic plasticity, including immediate early genes. These results suggest that mislocalization of FUS in adult neurons is sufficient to trigger defects relevant to FTD.

(j203) Involvement of oligodendrocytes in amyotrophic lateral sclerosis linked to Fused in Sarcoma protein.

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Aims: A mutation in the nuclear localisation signal (NLS) of the Fused in Sarcoma protein (FUS) leads to FUS cytoplasmic mislocalisation and early-onset ALS. FUS is a DNA/RNA binding protein that regulates gene expression and mRNA splicing. Increasing evidence suggests that not only motor neurons but also glial cells are affected by the disease. Especially abnormalities in oligodendrocyte lineage have been observed in ALS mouse models linked to SOD mutation. My PhD aims to understand the contribution of oligodendrocytes in FUS-ALS.

Methods: To conduct my project, we first used a murine model of FUS-ALS carrying a truncation of the NLS signal in the FUS protein to characterize the oligodendrocytes deficiencies. This mutation is very similar to the one found in FUS-ALS patients. We studied myelin ultrastructure using electronic microscopy in the cortex and corpus callosum at two-time points: presymptomatic stage (2 months of age) and symptoms' onset (10 months of age). We characterized oligodendrocytes' ability to proliferate and remyelinate the central nervous system after cuprizone-induced demyelination in our FUS-ALS model. Then, we developed two new mice models in which we can either induce the FUS mutation only in oligodendrocytes or rescue the mutation only in oligodendrocytes using a Cre-Lox recombination technique. We are currently investigating the pathological hallmarks and behavioural outcomes in those two models to determine whether this Fus mutation in oligodendrocytes is sufficient and/or necessary to induce ALS symptoms.

Results and discussion: The ultrastructure of the myelin sheath is altered in FUS-ALS model, as well as myelin production. This could be explained by the fact that the FUS protein binds to several myelin mRNAs, like Myelin Basic Protein (MBP) mRNA which is one of the most abundant proteins of the myelin. We suspect FUS to be necessary for MBP mRNA transport before its local translation in the myelin sheath. Moreover, FUS regulates the splicing of certain myelin proteins like MOBP and MAG. Also, cuprizone-induced demyelination leads to increased locomotor impairments in FUS-ALS mice. Molecular mechanisms are still under investigation at this time. Our two new mouse models may also enlighten the role of cytoplasmic FUS gain of function in oligodendrocytes of FUS-ALS, compared to previous experiments that have been conducted with FUS-KO in oligodendrocytes.

(j204) An arginine mono-methylation of TDP-43 regulates its function in translational control

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Methylation of the amino acid arginine is a post-translational modification that may change the localization, function, and/or activity of the respective protein. While methylation of FUS protein is well characterized and implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), very little is known about methylations of TDP-43. More than 50 high-throughput studies consistently detected a mono-methylation at arginine 293 (R293) of TDP-43 that has not been characterized yet. Strikingly, R293 of TDP-43 is highly conserved and represents the center of a hotspot of mutations causative for ALS/FTD (amino acid 287-298). Therefore, we hypothesized that ALS/FTD-linked mutations of TDP-43 in close proximity to R293 may affect this mono-methylation, and that this post-translational modification is involved in functions of TDP-43 relevant for the pathogenesis of ALS/FTD. Indeed, using in vitro and cellular approaches, we found that R293 of TDP-43 is mono-methylated by PRMT1, and that nearby ALS/FTD-linked mutations cause a decrease of methylated TDP-43. Methylation-deficient (R293K) and methylation-mimicking (R293F) variants of TDP-43 did not show considerably effects on the localization or solubility of TDP-43, neither under physiological, nor under stressed conditions. However, proximity proteomics revealed differential association of methylated/unmethylated TDP-43 with ribosomes. Interestingly, a recent study revealed regulation of ribosome association of TDP-43 by a conserved region in the low complexity domain (amino acid 321-340), another hotspot of ALS/FTD-linked mutations, including effects on global translation. Consequently, the impact of methylated/unmethylated R293 of TDP-43 on global translation is currently under investigation.

(j205) Lipid defects and enhanced ER stress susceptibility in iPSCs derived oligodendrocytes with an amyotrophic lateral sclerosis causing FUS mutation

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal, rapidly progressive neurodegenerative disorder, characterized by selective loss of motor neurons (MN). In 10% of cases, causative genetic mutations are underlying the disease, including mutations in the C9ORF72, SOD1, FUS and TARDBP genes. Oligodendrocytes, the myelinating cells in the central nervous system, are also affected in ALS and are hypothesized to contribute to MNs death. However, the role of mutant oligodendrocytes is not yet been fully understood. Here, we used induced pluripotent stem cells (iPSCs) from a FUSR521H ALS patient, as well as it's isogenic control, and a healthy control iPSC line wherein the FUSP525L mutation was introduced by CRISPR-Base Editing to address defects in oligodendrocytes, and eventually their impact on neuronal health. The FUS mutant iPSC showed cytoplasmic FUS mislocalisation, a key feature observed in FUS-linked ALS. By targeted recombinase-mediated cassette exchange, the transcription factor SOX10, controlled by a TET-ON promoter system, was integrated into the safe harbor locus AAVS1 of the FUS mutant as well as isogenic normal iPSC. This enabled the doxycycline inducible overexpression to make fast and efficient differentiation possible towards oligodendrocytes, as described before by our lab (Garcia et al, Stem Cell Reports, 2018; Garcia et al, Nature Protocols, 2021). Both mutant and control cells differentiated efficiently into oligodendrocytes. We performed RNA sequencing which suggested a lipid defect in mutant oligodendrocytes. Subsequent lipidomics studies demonstrated defects in particular myelin lipids, including the saturated long-chain fatty acids and monounsaturated fatty acids, which were dramatically reduced in mutant oligodendrocytes. Interestingly, oligodendrocytes from iPSC bearing the FUSP525L mutation, known to cause very early and severe ALS, displayed more pronounced lipid defects, compared to the FUSR521H mutant oligodendrocytes. Moreover, mutant oligodendrocytes demonstrated higher endoplasmic reticulum stress, when exposed to thapsigargin or tunicamycin. Taken together, these results demonstrated that FUS mutant oligodendrocytes might have a defect in lipid metabolism as well as ER stress, which could contribute to abnormal myelination and subsequent neurodegeneration. In addition, if and how ER stress and lipid metabolism are related is currently under investigation.

(j206) Proteomics suggest a general role of the fragile-X protein family in ALS-related protein aggregation

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The Fragile-X protein (FXP) family comprising FMR1, FXR1 and FXR2 has recently been implicated in several neurodegenerative diseases. Especially in amyotrophic lateral sclerosis (ALS), accumulating evidence points towards the FXP family as potential modifier of the degenerative phenotype that is independent of the underlying cause of the disease, and may link key events of ALS pathogenesis such as (micro)RNA dysmetabolism, protein aggregation and synaptic integrity. For example, overexpression of FMR1 mitigated the phenotype of FUS- and TARDBP-linked ALS in vivo models by restoring neuromuscular junction morphology and decreasing TDP-43 aggregation, respectively. Moreover, human ALS post-mortem spinal cord tissue revealed aberrant expression and aggregation of the FXPs in motoneurons, and loss of FXR2 expression correlated with the presence of pathogenic aggregates. Therefore, here, we aim at characterizing downstream events associated with the loss of expression of each individual FXP. Using CRISPR/Cas9-edited knockout cell lines, proteomic analyses of total and insoluble protein revealed shared and unique functions of the individual FXPs, but no effects on the expression or solubility of endogenous TDP-43, FUS and SOD1 were detected. However, our proteomic data suggests that loss of FXP expression is generally associated with aberrant protein aggregation and other pathogenic events in neurodegenerative diseases. Therefore, we hypothesize that loss of/reduced FXP expression represents one of several “hits” necessary for the death of neurons in multistep models of neurodegenerative diseases. The aggregation of TDP-43, FUS and SOD1 carrying causative ALS mutations, as well as potential consequences on stress granule dynamics in presence/absence of the individual FXPs is currently under investigation.

(j207) CNM-Au8 Gold Catalytic Activity Protects Neurons Against Degeneration and Death in Multiple in vitro Models of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disorder involving the progressive loss of motor neurons of the brain and spinal cord. Because ALS treatments available to date have largely failed to demonstrate substantial benefits, there is an exigent need for new, effective treatments. Recent discoveries have converged on metabolic and energetic dysregulation as potential drug targets for treatment of this disease. The hallmarks of energetic dysregulation in ALS include lipid dysregulation, insufficient oxidative respiration, mitochondrial dysfunction, and accumulation of excitotoxic and oxidative stress, leading to neuronal death.CNM-Au8 is a concentrated aqueous suspension of highly catalytic gold nanocrystals being developed as a neuroprotective therapeutic. CNM-Au8 is a concentrated aqueous suspension of highly catalytic gold nanocrystals being developed as a neuroprotective therapeutic. The blood-brain barrier penetrant, cell-permeant gold nanocrystals of CNM-Au8 both restore energetic homeostasis via mitochondrial complex I-like activity, as well as reduce oxidative stress via catalase-like activity. Here we demonstrate CNM-Au8's ability to promote neuronal survival and function in multiple independent in vitro models of ALS: (i) treatment of primary rat spinal motor neurons improves survival, preserves the neurite networks, and reduces cytoplasmic TDP-43 aggregate accumulation after either glutamate excitotoxic injury or exposure to beta-amyloid (Aβ 1-42) oligomers; (ii) treatment of spinal motor neurons from transgenic SOD1G93A rats protects motor neurons from death upon exposure to excitotoxic glutamate in a cAMP-dependent manner, and reduces SOD1 protein accumulation in a manner independent of cAMP; (iii) treatment of human induced pluripotent stem cell (iPSC)-derived neurons from C9ORF72 patients prevents their death in response to stress caused by mild neurotrophic factor withdrawal. Finally, we show (iv) survival and neurite outgrowth of human iPSC-derived motor neurons in co-culture with toxic, SOD1A4V ALS-patient derived astrocytes are substantially and dose-dependently improved with treatment of CNM-Au8. These results indicate that addressing the deficits of ALS with the energetic catalyst CNM-Au8 may be a promising new therapeutic strategy.

(j208) Selective Vulnerability of Tripartite Synapses in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder. Separate lines of evidence suggest that synapses and astrocytes play a role in the pathological mechanisms underlying ALS. Given that astrocytes make specialised contacts with some synapses, called tripartite synapses, we hypothesise that tripartite synapses could act as the fulcrum of disease in ALS. To test this hypothesis, we have performed an extensive microscopy-based investigation of synapses and tripartite synapses in the spinal cord of ALS model mice and post-mortem human tissue from ALS cases. We reveal widescale synaptic changes at the early symptomatic stages of the SOD1G93a mouse model. Super-resolution microscopy reveals subtle early-stage alterations in the size of postsynaptic nanostructures, followed by later stage loss of large multi-nanostructured synapses in ALS mice. Most surprisingly, tripartite synapses are selectively lost while non-tripartite synapses remain in equal number to healthy controls. Finally, we also observe a similar selective loss of tripartite synapses in human post-mortem ALS spinal cords. From these data we conclude that tripartite synaptopathy is a key hallmark of ALS.

(j209) Physiological tissue-specific and age-related reduction of mouse TDP-43 levels is regulated by epigenetic modifications

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TDP-43, a splicing factor belonging to the heterogeneous nuclear ribonucleoprotein family of proteins, is the main component in brain inclusions and is considered the histological landmark of amyotrophic lateral sclerosis and frontotemporal lobar degeneration. The TDP-43 cellular levels are tightly regulated, and experiments have shown that increases or decreases in these levels have deleterious effects in cells. The predominant mechanism responsible for the regulation of the TDP-43 levels is an autoregulatory negative feedback loop mediated by the binding of the TDP-43 protein to specific sensor sequences in the 3'UTR of its pre-mRNA. Previous observations have indicated the existence of additional physiological, age-related mechanism(s) that result in a reduction in the TDP-43 levels in specific tissues. This has been observed in distant species such as Drosophila and mice and could be relevant to the pathogenic mechanism leading to neurodegeneration. In this study, we identified an in vivo cause/effect relationship between TARDBP gene promoter methylation and specific histone modification and the TDP-43 levels in tissues of mice at different ages. These observations are pertinent for understanding the basic physiology of splicing during development and life of the organism and the pathogenic mechanisms leading to neurodegenerative diseases and pathologies in which an increase or decrease in the level of an RNA-binding protein has been implicated. For example, in amyotrophic lateral sclerosis, the formation of TDP-43-containing brain inclusions removes functional protein from the system. This phenomenon is continuous but compensated by newly synthesized protein. The balance between sequestration and new synthesis might become critical with ageing if accompanied by an epigenetic modification-regulated decrease in newly synthesized TDP-43. Sequestration by aggregates would then decrease the amount of functional TDP-43 to levels lower than those needed by the cell and thereby triggers the onset of symptoms.

(j210) The motoneuronal receptorome in ALS reveals adrenergic entry points to modulate MN excitability and firing

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Modulation (up- or downregulation) of motoneuron (MN) excitability and synaptic excitation constitutes an important entry point to affect MN degeneration in several MN diseases. We have previously demonstrated that chemogenetic interventions at the level of excitability and of PKA signaling exert profound beneficial effects on synaptic integrity and disease burden in ALS MN. In order to achieve a similar upregulation of PKA signaling and MN firing through natural receptor, we explored the PKA-coupled motoneuronal receptorome in ALS. Among the receptors prioritized by screening available databases (Allen Spinal Cord Atlans, GPCR database) in situ hybridization reveals that adenosinergic, histaminergic, cholinergic and several peptidergic receptors are downregulated, whereas beta-1 adrenergic receptor is distinctively upregulated and the expression of dopaminergic D5 and beta-2 and beta-3 adrenergic receptor are preserved. Importantly, activation of Dopaminergic and beta2/3 adrenergic receptors by selective agonists results in the increase in neuronal excitability and.... , suggesting a physiological role for dopaminergic and adrenergic inputs in the regulation of MN excitation.The ALS MN receptorome is nevertheless highly dynamic and all studied receptors are downregulated in advanced stages of disease. Notably, PKA stimulation and suppression of excitability are characterized by distinct receptoromes, with adrenergic beta-1 receptor systematically downregulated. Finally, the MN receptorome in ALS is substantially modified by pharmacological agents in clinical use. Our data show that MN display extensive entry points for modulation of their electrophysiological properties, which can be accessed with small molecules with translational potential for ALS treatment.

(j211) Post-transcriptional regulation of the fragile-x protein family in ALS

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Amyotrophic lateral sclerosis (ALS) is a highly heterogeneous disease and multiple pathogenic mechanisms contributing to the degeneration of motor neurons have been described. However, putative pathogenic cascades are frequently restricted to specific mutations or genes, and are not necessarily evident in patients with a different genetic cause or in sporadic cases. Little is known about converging disease mechanisms as well as early pathogenic events in ALS. Recent work suggests a role of the fragile-x protein (FXP) family (FMR1, FXR1 and FXR2) in ALS pathogenesis that is independent of the underlying cause of the disease. While human ALS spinal cord tissue revealed occurrence of pathogenic aggregates exclusively in motor neurons with low expression level of the FXPs, improved phenotypes of ALS in vivo models were reported upon overexpression of FMR1. Moreover, expression level of the FXPs were linked to the relative abundance of a subset of microRNAs downregulated in most ALS patients as well as in presymptomatic carriers of causative ALS mutations. Therefore, expression level of the FXPs may represent an early modifier of the ALS degenerative phenotype. Consequently, in this work, we aim at gaining insights into mechanisms regulating FXP expression. Considering that dysregulated FXP mRNAs have never been reported in ALS, we focus on post-transcriptional mechanisms. In a first step, we monitor FXP expression at the mRNA and protein level in HEK293 cells and mouse primary cortical neurons upon application of different stressors and upon overexpression of ALS-related proteins. In further experiments, conditions affecting FXP expression at the protein, but not at the mRNA level, will be used to identify factors regulating FXP translation.

(j212) Mislocalization induced changes in the TDP-43 interactome

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Introduction: TDP-43 is a DNA- and RNA-binding protein that is mainly located in the nucleus. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) it mislocalizes and is the predominant component of pathological cytoplasmic protein aggregates.

Objectives: In the present study, we investigated the interactome of wild-type TDP-43 and TDP-43 lacking nuclear localization signal (dNLS) mimicking a pathological condition in ALS.

Methods: We generated inducible mammalian cell lines stably expressing the recombinant fusion protein wtTDP-43 or dNLS-TDP-43 with the biotin ligase BioID2 and control cell line expressing only BioID2. After induction of BioID2 activity, the biotinylated proteins were isolated from the cell lysates by pull-down assay. The isolated proteins were then detected by Western blotting, silver staining, and mass spectrometry (MS). The MS analysis yielded a list of unique wtTDP-43 and dNLS-TDP-43 interactors that were validated by Western blot, immunocytochemistry, and proximity ligation assay (PLA). Cellular localization and function of the interactors were investigated using DAVID bioinformatics analysis.

Results: Proximity-dependent biotin identification (BioID) method followed by mass spectrometry revealed that wild-type TDP-43 interacts mainly with proteins of the ribonucleoprotein and spliceosome complexes, and with paraspeckles, whereas the interactors of mutant TDP-43 (dNLS-TDP-43) are components of cytoplasmic stress granules (SG) and processing bodies (P-bodies). Validation of selected interacting proteins (NONO, SFPQ, FUS, MAML1, PUM1, and ATXN2L) showed that MAML1 is unique TDP-43wt interactor, whereas NONO, SFPQ, and FUS are joint interactors of TDP-43wt and dNLS-TDP-43 and are more abundant in the TDP-43wt fraction. The interaction proteins ATXN2L and PUM1 are unique interactors of mutant TDP-43.

Conclusion: Protein interactions in the presence and absence of NLS resulted in a list of common and unique TDP-43 interacting proteins. Our results suggest that the pathological mechanisms leading to ALS may involve loss of regulatory functions related to transcription and/or paraspeckle function, on the one hand, and may be associated with SG and P-bodies due to their increased association with mutant dNLS-TDP-43 on the other hand. In addition, the novel interactors of TDP-43 identified in this study may also be involved in the aggregation mechanism or have neuroprotective effects.

(j213) Deciphering the effect of FUS mutations on motor neuron axonal pathology in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder with a lifetime risk of 1:400, and affecting upper and lower motor neurons. Loss of motor nerves leads to weakness of skeletal muscles, ultimately resulting in death 3-5 years after diagnosis due to respiratory failure. The most aggressive forms of ALS, associated with early-onset disease and rapid progression, are caused by genetic mutations in Fused in sarcoma (FUS; 5%). FUS is a RNA binding protein that regulates RNA metabolism, including transcription, splicing and RNA transport. Despite increasing knowledge of genetic mutations in ALS, the functional consequences of these mutations, such as in FUS, remain poorly understood. To unravel the downstream pathogenic mechanisms, this study explored the effects of FUS mutations on axonal pathology. To achieve this, induced pluripotent stem cell (iPSC) technology is combined with microfluidics. By using microfluidic devices that consist of two chambers that are connected via microchannels, motor neuron axons are separated from the soma. Therefore, the use of compartmentalized microfluidic devices allows specific analysis of axonal pathology, such as outgrowth defects, impaired axonal transport and changes in the axonal transcriptome. RNA sequencing of the somatodendritic and axonal compartment has demonstrated that the axonal transcriptome is distinct between the different cellular compartments. Biological processes upregulated in axons are related to membrane transport, while genes expressed in the cell soma are involved in RNA and protein metabolism. To study FUS toxicity in motor neurons, axonal RNA was isolated from motor neurons generated from iPSCs derived from FUS-ALS patients and healthy controls. Sequencing of these RNA samples will help us to determine the effect of FUS mutations on the axonal transcriptome that might contribute to motor neuron degeneration and ALS progression. Understanding these underlying pathways may open up new avenues for developing novel, effective treatments for ALS.

(j214) Protein Homeostasis with a Twist: Assessing the Role of Triple Helical Structures for Ubiquitin Recognition by the Ubiquitin Receptor Ubiquilin-2.

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Most ALS cases are sporadic, with only 5-10% of cases caused by an inherited genetic mutation. Mutations in Ubiquilin 2 (UBQLN2) are one such example, resulting in a rare form of juvenile X-linked ALS. UBQLN2 is a ubiquitin receptor involved in protein degradation through the Ubiquitin Proteasome System. Of interest is the presence of UBQLN2 positive aggregates in ALS patients with no genetic mutation in UBQLN2. This reaffirms the belief that UQBLN2 activity is central to cellular homeostasis and protein degradation within motor neurons. Proteins can be degraded through a variety of mechanisms, but the common factor is ubiquitin modifications. The type and length of ubiquitin modification governs the ultimate fate of the protein. For ubiquitin receptors to function correctly they must be able to bind to and differentiate between the various ubiquitin tags. This differentiation can be achieved through Ubiquitin-binding Domains (UBA Domains). Whilst some ubiquitin receptors contain numerous UBA domains to increase binding affinity and confer specificity, others form multimers. UBQLN2 contains only one UBA domain, which when studied in isolation displays poor affinity and no specificity to ubiquitin chains. This therefore begs the question of how UBQLN2 is functioning, at a molecular level, as a ubiquitin receptor. UBQLN2 contains a Proline rich repeat (PRR) region, the function of which is yet to be determined. Interestingly, most ALS-causing mutations reside in this region. Upon closer inspection, the PRR region bears similarities to bacterial collagen-like proteins, which trimerize via their PRR domains. We hypothesise that UBQLN2 trimerizes via its PRR region to modulate the affinity and specificity of ubiquitin binding. Circular Dichroism data indicates a previously undescribed secondary structure for the PRR region and investigations by FPLC, AUC, CD and Native PAGE suggest evidence of multimeric PRR regions, potentially similar to a collagen-like triple helix. CD data of the synthesised PRR region alone confirms a collagen triple helix for the WT sequence whilst the ALS mutant resulted in a largely unstable, disordered protein. Through protein engineering we have designed a novel, stable trimeric structure to aid in NMR spectroscopy, with the intention of determining the structure, inferring the function of the PRR region of UBQLN2 and elucidating its contribution to the development of ALS.

(j215) Positive effect of exosomes derived from permanently growing human MSC on primary murine ALS motor neurons

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Introduction & Objectives: In recent years, the neuroprotective potential of mesenchymal stroma-/stem-like cells (MSCs) as well as of MSC-derived exosomes has been intensively explored. Several studies have reported that MSC-derived exosomes have similar properties compared to MSCs, such as stimulation of tissue regeneration or reduction of inflammation. [1] Besides extracellular vesicles (EVs) MSCs release trophic factors and thereby modify cell-to-cell communication. These cell-free products may contribute to protect degenerating motor neurons (MNs) and represent a potential therapeutic approach for ALS. [2]

Method: EVs (microvesicles and exosomes) were isolated from permanently growing subconfluent human MSC544 cultures according to a modified protocol by Thery et al.[3] and the updated MISEV (minimal information for studies of extracellular vesicles) 2018 standards, to obtain enriched exosomes[4] . Protein aliquots of these exosomes were quantified using the colorimetric BCA assay.To investigate the effect of human MSC exosomes on murine primary MNs, we isolated MNs from spinal cord of G93A-SOD1 mice (E 12,5) using previously described protocols [5] to yield MNs and cultivated them for six days. Two days after seeding, MNs were incubated with 1,4*106 exosomes for 72h and then exposed to staurosporine (0,5µM) for 18h. We assessed the protective effects of exosomes by measuring cell viability and via Immunocytochemistry of wildtype and transgenic MNs.

Results: Initial results show that exosomes reduced the oxidative damage caused by staurosporine and preserve characteristic MNs morphology.

Conclusions: While ALS remains incurable, it is important to study potential novel therapeutic approaches to counteract neurodegeneration such as ALS. Thus, MSC-derived exosomes may exhibit distinct neuroprotective effects and provide advantages as cell-free products regarding handling and safety in clinical trials which is worth further preclinical evaluation.

References: [1] https://www.mdpi.com/1422-0067/15/3/4142[2] Frontiers | ALS Pathogenesis and Therapeutic Approaches: The Role of Mesenchymal Stem Cells and Extracellular Vesicles | Cellular Neuroscience (frontiersin.org)[3] https://pubmed.ncbi.nlm.nih.gov/18228490/[4] PMID: 33023058[5] https://www.nature.com/articles/nprot.2009.1

(j216) Novel Chemical Chaperones as a Potential Therapeutic Strategy for ALS

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease, characterized by the degeneration of upper and lower motor neurons (MNs) in the brain and spinal cord. About 20% of ALS familial cases are caused by mutations in superoxide dismutase (SOD1) gene. Although the mechanisms underlying SOD1 pathogenesis remain elusive, studies suggest SOD1 toxic gain of function through protein misfolding and formation of amyloid aggregates. One of the possible therapeutic strategies for these protein misfolding-related disorders are chemical chaperones. For instance, the chaperone sodium 4-phenylbutyrate acid (4-PBA), combined with taurursodiol, was recently tested in clinical trials for ALS. Here, we investigated the potential therapeutic effect of C4 and C5, two 4-PBA derivatives. By combining in vivo and in vitro techniques, we show that although C4 and C5 successfully inhibited amyloid aggregation of recombinant mutant SOD1 in a dose dependent manner, they failed to suppress the accumulation of misfolded SOD1 in vitro. Moreover, C4 or C5 daily injections to symptomatic SOD1G93A mice, had no effect on the accumulation of misfolded SOD1 or the neuroinflammatory response in the spinal cord and therefore failed to extend the survival of SOD1G93A mice or improve their motor symptoms. Our findings suggest that suppressing the accumulation of soluble misfolded SOD1 is likely required to slow down the progression of the disease in a mouse model of ALS.

(j217) Neurochondrin, an essential neural protein, has a potential role in Spinal Muscular Atrophy pathology. Could its relevance extend to other MNDs?

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Spinal muscular atrophy (SMA) is an inherited child motor neuron disease where the main pathological feature is progressive degeneration of motor neurons in the anterior horn of the spinal cord. The cause of SMA is a reduction in functional Survival Motor Neuron protein (SMN). Exactly why a reduction in SMN results in SMA is still under investigation, with two theories at the forefront of interest. Firstly, SMN forms part of the SMN complex which plays an essential role in small ribonucleoprotein (snRNP) assembly. Specifically, SMN is involved in the addition of the Sm family of proteins to splicing snRNPs. Splicing snRNPs are required for correct splicing of mRNA – therefore mRNA processing defects have been proposed to cause SMA. Secondly, SMN has also been linked to mRNA transport along the axons of motor neurons for local translation – therefore defects in neural mRNA trafficking have also been proposed to cause SMA. It is conceivable that loss of SMN affects multiple pathways that converge upon SMA pathogenesis, as SMN is a multi-functional RNA binding protein. Here we show NCDN interacts with Sm proteins (core snRNP proteins) and SMN in trafficking vesicles of neural SH-SY5Y cells and mouse brain with additional evidence of NCDN being enriched in the motor neurons of mice spinal cord. We also demonstrate NCDN is required for the correct localisation of SMN and vice versa, suggesting both NCDN and SMN are required for formation and transport of neural trafficking vesicles. These results identify a potentially important role for NCDN that may be implicated in SMA. Interestingly, Nicolas, G. et al (2022) report an interaction and co-dependency for correct localisation within the cell between NCDN and FUS, a protein implicated in Amyotrophic lateral sclerosis (ALS), aka motor neurone disease, and other neurodegenerative diseases such as Frontotemporal dementia. This recent finding combined with previous reports of an interaction between SMN and FUS (e.g., Yamazaki, T. et al (2012)) could support some common links between various MNDs. Further investigation into the functions of Neurochondrin could reveal additional relevancies of this protein in MNDs and in turn provide a novel target across MNDs.

(j218) TDP-43 M337V mESC-derived motor neurons demonstrate impaired mitochondrial respiration and glycolysis in response to oxidative stress

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Dysregulation of cellular energy metabolism has become increasingly recognised as a hallmark of ALS. Impaired mitochondrial respiration has been reported across a number of ALS models, with upregulation of glycolysis thought to be neuroprotective. Mouse embryonic stem cell-derived motor neurons (mESC-MNs) provide an excellent model for high-throughput phenotypic screening. Our group has previously developed a BAC transgenic mESC-MN model that expresses TDP-43 WT or TDP-43 M337V at low levels. In this model, TDP-43 M337V mESC-MNs have been shown to demonstrate altered TDP-43 interactions, dysregulated stress granule characteristics and reduced survival in response to oxidative stress compared to controls. Here, we aim to investigate dysregulation of cellular energy metabolism in TDP-43 M337V mESC-MNs, and to examine the effects of a pro-survival drug on mitochondrial respiration and glycolysis. Mouse ESCs were expanded as embryoid bodies and differentiated to MNs. Analysis of the TDP-43 M337V interactome and Western blotting were performed to assess TDP-43 interactors and the expression of mitochondrial and glycolytic proteins. Mitochondrial respiration and glycolysis were examined in unstressed and oxidative stress conditions, using the Seahorse XF Analyser. To examine the effects of a pro-survival drug, mESC-MNs were treated at optimised concentrations 24-hours prior to analysis. Examination of the TDP-43 M337V interactome revealed dysregulated interactions with transcripts related to mitochondria and glycolysis. We also observed upregulation of certain mitochondrial proteins and glycolytic enzymes in TDP-43 M337V mESC-MNs. We observed no differences in mitochondrial respiration or glycolysis in unstressed conditions, however following oxidative stress we observed a significant reduction in basal glycolysis, compensatory glycolysis, maximal respiration and spare respiratory capacity in TDP-43 M337V mESC-MNs relative to controls. Treatment with a potential therapeutic agent significantly increased cell viability and basal glycolysis, whilst significantly reducing basal respiration in TDP-43 M337V mESC-MNs. In this study we have identified metabolic dysregulation in TDP-43 M337V mESC-MNs following oxidative stress. Treatment with a pro-survival drug leads to a significant increase in basal glycolysis accompanied by a significant decrease in basal respiration, suggesting this drug may induce neuroprotective effects through inducing a metabolic shift.

(j219) Localization and interactions of hnRNPH in nuclear C9ORF72 G4C2 foci and cytoplasmic stress granules

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hnRNPH is a member of a large protein family of RBPs with important functions in nucleic acid metabolism in normal and diseased mechanisms. They are involved in regulation of alternative splicing, mRNA stabilization, transcription, and translation. In amyotrophic lateral sclerosis (ALS) brain tissues, hnRNPH colocalizes with nuclear RNA foci derived from the G4C2 hexanucleotide repeat expansion in the C9orf72 gene. Under cellular stress conditions, the protein localizes to cytoplasmic stress granules. Sequestration of hnRNPH in insoluble RNA aggregates correlates with dysregulation of splicing and may contribute to neurodegeneration. Nuclear foci share a group of interacting proteins with stress granules and their simultaneous presence in ALS neurons may have further pathological implications. The aim of this study was to decipher which domains of hnRNPH determine its localization in the G4C2 foci and stress granules. A series of hnRNPH1 protein constructs were designed based on its domain structure and individual qRRM domains were mutated to disable their RNA binding activity. Quasi (q)RRM2 and qRRM3, but not qRRM1, are sufficient for the localization of hnRNPH in stress granules. Localization of hnRNPH in G4C2 foci is independent of the RNA-binding activity of any individual qRRM domain. Using RBDmap, we provide evidence for RNA binding activity of the putative ZnF domain of hnRNPH. Surprisingly, hnRNPH protein still localized to G4C2 foci even when the RNA-binding activity of the qRRM and ZnF domains was genetically ablated, suggesting that RNA-binding activity is not required for sequestration of hnRNPH in G4C2 foci. These results suggest that RNA binding may not be the only driving force for sequstration of hnRNPH into the G4C2 foci associated with C9orf72 ALS.

(j220) C9ORF72-ALS patient-derived iPSC microglia show C9orf72 haploinsufficiency, express Poly(GA)/(GP), and have a pro-inflammatory profile

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Introduction: A growing body of evidence supports a role for neuroinflammation in ALS pathophysiology, with microglia particularly implicated (1). In C9ORF72-ALS patients, widespread microglial activation correlates with disease progression (2). C9orf72 knock-out mice show a pro-inflammatory state in myeloid cells and microglia (3, 4). However, it remains unclear whether these results are recapitulated in human microglial cells, in which the hexanucleotide repeat expansion in C9ORF72 is thought to result in C9ORF72 haploinsufficiency and RNA foci and dipeptide repeat formation.

O**bjective:** This project aims to analyse the phenotype of C9ORF72 iPSC-derived microglia.

Method: We differentiated iPSC-derived microglia from three C9ORF72-ALS patients, three healthy controls, and one isogenic line (5) as described previously (6), with and without LPS treatment. The presence of Poly(GA)/(GP) was determined by MSD ELISA. RNA-sequencing was performed to identify transcriptomic changes. pHrodo zymosan particles were used to analyse phagocytic activity.

Results: iPSC-derived microglia displayed microglial morphology with no morphological differences between C9ORF72 and control microglia. All lines expressed key microglial markers in RNA-sequencing and by qPCR, with reduced expression of the homeostatic marker P2RY12 in C9ORF72 microglia. Poly(GA)/(GP) were detectable in C9ORF72 microglia but absent in healthy and isogenic controls, while C9ORF72 protein expression was significantly reduced in C9ORF72 microglia compared with healthy controls, indicating C9ORF72 haploinsufficiency. Transcriptomic analysis demonstrated enrichment of several pathways in C9ORF72 microglia including immune cell activation, chemo-/cytokine activity, and phago-/endo-/lysosomes. Specifically, we found altered gene expression of several key inflammatory chemo-/cytokines such as IL1a, IL1b, IL6, IL10, and CHIT1 in C9ORF72 microglia. Finally, C9ORF72 microglia showed enhanced uptake of pHrodo zymosan particles, indicating increased phagocytosis.

Conclusion: These findings provide preliminary evidence for a pro-inflammatory phenotype in C9ORF72 microglia, possibly through the combination of C9ORF72 loss-of-function and gain-of-function toxicity.

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(j221) Pathological aggregation and exovesicle concentration in patient derived ALS model are reduced upon treatment with TDP-43 modulators

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Patient derived models of neurodegenerative diseases allow to advance towards personalized diagnosis and treatments by using specimens directly obtained from patients.[1] Amyotrophic Lateral Sclerosis (ALS) lymphoblasts have shown to recapitulate TDP-43 pathology and are an ideal model to further study the disease and test novel compounds. [2]

Here we have studied two prominent features in ALS: protein aggregation and the role of extracellular vesicles. First, using a simple turbidimetric assay, we have studied the total protein aggregation in healthy controls and patients lymphoblasts, and its variation upon TDP-43 modulators treatment. Results show how patients present a higher protein aggregation compared to healthy controls. In addition, we also prove that this pathological aggregation can be rescued after a treatment with different TDP-43 modulators. This methodology enables a rapid evaluation of promising drug candidates, and can be further used as a drug screening platform.

In order to study the spreading of the TDP-43 pathology, we have analyzed the extracellular vesicles secreted from this model. We have observed how ALS lymphoblasts have a higher concentration of extracellular vesicles, and how the treatments with TDP-43 modulators are able to recover this pathological profile. Finally, TDP-43 has been quantified using a TR-FRET assay showing a correlation with both pathological events that is also modulated upon pharmacological recovery.

(j222) TDP-43 function is relevant in MAM-dependent phospholipid synthesis

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Introduction: TDP-43 could contribute to amyotrophic lateral sclerosis in different cellular compartments like mitochondria. Mitochondria show intimate contact with endoplasmic reticulum (ER) domains termed mitochondrial-associated membranes (MAMs). These contacts between both organelles are involved in several cellular functions, such as phospholipid synthesis. Our recent finding that TDP-43 is located in MAMs led us to evaluate the possible implication of this protein in phospholipid synthesis.

Objective: To evaluate the potential implication of TDP-43 in MAM-dependent phospholipid synthesis and infer its possible dysfunction in ALS.

Method: We have evaluated the MAM-dependent phospholipid synthesis by metabolic (PtdSer to PtdEtn conversion) in different ALS models: transgenic B6N.Cg-Tg(Prnp-TARDBP*Q331K)103Dwc/J (TDP-43 Q331K) mice (both brain and spinal cord), mouse embryonic fibroblasts different TDP-43 mutations (TDP-43Q331K, TDP-43F210I , TDP-43M323K), and a HeLa-PLKO Dox system to induce TDP-43 silencing.

Results: Our results show that both TDP-43 mutations and expression can affect the correct function of MAMs. The decrease of TDP-43 levels in HeLa-PLKO cells induced a severe reduction of PtdSer and PtdEtn levels. In the same way, mouse fibroblasts showed mutation-dependent changes in these activities. Of note, transgenic mice overexpressing human TDP-43Q331K show tissue and age-dependent differences in this MAM activity.

Conclusion: Abnormalities in TDP-43 affect the correct function of phospholipid biosynthesis residing in MAMs, both in transgenic mice and in in vitro models of ALS, potentially contributing to dearrangements in membrane lipids.

(j223) Defining the contribution of TBK1 activity in cellular response to cytotoxic stress

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Mutations in serine/threonine protein kinase TANK Binding Kinase 1 (TBK1) are associated with familial and sporadic forms of amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and co-existent ALS/FTD. Familial mutations in TBK1 result in reduced expression of the affected allele, suggesting a loss of critical function of this kinase underlies neurodegeneration onset or progression. TBK1 contributes to a broad repertoire of molecular signalling cascades, with defined targets including proteins associated with autophagy, mitophagy and immune response, processes with essential roles in protecting against neurodegeneration relevant insults. Defining which of these TBK1 regulated mechanisms significantly contribute to pathology will be essential in understanding and therapeutically intervening in ALS/FTD patients with mutations in this gene. Focusing on neuron relevant biology, we first demonstrated that pharmacological inhibition of TBK1 kinase activity does not significantly impact on baseline viability, morphology and proteome of neuroblastoma cells. As these findings suggest loss of TBK1 activity alone is insufficient to induce degenerative phenotypes, we next aimed to understand how loss of TBK1 function may render cells vulnerable to neurodegeneration relevant cytotoxic stresses. We conducted an microscopy based live-dead cytotoxicity screen in neuroblastoma cells pharmacologically inhibited for TBK1 activity. Our screen revealed a role from TBK1 in protecting neurons from cell death induced by disruption of lysosomal homeostasis and endoplasmic reticulum calcium stress. We are currently working to define the molecular and functional contribution of TBK1 in regulating these critical cellular processes, and generating cells genetically disrupted from TBK1 expression. Our findings suggest TBK1 contributes to the regulation of important cellular processes required to protect against specific forms of cytotoxic stress, suggesting potential future targets for therapeutic intervention.

(j224) Restoring ER-mitochondria tethering rescues TDP-43-linked damage to Ca2+ signaling

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Signaling between the endoplasmic reticulum (ER) and mitochondria regulates many of the functions that are damaged in ALS. These include mitochondrial bioenergetics, lipid metabolism, axonal transport, and synaptic function. This signaling is facilitated by close physical contacts between the two organelles that are mediated by the VAPB-PTPIP51 "tethering" proteins. VAPB is an integral ER protein that binds to the outer mitochondrial membrane protein PTPIP51 to form these "tethers". A number of familial genetic ALS insults have now been shown to disrupt ER-mitochondria contacts and signaling; these include mutant TDP-43, FUS, C9ORF72, Cu/ZnSOD1 and Sigma1 receptor. For TDP-43, C9ORF72 and FUS, the disruption has been shown to involve breaking of the VAPB-PTPIP51 tethers. A primary function of the VAPB-PTPIP51 tethers is to facilitate IP3 receptor mediated delivery of Ca2+ from ER stores to mitochondria. This is important as it regulates the ability of mitochondria to generate ATP since dehydrogenases in the TCA cycle are Ca2+ dependent. Indeed synaptic activity which is one of the most energy dependent functions in mammals, is dependent on the VAPB-PTPIP51 tethers. To determine whether restoring proper ER mitochondria contacts can rescue TDP-43 induced damage to IP3 receptor mediated delivery of Ca2+ to mitochondria, we monitored mitochondrial Ca2+ levels in neuronal cells expressing TDP-43 and either VAPB, PTPIP51 or an artificial tether. Increased expression of VAPB, PTPIP51 or the artificial tether have all been shown to increase ER-mitochondria contacts. We find that restoring tethering, rescues TDP-43 linked damage to mitochondrial Ca2+ levels. Our results have important implications for drug discovery programmes that aim to restore mitochondrial bioenergetic potential in ALS.

Supported by grants from the MRC, Alzheimer's Research UK and the Alzheimer's Society.

(j225) Maintaining ER- mitochondria contacts is neuroprotective in preclinical models of ALS

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The mechanism underlying neurodegenerative diseases (NDs) remains elusive and it has been challenging to find effective therapeutic targets. Several cellular processes have been connected to neurodegeneration such as altered RNA processing, protein aggregation, mitochondrial dysfunction and axonal transport defects, nevertheless it has been difficult to delineate causal from consequential pathogenic alterations. Previous studies from our group have shown strong evidence that motoneurons (MNs) in ALS are selectively vulnerable to endoplasmic reticulum (ER) stress and mitochondrial dysfunction. A critical question that we have examined is whether homeostatic changes in the ER affects other cellular organelles, thereby directly contributing to the disease stage dependent pathological changes. Here, focusing on the early intrinsic vulnerability of motoneurons to ER stress and its repercussion on the mitochondrial-ER associated membranes (MAMs), that has been reported to be disrupted in several NDs. Using MNs differentiated from C9ORF72 patients induced pluripotent stem cells (iPSCs), we have identified a MAM linker molecule GRP75 that serves to not only physically connect the two cellular compartments, but whose transient enhanced expression has a neuroprotective effect during the progression of the pathology. Moreover, taking advantage of the C9orf72 pre-clinical rodent model, we performed pharmacological and viral mediated modulation of ER stress and provide evidence that the ER compartment is critical for optimal mitochondrial function and restoring the physiological levels of ER stress-mitochondria contacts is able to rescue mitochondrial deficits. Lastly, we validate the therapeutic potential of GRP75 via gene therapy using adeno-associated viruses (AAVs) thereby highlighting a promising therapeutic target for ALS.

(j226) C9orf72 ALS/FTD dipeptide repeat protein levels are reduced by small molecules that inhibit PKA or enhance protein degradation

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Intronic GGGGCC (G4C2) hexanucleotide repeat expansion within the human C9orf72 gene represents the most common cause of familial forms of amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) (C9ALS/FTD). Repeat-associated non-AUG (RAN) translation of repeat-containing C9orf72 RNA results in the production of neurotoxic dipeptide-repeat (DPR) proteins. DPR proteins misfold and aggregate into cytoplasm or nuclei of motor neuron. Here they can alter the proteotoxic response machinery. The protein quality control (PQC) system maintains protein homeostasis by re-folding (by chaperone) or by degradation (by autophagy or proteasome) of misfolded proteins to counteract proteotoxicity. We developed a high-throughput drug screen for the identification of positive and negative modulators of DPR levels. In NSC34 cells we evaluated the role of the selected compound in the regulation of the two main degradative pathways of PQC. Using RT-qPCR, WB and IF analysis, we observed that none of the compounds were able to modulate TFEB, SQSTM1/p62, and LC3 expression and localization. Nevertheless, the reduction of DPR levels observed in cells treated with geldanamycin (an HSP90 inhibitor) and with spironolactone (an aldosterone antagonist) is counteracted by autophagy and proteasome inhibitor suggesting that these compounds promote DPR proteins degradation via the proteasome and autophagy pathways respectively. Surprisingly, cAMP-elevating compounds boosting protein kinase A (PKA) activity increased DPR protein levels. Inhibition of PKA activity, by both pharmacological and genetic approaches, reduced DPR levels in cells and rescued pathological phenotypes in a Drosophila model of C9ALS/FTD. Moreover, knockdown of PKA-catalytic subunits correlated with reduced translation efficiency of DPRs, while the PKA inhibitor H89 reduced endogenous DPR protein levels in C9ALS/FTD patient-derived iPSC motor neurons. Together, our results suggest new and druggable pathways modulating DPR protein levels in C9ALS/FTD.GRANTS: FONDAZIONE CARIPLO, FONDAZIONE ARISLA, FONDAZIONE TELETHON, and Kennedy's disease association.

(j227) Dynamic Expression Profiles of Stressed iPSC-MNs by Translating Ribosome Affinity Purification (TRAP) from C9orf72-ALS Patients

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Introduction: The G4C2 Hexanucleotide Repeat Expansion (HRE) in the gene C9orf72 is the commonest genetic cause of Amyotrophic Lateral Sclerosis (ALS). The intronic repeat RNA accumulates intracellularly as RNA foci and is translated non-canonically into dipeptide repeat proteins, both of which have been shown to affect RNA metabolism. Furthermore, impaired global translation and perturbed stress granule dynamics have been associated with C9orf72-ALS. We hypothesize that the C9orf72 HRE leads to differences in translating mRNAs (translatome) in motor neurons at baseline, after transient oxidative stress or recovery.

Methods: In this study, we captured the translatome by Translating Ribosome Affinity Purification (TRAP), which immunoprecipitates ribosome-bound mRNAs by a tagged exogenous ribosomal protein (RPL-22) introduced by lentiviral transduction. We optimized TRAP with a ubiquitous promoter in iPSC-MNs from three C9orf72-ALS patients and three age- and gender-matched healthy controls. Oxidative stress was induced by 0.5 mM sodium arsenite treatment for one hour, and then removed for recovery for up to 24 h prior to harvesting.

Results: We validated expression of exogenous proteins in iPSC-MNs and showed little impact from lentiviral transduction on the transcriptome. Enriched RNA pull-down was observed in the TRAP samples (IP) of the transduced group versus the non-transduced group. RNA-Seq on whole RNA samples also confirmed that the IP samples were depleted of certain types of non-coding RNAs as well as mitochondrial RNAs, which are translated by mitochondrion-specific ribosomes. Compared with the transcriptomic input fraction (IF) samples, the IP samples reflected earlier changes after stress and recovery for 2 h. Dysregulated global translational activity after 4 h and 24 h of recovery was observed in one C9orf72-ALS line versus control measured by modified SUnSET assay ($p = 0.025$ and $p= 0.003$ respectively, independent t test).

Conclusion and Plans: This study successfully applied TRAP in iPSC-MNs. This method provides a tool to investigate the dynamic changes in translatome of C9orf72-ALS iPSC-MNs at baseline, after stress and recovery.

(j228) Senescent astrocytes drive neurodegeneration via extracellular vesicles in ALS-FTD.

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ALS is a non-cell autonomous neurodegenerative disease characterized, among other factors, by altered intercellular communication and nucleocytoplasmic transport defects. Glial cells contribute to ALS pathology and play a key role in the progression of the disease, but we still lack a thorough understanding of the underlying molecular mechanisms. Cell cycle dysregulation and senescence are gaining increasing attention in the field of neurodegenerative diseases. In this context, a recent publication in Cell has identified p53, a transcription factor involved in cell cycle regulation, as a driver of neurodegeneration in several C9orf72 ALS models. In the last years, our lab has been investigating the molecular mechanisms of glia-to-neuron miscommunication in a model of ALS. One of the possible ways in which cells communicate with each other is through extracellular vesicles (EVs), nanoparticles that transport proteins, lipids, and nucleotides from one cell to the other. Using transgenic mice expressing mutant TDP-43 Q331K and RNA sequencing, we observed that EVs derived from mutant astrocytes are sufficient to induce DNA damage and death in wild-type (WT) neurons. We analyzed the proteome of EVs derived from WT and mutant astrocytes by mass spectrometry and found that the protein cargos differ significantly between control and disease conditions. Interestingly, many differentially enriched proteins are linked to cell cycle progression. Importantly, TDP-43 Q331K astrocytes show reduced proliferation in vitro and increased senescence-associated beta-galactosidase (SA-beta-gal) compared to WT astrocytes suggesting that prematurely senescent glial cells might participate in neurodegeneration.

(j229) AMPK regulates ER-mitochondria tethering

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An estimated 5–20% of mitochondria are closely associated with a subdomain of the ER called mitochondria-associated ER membranes (MAMs). Inter-organelle communication at these contact sites has been shown to regulate several physiological processes including calcium homeostasis, autophagy/mitophagy, mitochondrial dynamics, phospholipid synthesis, the unfolded protein response, apoptosis, and inflammasome activation. Perturbations to ER–mitochondria contact sites are a common phenomenon in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We and others have demonstrated reduced ER–mitochondria coupling in mutant SOD1, TDP-43, FUS, C9orf72, and Sigma-1R-associated ALS/FTD. How ER–mitochondria tethers are formed and regulated is still poorly understood, but we and others have been identified a number of the proteins involved in their formation and maintenance. ER–mitochondria contacts are established by protein tethers that physically link the opposing faces of the ER and the outer mitochondrial membrane (OMM). We have shown that a complex made up of vesicle-associated membrane protein-associated protein B (VAPB) on the ER membrane and protein tyrosine phosphatase-interacting protein 51 (PTPIP51) on the OMM is a MAM tether that physically links the two organelles. The VAPB/PTPIP51 tether appears to be of particular importance in ALS/FTD because reduced VAPB/PTPIP51 interaction has been observed to be the direct cause of the observed disruption in ER–mitochondria communications. The aim of this study was to investigate regulatory mechanisms that govern ER–mitochondria interactions via the VAPB/PTPIP51 tether. Our results reveal a novel role of AMP-activated protein kinase (AMPK), a key energy sensor that is involved in the maintenance of energy homeostasis, in the regulation of ER-mitochondria contacts. We showed that VAPB/PTPIP51 interaction increases in an AMPK-dependent way under energy stress conditions caused by mitochondrial dysfunction. Furthermore, activation of AMPK was sufficient to drive increased VAPB/PTPIP51 interaction in the absence of energy stress or mitochondrial damage. Pharmacological modulation of AMPK, and consequent alterations in ER–mitochondria contacts, may represent a promising therapeutic target for ALS/FTD.

(j230) Chemical Tools to Investigate the Molecular Mechanisms of HDAC6 in Health and Disease

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Histone Deacetylase 6 (HDAC6) is localized in the cytoplasm and deacetylates various, non-histone proteins such as α-tubulin, Miro1, HSP90, peroxiredoxins and cortactin. In addition, it is also binding with high affinity to ubiquitinated proteins as well as motor proteins. Pharmacologically inhibiting the deacetylating function of HDAC6 rescues axonal transport deficits in several preclinical models of ALS, as well as peripheral neuropathies. However, the underlying mechanisms and exact expression patterns of HDAC6 are still unknown. Therefore, the aim of this study was to design and synthesize a library of fluorescent HDAC6 ligands based on the structure-activity relationship of HDAC6 inhibitors. The compounds still inhibited to a certain degree the deacetylase function of HDAC6 as demonstrated by increased levels of α -tubulin in neuronal-like cells and biochemical in vitro assays. The fluorescent ligands showed a cytoplasmic staining in fixed and living cells and optimization of the imaging properties (i.e. signal-to-background ratio and brightness) allowed the visualization of endogenous HDAC6 in living cells. We detected an increased signal when overexpressing HDAC6 in cells, confirming the HDAC6-selectivity. High resolution imaging revealed a microtubule-associated signal of the HDAC6-binding compounds. Live imaging of the fluorescent compound visualized HDAC6 movements between the cell soma and neurites in patient-derived iPSC motor neurons (FUSP525L). The signal was less intense in the mutant motor neurons in comparison to their isogenic controls, indicating subtle differences in HDAC6 localization. In conclusion, we have developed a fluorescent compound which is able to visualize the localization and movement of HDAC6 in cells. Further research in disease-relevant, cell-based and animal-based models enable to monitor endogenous HDAC6 localization, distribution and dynamics in a live cell environment. This will lead to a better understanding of the mechanisms of HDAC6 inhibition, its rescuing effect on axonal transport deficits and its therapeutic potential in ALS.

(j231) In ALS dysfunction of nucleoporin 107 impairs autophagy contributing to TDP-43 aggregation

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Nucleocytoplasmic communication is altered in ALS. Nucleoporins (NUPs)are essential components of this transport. Previous results indicate that NUPs homeostasis may be changed in ALS. This work has studied the potential relationship between protein aggregation (using TDP-43 as a paradigm), NUPs subcellular distribution, and cellular stress response. To do this, we analyzed the levels of several NUPs by immunodetection techniques in isolated tissues and nuclei extracted post mortem from ALS patients and a transgenic murine model of ALS in several stages of the disease and both genders. In addition, we performed cell culture studies to elucidate the possible mechanisms that influence NUPs-mediated TDP-43 dysregulation. The results demonstrate changes in the levels of NUPs involved in recognizing transporter proteins in both post-mortem tissues from ALS patients and in mice showing MN demise. On the other hand, the silencing of one of the NUPs, NUP107, caused an increase in the levels of TDP-43 and its phosphorylation and an increase in the formation of its cytoplasmic aggregates. In addition, this was associated with autophagic response alterations, evidenced by the rise of LC3II, p62, and the levels of general protein ubiquitination. Similarly, oxidative stress and osmotic stress in vitro caused an increase in the pathological characteristics of TDP-43 mentioned above, an increase associated with changes in the expression of NUPs and their cellular distribution. These findings demonstrate that the deterioration of NUPs in the ALS framework may contribute to the alteration of intracellular traffic resulting in the aggregation of the protein involved in motoneuronal neurodegeneration, such as TDP-43.

(j232) Mutations in the ALS-causative genes FUS and TDP-43 cause distinct dysregulation of somatic and axonal transcriptomes

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Most amyotrophic lateral sclerosis (ALS) cases appear sporadic with unknown etiology, but ALS can also be caused by mutations in different genes. Even if the diverse ALS causative genes result in distinct intracellular mechanisms that leads to motor neuron loss, they all share an early denervation at the neuromuscular junction (NMJ) with degeneration of the distal axon preceding the loss of the somas. This indicating a dysregulation that may start or end in the axons but offer us a potential common therapeutic target across mutations. Indeed, the mechanisms governing this process are largely unknown, but we hypothesize that an early dysregulation in the axon transcriptome can be involved in the axonal loss. To reveal the mechanisms driving early axonal pathology, we conducted RNA sequencing of motor axons and somas of human motor neurons grown in microfluidic devices. We used human isogenic, induced pluripotent stem cell (iPSC) lines with homozygous, ALS-causative FUSP525L and TDP-43M337V mutations introduced by CRISPR/Cas9. We demonstrate that the ALS-causative mutations in FUS and TDP-43 induce mostly unique patterns of RNA dysregulation that are distinct between soma and axon. This reveals that different pathways are involved in the initiation of motor neuron pathology due to FUS and TDP-43 mutations and that axons and somas may need to be treated individually in disease for optimal protection of the motor neurons.

(j233) Dysregulation of extracellular vesicle formation and release in astrocytes from ALS patients.

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Introduction: In ALS, astrocytes exhibit a toxic phenotype that mediates motor neuron degeneration. In vitro, astrocytes exhibit toxicity through both cell-to-cell contact and secreted factors, thus indicating that inter-cellular communication is dysregulated. Extracellular vesicles (EVs) represent one of many modes of communication between astrocytes and neurons. The 4G2C hexanucleotide repeat expansion in the gene C9orf72 represents the most common genetic cause of ALS. Work published by our group shows that astrocytes generated from C9orf72-ALS patient fibroblasts have impaired secretion of EVs, that mediate neuronal toxicity compared to healthy control astrocytes. More recently C9orf72 has been reported to interact with proteins associated with endosomal trafficking and EV secretion.

Aim: We aim to determine which part of the EV formation and secretion pathway is impaired in C9orf72-ALS patient derived astrocytes.

Methods: Human induced astrocytes were generated from three unaffected individuals and three individuals carrying the hexanucleotide repeat expansion in the C9orf72 gene. The protein levels of early and late endosomal markers were measured via western blotting. The morphological characterisation of early endosomes was conducted using confocal microscopy, whilst multivesicular bodies and lysosomes were manually quantified blinded based on the morphological characteristics using electron microscopy.

Results: We found that C9orf72-ALS astrocytes display an increase (fold-change 1.5) in the early endosomal regulator protein EEA1 compared to astrocytes derived from healthy controls. This increase is accompanied by early endosome morphological changes that we are currently quantifying whilst blind using confocal microscopy. Consistent with the potential accumulation of early endosomes and the inability to transition to late endosomes, C9orf72-ALS astrocytes display a reduction in the number of multivesicular bodies. Interestingly, this phenotype is also accompanied by clustering and accumulation of lysosomes, indicating that multiple blockage points might be present in vesicle formation and degradation.

Conclusion: Our findings provide evidence that the phenotype of reduced EV secretion that we have previously observed may be the result of increased trafficking towards the lysosomal compartment and reduced maturation of early endosomes into multivesicular bodies.

(j234) Disruption of nucleocytoplasmic transport in SOD1 ALS

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Impaired nucleocytoplasmic transport (NCT) has emerged as a common mechanism shared by multiple neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Studies regarding C9orf72, TDP-43, and FUS-related ALS mutations have been implicated in the disruption of protein trafficking through the nuclear pore as a central target for toxicity. Here, we investigate whether misfolded SOD1, the first protein that was found to cause familial ALS (FALS), affects this pathway. We found that expression of mutant SOD1 interferes with nuclear protein import and export and leading to mislocalization of transport factors in SHSY5Y neuronal cells. RanGAP1, Ras-related nuclear protein (Ran), and XPO1, important key regulators of protein import and export of NCT machinery, were found to be mislocalized in spinal cord sections of mutant SOD1 transgenic mice supporting our observed NCT defects. However, no morphological impairment was detected. Moreover, we showed that cells transfected to express the double mutant SOD1G93A/L38R, which disrupts the binding to XPO1, are protected from NCT disruption. Finally, we found abnormal accumulation of RanGAP1 on the nuclear envelope in human fibroblasts and postmortem tissues from ALS cases involving mutations in SOD1. In summary, this research joins the list of studies presenting NCT defects in ALS cases and thus suggests this pathway as fundamental in the search for the cure of this fatal disease.

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