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A Blinded Interim Update on RESCUE-ALS: A Randomized, Placebo-Controlled, Phase 2 Study to Determine the Effects of CNM-Au8 to Slow Disease Progression in Amyotrophic Lateral Sclerosis

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Introduction:

Bioenergetic dysmetabolism in motor neurons and glial cells leads to cell death and the resulting clinical sequalae of amyotrophic lateral sclerosis (ALS). CNM-Au8, a bioenergetic nanocatalyst, is a suspension of clean-surfaced, faceted gold nanocrystals that enhance the bioenergetic capacity of motor neurons, resulting in significant neuroprotection and neurorepair in preclinical models.

The Phase 2 RESCUE-ALS study is a randomized, placebo-controlled trial utilizing a novel electrophysiological measure of motor unit estimation to measure clinical efficacy. Here we present interim blinded data from this fully enrolled trial.

Objective:

To investigate the clinical effects of the bioenergetic nanocatalyst, CNM-Au8, as a disease-modifying treatment for patients with ALS.

Methods:

ALS participants meeting all inclusion/exclusion criteria were randomized (1:1) to receive 30 mg CNM-Au8 or placebo (p.o.) over 36-weeks of double-blind treatment. The primary efficacy endpoint is change in the summed estimated motor unit number index (MUNIX) for the abductor digiti minimi, abductor pollicis brevis, biceps brachii, and tibialis anteriorcompared to baseline. Secondary and exploratory outcome measures include forced vital capacity, electromyography endpoints, the ALSFRS-R, ALSSQOL-S, and pharmacodynamic biomarkers.

Results:

The study enrolled 45 participants. Baseline characteristics include [mean (SD)], MUNIX(4) sum: 373.5 (178.4); FVC % predicted: 81.5 (16.7); ALSFRS-R: 38.7 (6.0); time from diagnosis: 4.9 (4.8) months; riluzole background treatment: 89%; indicating a study cohort early in the disease course. Analyses of completers at weeks 12, 24, and 36 indicate the enrolled cohort performed better than anticipated in terms of MUNIX(4) change from baseline compared to metrics from longitudinal studies with 34%, 26%, and 18% of the overall population demonstrating improvement versus baseline at each timepoint, respectively. Interim blinded results for MUNIX, FVC, and ALSFRS-R, will be presented.

Conclusion:

As the first therapeutic nanocatalyst in development for neurodegenerative diseases, CNM-Au8 has a unique multi-modal mechanism of action that addresses disease-related bioenergetic failure, oxidative stress, and proteostasis dysregulation. This study aims to establish cellular bioenergetic improvement as a therapeutic target for ALS, and to support the validation of electromyography endpoints as biomarkers for ALS disease progression.

Study Supported By:

FightMND, an Australian-based non-profit, and Clene Nanomedicine, Inc.

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. Machine learning models trained on the available 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 investigat 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. 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.
We also developed DGLinker, a webserver that implements and generalizes our machine learning method. DGLinker 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. 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 prioritisation in a genome-wide association study. As our predictions were based on data released in 2019, in 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 2019 and 2021 were predicted by our method. Five out of seven discovered genes were present among our predictions ($p = 0.012$).

Conclusions:

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.

A national survey exploring the nutritional management of people with ALS delivered by Dietitians in the UK

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Introduction

People living with Amyotrophic Lateral Sclerosis (ALS) face many challenges to taking adequate nutrition, which accounts for the high prevalence of malnutrition. In the face of growing evidence that weight loss is an independent prognostic indicator of survival, there is a paucity of research focusing on the current nutritional management of people with ALS.

Objectives

The objectives of this study were to understand the practice of UK dietitians supporting people with ALS to inform recommendations for practice and the development of nutritional interventions.

Method

A mapping review of the literature and stakeholder workshops informed the development of a national online survey. A snowball sampling technique was used to distribute the online survey to UK healthcare professionals, between September and November 2018. The survey examined the nutritional management of ALS. Only the responses of dietitians are reported in this paper.

Results

In total, 130 dietitians responded to the survey, with 87% currently providing dietetic care to pwALS. Dietitians most frequently reported (66%) that people with ALS comprised less than a 20% of their total patient caseload. Less than half (42%) of dietitians reported that nutritional screening took place in their organisation. Half of dietitians reported that patients were referred for dietetic assessment at 'about the right time' although 44% reported referrals were made too late. With regards nutritional assessment, the majority (83%) used predictive equations for resting energy expenditure (REE) that were not validated in ALS. The majority of dietitians would set weight maintenance goals if the person with ALS had a BMI>18.5kg/m2. Only about a quarter (23%) of dietitians reported that the 'food first' approach was effective in ALS. Dietitians most frequently (43%) reported that people with ALS were not weighed frequently enough.

Conclusions

This is the largest published survey of UK dietitians practice with regards the nutritional management of people with ALS. The reported late referral for dietetic advice and infrequency of nutritional monitoring may be a possible explanation for the prevalence of malnutrition in ALS. The use of predictive equations to estimate REE not validated for use in ALS and aiming for weight maintenance is consistent with the evidence base suggesting weight gain improves prognosis. A UK research group have developed the online complex intervention, OptiCALS, to promote a high calorie diet in people with ALS. The intervention development was informed by a series of research identifying the barriers and facilitators to following a high calorie diet for people with ALS, including the findings of the survey reported here. Complex interventions designed to improve nutritional status in people with ALS need to be evaluated to support the impact nutrition can have on the outcomes of people with ALS.

A Phase 1, Multicenter, Open Label, Single-Ascending Dose Study to Evaluate Safety, Tolerability, and Pharmacokinetics of AP-101 in Familial and Sporadic Amyotrophic Lateral Sclerosis (ALS)

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Introduction :
Mutations in SOD1 result in misfolding of the SOD1 protein. This was the first genetic variant associated with ALS. Misfolding of SOD1 also occurs in the absence of mutation. Mis-folded SOD1 can be detected in the majority of ALS patients suggesting that SOD1 is a common pathogenic driver across familial and sporadic forms of ALS. AP-101 is a fully human IgG1 antibody with high affinity and selective binding to pathogenic misfolded SOD1 protein. In transgenic mouse models of ALS, a murine version of AP-101 was able to attenuate loss of spinal cord motor neurons and motor function as well as prolong overall survival.
Objectives:
To determine the safety and tolerability of AP- 101 in patients with ALS.
Method:
Given the rapidly fatal nature ALS and the high unmet need, an efficient accelerated dose escalation study was performed using an open label oncology style 3+3 design (ClinicalTrials.gov Identifier: NCT03981536). Specifically, AP-101 was administered to patients via intravenous infusion over 1 hour at increasing dose levels of 100, 500 or 2500mg. At each dose level, a sentinel patient was observed before recruitment of two additional patients into the cohort. After the observation period and in the absence of any drug related toxicity the next dose cohort was opened for recruitment. If any safety signal was observed in the first 3 patients, an additional 3 patients would have been recruited at the same dose level. Dose limiting toxicities in 2 or more patients at any dose level would result in a declaration of maximum tolerated dose. The overall goal was to assess safety, tolerability, and pharmacokinetics of AP-101 after intravenous administration in fALS and sALS patients. Cerebral spinal fluid was also collected from patients
Results:
The top dose of 2500mg was achieved with no dose-limiting toxicities effects or any safety or tolerability concerns related to AP-101. The most common adverse events are related to the lumbar puncture associated with patient screening. Results are being used to guide the design of a proof-of-concept study to examine the efficacy potential for AP-101.
Conclusion:
AP- 101 is safe and well tolerated in patients with Amyotrophic Lateral Sclerosis. A proof of concept /MAD study is launched in 5 jurisdictions internationally

A preliminary comparison between ECAS and ALS-CBS in classifying Amyotrophic Lateral Sclerosis cognitive-behavioural changes in a cohort of non-demented patients.

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease mainly characterized by degeneration of the upper and lower motoneurons and associated to a frontotemporal dysfunction that can occur in up to 40% of cases. Up to now, two screening tools to define the presence and type of frontotemporal dysfunction have been largely used: the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), mainly used in Europe, and the ALS Cognitive Behavioural Screen (ALS-CBS), mainly used in the United States of America.

Objective: To compare the ability of ECAS and ALS-CBS in classifying non-demented ALS patients according to Strong criteria.

Methods: One-hundred and fifty-four in- and out-patients with an age > 18 and a definite or probable ALS diagnosis were recruited between September 2019 and February 2020 at NeMO Clinical Centre and at Istituto Auxologico Italiano in Milan and underwent the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) and the ALS Cognitive Behavioural Screen (ALS-CBS).

Exclusion criteria involved patients with a diagnosis of FTD, with a severe cognitive deterioration and/or an important behavioural impairment, with a significant psychiatric disorder or with the co-presence of another significant illness.

Results: The distribution of patients according to Strong criteria was different for ECAS and ALS-CBS and the degree of agreement between the two tests in terms of Cohen's Kappa coefficient resulted equal to 0.2047 with a 95% confidence limits interval between 0.1122 and 0.2973.

Conclusions: This study for the first time compares the ability of ECAS and ALS-CBS in stratifying ALS patients. Even though both instruments showed to be useful in stratifying our cohort of patients in the different diagnostic categories, some differences emerged. As shown by the Cohen's Kappa coefficient, these two tests showed a minimal level of agreement, meaning that they classified our cohort following different principles. We assume that some differences found in identifying both ALSci and ALSbi patients may have depended on the different structure of these two screening tests. Regarding the ALSci differences, while ECAS is structured as a multi-domain test with multiple subscores investigating different cognitive functions, ALS-CBS has only a total score that accounts for dysfunctions that typically occur in ALS. Moreover, regarding the ALSbi category, while ECAS caregivers' questions are based on the criteria for FTD diagnosis, ALS-CBS caregivers' questions are based on behavioural changes typically found in ALS patients, determining a not full overlap between the two behavioural questionnaires. However, further studies will be conducted to better understand the reasons underlying the differences between these two tests in classifying the different subtypes of fronto-temporal dysfunction in ALS.

A systematic review of the utility of wearable devices for monitoring motor progression in amyotrophic lateral sclerosis.

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Introduction: Amyotrophic lateral sclerosis (ALS) is the most common subtype of motor neuron disease (MND). 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 of physical functioning. Wearable devices have potential as a sensitive alternative for assessing motor progression in both clinical practice and trials.
Objectives: This review will explore the current landscape of wearable devices and their use in people with ALS to evaluate the progression of physical symptoms.

Methods: We reviewed articles evaluating the utility and suitability of wearable sensors to evaluate disease progression people with ALS (pwALS). We systematically searched Google Scholar, PubMed, and EMBASE. No language or date restrictions were applied. We extracted information on devices and assessments.

Results: 15 studies, involving a total of 629 (median 25) pwALS were included. Sensor type included accelerometers (n = 10), activity monitors (n = 6), smartphones (n = 5), gait (n = 1) or kinetic sensors (n = 2). 14 (93%) of studies used the ALS-FRS-R to evaluate concurrent validity. Participant feedback on sensor utility was generally positive. All studies showed initial feasibility, warranting larger longitudinal studies to compare device sensitivity with ALSFRS-R.

Conclusions: Measurement of physical symptom progression using wearables sensors is an emerging, and seemingly promising, area of ALS research. Further well-powered longitudinal validation studies are needed.

Abnormal microstate resting-state EEG characteristics in ALS disease

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Introduction: Resting-state electroencephalography (EEG) has shown great promise in highlighting network-level abnormalities in ALS [1-2]. Furthermore, episodes of dynamic changes in EEG, also called microstates and described as 'building blocks of spontaneous thinking' [3], can provide potential biomarkers of altered network interactions in neurodegenerative diseases [4–6], but have not been investigated in ALS to date. This study reports preliminary findings on potential abnormalities of EEG microstates in ALS patients.

Objectives: To assess the alterations in dynamic resting-state EEG, as potential prognostic biomarkers of ALS.

Method: High-density resting-state EEG data from 125 patients and 81 healthy controls (HC) were recorded. First, the spatial topographies of resting-state EEG (1-30Hz) were found for the HC at the peak times of Global Mean Field Power (GFP) to derive 4 microstates prototypes (optimal number based on previous studies [7]). All EEG samples were then backfitted for each subject group, based on the microstate prototype they were more similar to, and three microstate properties were calculated for each of the 4 class, i.e. duration, occurrence and global explained variance (GEV). These properties were statistically compared between the HC and ALS (Mann-Whitney U test, FDR correction), and correlated with clinical scores, for patients.

Results: The 4 microstate classes (A, B, C, D) extracted from the HC EEG were similar to the ones reported in the literature [7]. The properties of the microstate classes showed significant differences between the two groups. Higher occurrences were observed for microstate classes B, C and D ($p = 0.01$, $p = 0.03$, $p = 0.08$; FDR at 0.1) and a lower duration was found for microstate class A ($p = 0.004$; FDR at 0.05) in ALS patients compared to HC. Furthermore, the GEV of microstate class D negatively correlated with survival time ($p = 0.008$, $\rho = -0.3$, $1-\beta = 0.77$) and ALSFRS-R subscores progressions, estimated by linear mixed model (bulbar ALSFRS: $p=0.02$, $\rho = -0.3$, $1-\beta = 0.63$; lower: $p = 0.01$, $\rho = -0.3$, $1-\beta = 0.74$; upper: $p= 0.0007$, $\rho = -0.4$, $1-\beta =0.94$).

Conclusions: This study showed altered resting-state EEG microstates characteristics in ALS. More specifically, microstate class D, which correlates with clinical sub-scores, has also been found to be abnormal in Huntington's disease [5]. Additional analysis of microstates, at the level of brain sources, would provide further insights into dynamic interactions of the brain networks in ALS. The study confirms the potential of dynamic resting-state EEG measures, as quantitative functional biomarkers that can be instrumental for future clinical trials.

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Adoption and Implementation of the Sheffield Telemedicine in MND (TiM) System in the Irish MND Service

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Introduction: Routine review visits to the national MND clinic at Beaumont Hospital were curtailed due to COVID-19 restrictions. A remote monitoring app developed for use in MND, provided a potential solution (Hobson et al 2018,2019). On receipt of COVID response funding, we implemented the Sheffield Telemedicine in MND (TiM) system, hosted on MyPathway, and developed by the software company ADI. It collects patient/carer reported outcome measures (PROMS) through standardised measures used in clinical care.

Objectives: We aim to outline the process of adaptation of TiM on My Pathway with reference to the regulatory, governance and stakeholder consultation processes required for implementation in the Irish MND Service.

Method: Baseline service data was gathered including clinic time-in-motion studies and metrics on successful pre-identification of patients requiring specific services during a planned clinic visit.

Local Regulatory and Governance procedures were followed which included approvals by the Data Protection Officer, consultation with the Information Technology services, and a Data Sharing Agreement between the hospital and ADI.

Questionnaires and metrics on the Sheffield TiM on My Pathway system were compared to Irish practice norms and required adjustments identified to align to the national service needs. A demo system based on these initial adjustments was provided by ADI.

Patient and participant involvement (PPI) included consultations with patients and their carers and with the healthcare professionals working in the MND service. Consultations were conducted using Video conferencing. The app was demonstrated in real time and feedback was documented.

Results: PPI feedback suggested modifications to wording, usability, and frequency of outcome measures. The issues were systematically recorded and provided to the software development company for remediation. Additional localisation issues included adding links to online resources hosted by the Irish Motor Neurone Disease Association (IMNDA) through TIM.

Since January 2021 20 patients have been onboarded to the app and are actively using the service. Initial feedback on usability and acceptability is positive and the data is being used to plan clinic visits for the patients.

Conclusions: Systems allowing patients to complete PROMs from home using a telehealth system such as TiM, via a phone, laptop or tablet show promise of practical utility in MND. However, differences in service structures and practices and cultural norms mean that a 'one size fits all' system is not practical, and localisation is required. Regulatory and governance approvals are time consuming but necessary aspect of the implementation process. Extensive evaluation of service efficiency and user experience and ongoing developments in partnership with the original developers in Sheffield are planned.

Age-dependent cytoskeletal shifts in human sensory nerve fibers

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Introduction:
The neuronal cytoskeleton is essential to maintain axonal homeostasis. Neurofilaments regulate caliber and therefore indirectly affect electrical pulse velocity, actin is involved in shaping the axon, while microtubules provide the basis for organelle transport. Microtubules are dynamic structures and decorated by post-translational modifications, which in turn recruit microtubule associated proteins and influence their functionality. In order to better understand how cytoskeletal aberrations contribute to axonal pathology in amyotrophic lateral sclerosis and why aged individuals are more susceptible to motor neuron diseases, we investigated changes of the cytoskeleton during aging.

Objectives:
We chose human skin biopsies as a model to study cytoskeletal changes in neurons. Available to us are biopsies of 84 healthy individuals ranging from 23 to 79 years, 62 males, 22 females. The results presented here build the groundwork of cytoskeletal analyses in motor neuron disease patients.

Method:
Skin biopsies of 27 men (mean age 54.2 ± 18.9 a, range 24 to 79 a) were immunostained for neurofilaments (non-phosphorylated neurofilament heavy chain (npNfH)), microtubules (βIIItubulin), and actin (phalloidin). Confocal stacks of the dermis were recorded and nerve endings were analyzed for their mean gray values and diameter using ImageJ. Statistics were calculated using GraphPad Prism.

Results:
The expression levels of cytoskeletal component drastically increased until age 65, but then plateaued or even declined. NpNfH increased 2.7-fold and βIIItubulin 3.2-fold with a maximum at 75-year-old individuals. Similarly, actin expression peaked at 62-year-old controls with a 3.8-fold change. In contrast, sensory axon caliber increased linearly, reaching a maximum at age 78/79.

Conclusions:
We assume an age-driven imbalance of the axonal cytoskeleton in peripheral sensory nerve endings regarding the expression of its components and the caliber. The axonal diameter is further increasing, while the cytoskeletal expression stagnates in higher age. This imbalance might serve as a risk factor regarding motor neuron diseases. The results will be corroborated in testing more individuals. We further plan to analyze the length dependency of biopsies obtained from thigh and ankle. Extending our study to motor axons of aged mice (up till 24 months) will show if this cytoskeletal aging phenomenon is conserved in neurons and across species.

ALS in Emilia Romagna Region: epidemiological, clinical and genetic features in a prospective population-based study in the last decade.

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Background: increased incidence rates of MND has been reported in the last decades across different Western Countries, although geographic variations among different areas in term of incidence, clinical features, trends, and genetics need to be further explored. In this study we describe demographic, clinical features and genotype-phenotypes correlation of incident ALS cases in Emilia Romagna Region in the last decade.

Method: we performed a prospective, population-based, epidemiological study in Emilia Romagna Region of northern Italy (population: 4.5 million inhabitants) where an ALS register is still collecting newly diagnosed and deeply phenotyped ALS patients since 2009.

Results: from 1 January 2009 to 31 December 2019, 1398 patients received a new diagnosis of ALS in our Region; crude incidence rate was 2.86/100,000 (M/F ratio: 1.33) whereas adjusted incidence rate was 2.71/100,000 population. Mean age at diagnosis was 67.69 years (SD: 11.35), with a higher age for women and bulbar and respiratory phenotypes, and a lower mean age at diagnoses for carriers of C9orf72 expansion. Incidence rates increased by 3,91% from the period 2009-2014 to the years 2015-2019, with a preserved incidence peak at 70-75 years, followed by a sharp decrease, and, only for women, by a new progressive increase after 90 years old. Approximately 15% of cases were familial and, among these, 22.4% of patients carried a gene mutation related to ALS; 2/3 of patients with c9orf72 expansion had a family history for ALS or dementia.

Discussion and conclusions: if our population-based registry confirms the already reported relationship among phenotype, sex, age, and gene mutations, we found incidence rates slightly higher to those described in recent European population-based studies, probably due to the aging population characterizing our region, as well as to the structure of our regional health care services, that enhance patient's identification and access to services. A slight increase through the years of the study was mainly due to an increase in bulbar and classic phenotype in men. We found also an increasing age at diagnosis, that, together with the other highlighted clinical features, may suggest that environmental factors can contribute to the heterogeneity and trends of disease presentation and progression.

ALS subgroups revealed by clustering neuroimaging patterns of brain degeneration

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Introduction: The clinical and genetic heterogeneity of ALS imposes multiple challenges in search of the cure for this devastating disease. This heterogeneity has provoked researchers on questioning whether to treat ALS as a single disease or a collection of multiple disease subtypes.

Objective: In this study we aim to identify subtypes of ALS by comparing patterns of neurodegeneration in the brain, measured by MRI.

Methods: We acquired T1 and DTI images from 488 ALS patients and 338 control subjects. Whole brain cortical thickness measurements and fractional anisotropy of the white matter brain network were acquired and corrected for age, sex and two principal components derived from control subjects. We estimated similarity between pairs of subjects using Pearson's ρ and created a mathematical similarity network comprising all patients. A clustering algorithm divided patients into subgroups that displayed higher degrees of similarity in terms of neurodegeneration patterns. Clinical characteristics and cognitive profiles were assessed for each subgroup. Longitudinal data was used to validate the results of the clustering algorithm and identify possible transitions between subgroups over time.

Results: The algorithm divided ALS patients in three subgroups of 187, 163 and 138 patients. The brain phenotypes of these subgroups all displayed involvement of the precentral gyrus and are respectively characterised by (1) patients with a pure motor phenotype (PM), (2) patients with orbitofrontal and temporal involvement (FT) and (3) patients with involvement of the posterior cingulate cortex, parietal white matter and temporal operculum (CPT). These subgroups had distinct clinical characteristics: patients in the PM subgroup had a lower age at onset ($p<0.001$) and lower rates of bulbar onset ($p<0.001$), the FT subgroup had higher rates of FTD at diagnosis ($p=0.001$). All subgroups had similar motor symptom severity in terms of King's stages, and survival rates did not differ between the subgroups. Compared to controls, both the FT and CPT subgroups revealed higher rates of cognitive impairment on the ECAS (both ALS-specific and non-specific scores), but this was not the case for the PM subgroup. Longitudinal analysis showed that clustering remained largely stable over follow-up scans, with patients remaining in the same subgroup in 90.4% of the follow-up visits.

Conclusions: We demonstrate that three distinct manifestations of ALS can be found in the brain, that are each associated with distinct clinical characteristics and cognitive profiles. A new neuroimaging phenotype has emerged besides the pure motor and FTD-like variants of ALS, which is characterised by posterior cingulate, parietal and temporal involvement.

ALSFRS-r scale increases arterial blood gas analysis' sensitivity in assessing pulmonary function in Amyotrophic Lateral Sclerosis.

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Introduction
Spirometry is commonly used to monitor respiratory function in Amyotrophic Lateral Sclerosis (ALS); however, its use in patients with facial weakness or cognitive impairment is poorly accurate. Arterial blood gas analysis (ABG) correlates moderately with forced vital capacity (FVC). The ALSFRS-r is a validated clinical rating scale that accurately monitor progression of patients' disability in ALS, but the capability of its respiratory domain to reflect the real pulmonary function has been questioned.
Objectives
To investigate if clinical evaluation of respiratory symptoms, assessed by using the ALSFRS-r respiratory items (10 and 11), increases the correlation between ABG and FVC in a cohort of ALS patients.
Method
We selected the first ABG performed before non-invasive ventilation by ALS patients diagnosed from 2000 to 2015 in Turin ALS Centre. To find out the best combination to predict FVC, we assessed the correlations of different combinations of ABG parameters (carbon dioxide, pCO2; carbonate, HCO3-) and respiratory symptoms with FVC%. Dyspnea and orthopnea were considered present if ALSFRS-R item 10 and 11 were <4, respectively. Patients were then grouped according to ABG values (pCO2 and HCO3- were increased if >45 mmHg and 26 mmol/L, respectively) to compare clinical and epidemiological characteristics between patients with and without respiratory symptoms. Binary logistic regression was used to evaluate if cognitive function influenced the complaining of respiratory symptoms.
Results
A total of 488 ABGs were collected. The best combination to predict FVC was: pCO2 + HCO3- + ALSFRS-R item 10 (R=0.430, p <0.001). At equal values of pCO2 and HCO3-, the presence of dyspnea was associated with a more severe general impairment, a higher disease progression rate and lower FVC values. Patients with normal ABG who complained dyspnea showed a reduced survival than patients without dyspnea (0.91 years, IQR 0.46-1.91 vs 1.46 years, IQR 0.89-2.29, p=0.002). Type of onset did not differ between patients with and without dyspnea and its complaining was not influenced by cognitive dysfunction (OR 1.009, 95% CI 0.837-1.215, p=0.927).
Conclusions
Combining ABG with clinical evaluation of dyspnea can be a sensitive tool to assess pulmonary function in ALS, especially for patients with an early respiratory impairment and in patients with bulbar or cognitive impairment whose evaluation by the routinely used spirometry can be demanding and poorly accurate.

Amyotrophic lateral sclerosis – Riga East University Hospital patients data characteristics.

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Introduction
Amyotrophic Lateral Sclerosis (ALS) is a progressive, neurodegenerative disease accounting for approximately 10,000 deaths annually in Europe. ALS is a rare, rapidly progressive and always fatal motor neuron disease. Usually, the time to diagnosis is 10 to 16 months and the median survival of patients after diagnosis is mainly within 14 months.
Objectives
To collect and evaluated data of the epidemiological and demographic status, clinical presentation, examinations and prescribed therapy.
Method
Retrospective data analysis of all ALS cases treated at Riga East University hospital from 2015 to 2020. Descriptive statistics of the group was calculated using Excel.
Results
We collected 103 ALS patient's data from medical records (medical histories, outpatients cards, councilium conclusions) of Riga East University Hospital from 2015 to 2020. Out of 103 included patients 57 (55%) were females. The average age of ALS symptoms onset was 65 +/- 12.6 SD years (19-86). Median diagnostic delay in this study was 17.33 +/- 22.13 SD months (60 patient data). The most common clinical symptoms were bulbar syndrome - 66%, upper limbs paresis - 88%, lower limbs paresis - 96% and axial muscles paresis - 34% of cases. Muscle fasciculations were observed for 56.5% of patients. Weight loss (>10% body mass) in 37% at the time of diagnosis. The data of concomitant diseases were available for 73 of all patients: 33% patients had cardiovascular disease, 11% patients had cancer, 10% diabetes, spondylosis in 10% of patients. We had EMG examination data from 53 hospitalized patients - 15% of them had negative EMG findings in the beginning of the disease due to very mild and recent onset of symptoms. 6% of hospitalized patients needed consultation of a psychologist, 23% - speech therapist, 22% - nutrition specialist; 64% - physical medicine and rehabilitation physician. No genetic testing was done for the patients. In 81% of patients Tab. Riluzole 50 mg x 2 was prescribed as a first-choice therapy. Symptomatic treatment was used in 73% of all the cases: antidepressants 29%, painkillers 22% of cases and 47% therapy for other comorbidities. The mean time from first symptoms to percutaneous endoscopic gastrostomy was 23.8 +/-23.2 SD months (7-84 months, 10 patient data). The mean time from first symptoms to tracheostomy was 30.4 +/- 19.2 SD months (2-48 months, 5 patient data). The mean survival time from onset to death was 32 +/-8 SD months (10 patient data). In this study, 66% of patients were hospitalized.
Conclusions
The epidemiological data, clinical manifestations are similar to findings from other studies with regards to age of onset, sex ratio and survival. The treatment of amyotrophic lateral sclerosis requires multidisciplinary approach and should include physicians, physical therapists, speech pathologists, pulmonary therapists, medical social workers and nurses.

Antibodies-based therapy to overcome TDP-43 proteinopathy.

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Introduction: TDP-43 proteinopathy is an event characterized by a consistent cytoplasmic mislocalization and aggregation of the protein TDP-43, and a pathological hallmark of Amyotrophic Lateral Sclerosis (ALS) and FrontoTemporal Lobar Degeneration (FTLD). Different studies highlighted the sensitivity of the RRM1 domain in inducing TDP-43 proteinopathy (Chang et al. 2013; Shodai et al. 2013), and its role in activating the NF-kB pathway (Swarup et al. 2011).

Objectives: To overcome this toxic effect, we developed two antibody-based therapeutic approaches, specifically directed against the RRM1-domain of TDP-43.

Methods: We generated a monoclonal full-length antibody (named E6) and tested its target specificity, and therapeutic efficacy in the TDP-43 A315T mouse model (Pozzi et al. 2020). From E6 full-length monoclonal antibody we also derived a single chain antibody (named VH7Vk9) that we virally delivered into two mutant TDP-43 mouse models (Pozzi et al. 2019).

Results: We observed that the full-length antibody recognizes specifically the cytoplasmic fraction of TDP-43 in cells, animal models and human tissues. We demonstrated that in neuronal cells the antibody can reduce cytoplasmic TDP-43 levels by activating the TRIM-21/proteasome degradative pathway. We delivered the antibody by repeated intrathecal injections in TDP-43 A315T mice and demonstrated a wide diffusion in the spinal cord and a specific uptake by large neurons and microglial cells. In tissues of treated mice, we measured the levels of cytoplasmic TDP-43 and nuclear p65, the active subunit of NF-kB, observing a significant reduction of both events in conditions where E6-antibody was administrated. The delivery of the single chain antibody demonstrated to rescue motor and cognitive impairments in animal models. It reduced the amount of cytoplasmic accumulated TDP-43 by targeting the protein to degradative pathways and it blocked the interaction between TDP-43 and p65, decreasing neuroinflammation in mice.

Conclusions: We therefore demonstrated for the first time the feasibility and efficacy of two antibody-based approaches against the RRM1-domain of TDP-43 in reducing TDP-43 proteinopathy and rescuing motor and cognitive deficits in ALS/FTLD mouse models.

Anxiety predicts survival more than depression in Amyotrophic Lateral Sclerosis

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Introduction

Psychological status has already been related to clinical outcome in ALS (1), as well as in other neurological disease, without considering the specific role of anxiety and depression.

Objectives

Methods. We collected all Hospital Anxiety and Depression scale (HADS A and D) questionnaires administered to patients at diagnosis from 2008 to 2018 in the Turin ALS Centre during the full neuropsychological evaluation. We managed to assess the prognostic role of anxiety and depression in ALS patients, adjusting for different motor and cognitive confounders. Survival analysis were performed for both depression (HADS-D) and anxiety (HADS-A) domains, using Cox proportional hazard models, adjusting for age, sex, education, ALSFRS-R score, ΔALSFRS-R, onset-diagnosis interval, cognitive categories and Kaplan-Maier curves.

Results. Five hundred sixty-nine patients with HADS questionnaire and all complete data were collected. Median HADS-A score was 7 (IQR 5-10) and median HADS-D score was 5 (IQR 3-8): both scores showed moderate correlation (0.485, $p<0.001$). Depression was significantly correlated to age, total ALSFRS-R score and cognitive impairment, while anxiety showed only a minimal correlation to ALSFRS-R score (-0.108, $p=0.022$). Cox proportional hazard model using raw scores showed that HADS-A, was related to overall survival (HADS-A: HR 1.040, CI 1.012-1.069, $p=0.005$; HADS-D: HR 1.013, CI 0.976-1.052, $p=0.497$) but not HADS-D. The best discriminating cut-off for both HADS-A was 6 (HR HADS-A>6 1.453, CI 1.151-1.835, $p=0.002$; log-rank test $p=0.012$): interestingly, HADS-D raw scores resulted to be prognostic only in patients with anxiety (HADS-A>6).

Conclusions

We confirmed that anxiety is more frequent than depression in ALS patients at diagnosis (2) and, unlike depression, seems not to be related to motor and cognitive features and to disease duration. We pointed out that anxiety is not a simple epiphenomenon related to diagnostic challenge, but rather an independent prognostic factor, similarly to what is found in other non-neurological disease (3).

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APPLICATIONS OF SURFACE EMG ARRAYS FOR IDENTIFICATION AND TRACKING OF MOTOR UNITS IN AMYOTROPHIC LATERAL SCLEROSIS

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Background: Recording the electrical activity of motor units (EMG) within a muscle using invasive needle electrodes is an important step in the diagnosis of amyotrophic lateral sclerosis (ALS). Recent advancements in EMG technology have enabled individual motor units to be detected from non-invasive recordings from the skin surface using high-density surface EMG arrays. The potential applications of this new technology to study changes in brain-muscle communication in ALS, however, have yet to be fully realised.

Objectives: The aim of this study is to highlight how information on motor unit populations, obtained using surface EMG arrays, could be used to interrogate motor unit networks in ALS.

Methods: We have evaluated two novel methods for analysing motor unit data from surface EMG arrays: 1) multi-variate coherence estimation, and 2) motor unit tracking in multi-dimensional space. The multi-variate coherence method provides an accurate assessment of rhythmic patterns present in the motor unit firing activity. The synchronous discharge of motor units, particularly in the beta-band frequency range (15-30 Hz), has been linked to oscillatory cortical and sub-cortical processes. The collective synchrony of the motor unit population can thus provide insight into changes in the communication between brain and muscle. The second method is a technique that can be used to reliably track the same motor unit across different experimental sessions. The action potential waveform of each motor unit is characterised in multi-dimensional space, incorporating information from all channels of the surface EMG array. This technique opens up the possibility of tracking motor units longitudinally in ALS patient groups, whereby changes in the activity of individual motor units over time could be directly assessed.

Results: In young, healthy subjects (N=18) the multi-variate coherence method detected a decrease in beta-band and a novel increase in gamma-band motor unit coherence at higher muscle contraction forces. When applied to simulated datasets, the motor unit tracking method identified action potentials from the same motor unit with 100% accuracy (compared with cross-correlation methods which yield high rates of false positives, 40-60%).

Conclusions: The ability to accurately assess collective motor unit synchrony and reliably track the same motor unit over time could offer new insights into brain-muscle connectivity in ALS patient groups, and be used to explore how this communication is altered as the disease progresses. The information gained from high-density surface EMG recordings is a potential non-invasive biomarker of disease progression in ALS.

Are psychiatric symptoms in people with MND and their kindreds associated with cognition and behaviour in people with MND?

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Introduction Cognitive and behavioural impairment (MNDci and MNDbi) occurs in up to 50% of people living with MND (pwMND), characterised by deficits in language, executive functioning, verbal fluency and apathy. Psychiatric disorders occur at high rates in MND kindreds, but it is unclear if they relate to cognitive and behavioural symptoms in pwMND.

Objectives: To determine whether psychiatric symptoms and disorders in MND kindreds are associated with cognitive and behavioural impairment in pwMND.

Methods: pwMND and their first and second-degree relatives were recruited in Scotland and Ireland. Current and lifetime, clinical and subclinical symptoms of mood (depression, mania), neurotic (anxiety, obsessive compulsive disorder), psychosis, suicidal thoughts, autism and attention deficit hyperactivity disorder (ADHD) were assessed in kindreds using a comprehensive series of self-report questionnaires. Cognition and behaviour in pwMND were assessed using the Edinburgh Cognitive and Behavioural ALS screen (ECAS) which includes an ALS specific (executive+language+fluency) and ALS non-specific score (memory+visuospatial).

Results: In total, 125 pwMND with ECAS and kindred psychiatric data were included. For psychiatric symptoms in pwMND, current depression and anxiety was associated with poor language (exp(b)=0.62, p=0.02 and exp(b)=0.53, p=0.03). Current initiation apathy and impulsivity was associated with poor memory (exp(b)=0.98, p=0.04 and exp(b)=0.98, p=0.04) and ALS non-specific scores (exp(b)=0.98, p=0.01 and exp(b)=0.99, p=0.01). An episode of mania or psychosis across the lifespan was associated with hyperorality (OR=8.67 [2.06 to 37.37], p=0.003 and OR=6.80 [1.48 to 30.15], p=0.01).

In 99 MND kindreds, more impulsivity symptoms were associated with poor memory (exp(b)=0.98, p=0.02) and ALS non-specific scores (exp(b)=0.98, p=0.03) in pwMND. Several associations between family psychiatric symptoms and better cognitive functioning in pwMND were also shown. More current anxiety symptoms were associated with increased apathy (OR=1.40 [1.07-1.97], p=0.03); autism with disinhibition (OR=1.29 [1.06-1.67], p=0.02); and ADHD with hyperorality (OR=3.49 [1.59-10.76], p=0.01).

Conclusion: Current psychiatric symptoms in MND kindreds (depression, anxiety, impulsivity) are associated with poor cognition in pwMND, while lifetime psychiatric symptoms and disorders (anxiety, mania, ADHD, autism) show associations with behavioural changes, indicating a possible pleiotropy.

ATXN2 intermediate CAG expansion in Catalonian ALS population

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Introduction:
Amyotrophic Lateral Sclerosis (ALS) in a neurodegenerative disease of motor neurons. Over 10% of ALS cases are familial, and there is a genetic cause in up to 5% of sporadic cases.
In ATXN2 gene, expansions of CAG nucleotides bigger than 35 repeats cause Spinocerebellar Ataxia type 2 (SCA2). But intermediate expansions have been related to an increased risk of ALS, especially between 29 to 34 repeats. Only 2% of global population has more than 24 repeats.
Objectives:
We want to communicate the epidemiological and clinical data of our ALS patients with intermediate expansion of CAG in ATXN2.
Methods:
We assessed C9orf72, SOD1 and SCA2 in all ALS patients visited at Bellvitge Universitary hospital between November 2017 and December 2020.
Only clinical data of patients who has given written informed consent has been included in the study.
Results:
We studied 250 patients with ALS. There were 19 patients (7.6%) with an expansion in ATXN2 between 27 and 33 repeats, 9 (3.6%) of them with 30 or more repeats. No patient with c9orf72 hexanucleotid expansion or a pathogenic mutation in SOD1 has an intermediate expansion in ATXN2. Mean age at onset was 60.85 years (33-77) in the ATXN2 group, and 63.18 years in the rest of ALS patients. 50% were males, and there was ALS family history in 1 patient, and familial ataxia history in another one. 5 patients had a bulbar onset, 2 has a progressive muscle atrophy phenotype (27 and 32 repeats) and 1 has a flail arm phenotype (33 repeats). Frontotemporal dementia was present in 2 patients, both with 28 repeats. 7 patients have died (3 of them with more than 30 repeats) at the moment of the analysis, with a mean survival of 23 months. Mean follow-up on those still living was 29 months. We exclude 1 patient (28 repeats) of the survival analysis because of very long survival (more than 10 years).
Conclusions:
ATXN2 intermediate expansion is present in 7.6% of our patients, most of them sporadic. There was no phenotypical difference between the more and less than 30 repeats group but for the absence of any patient with frontotemporal dementia in the more than 30 repeats group. More studies are needed to understand the role of the intermediate ATXN2 expansions in ALS.

ATXN2 phenotype-genotype study in ALS Spaniard patients

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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

Samples from 375 patients were analyzed (226 ALS patients and 149 FTD patients) as 264 age- and sex-matched controls. 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 (22) were found to harbor intermediate repeats (≥ 28) 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 (0.0097) for intermediate repeats (≥ 28) is statistically significant, and slightly lower comparing to that obtained with the directly higher number of repeats (≥ 29; p= 0.0139).

It is relevant to note that there are more patients presenting FALS (50%) compared to those with SALS. 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

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Benchmarking and using bioinformatics tools for the detection of HERVK insertions in ALS whole-genome sequencing data.

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Introduction
There is emerging evidence supporting a role for 'human endogenous retroviruses' (HERVs) in ALS. HERVs are a type of transposable element which make up approximately 8% of the genome, some are known to be polymorphic and their frequencies in the human populations vary. One HERV subgroup, called HERVK, has been linked to autoimmune diseases and cancer. Research has shown that HERVK protein may be toxic for motor neurons. However, we are still far from a clear understanding of the role of HERVK in ALS.

Objectives
1) Evaluate the performance of the available tools for the detection of HERVK in whole-genome sequencing data.
2) to use the best performing tool to characterise the HERVK genomic landscape in ALS cases and controls in order to test if any HERVK insertion is associated with the risk of ALS or might act as disease modifier.

Methods
We first ran a benchmarking experiment to identify the most reliable WGS HERVK detection tool. We tested six tools on four simulated genomes with known HERVK insertions to estimate sensitivity and specificity. We also applied the tools to a set of fifty human genomes and calculated the proportion of predicted insertions that were previously reported in the literature. Finally, we applied the tools to six genomes which had matched long and short read sequencing. The long-read sequencing was used to validate HERVK insertions found in the short-read sequencing data. We then applied the best performing tool (the 'Wildschutte pipeline') to a set of genomes from the UK ProjectMine dataset (196 cases and 84 controls). The HERVKs detected by the pipeline were tested using logistic regression to assess their association with ALS. We also tested if any of the HERVKs were predictors of survival using the Cox proportional hazards model.

Results
The benchmarking study showed that the performances of the HERVK detection tools vary greatly and allowed us to select the Wildschutte pipeline as the top performing method. When applied to 50 genomes, 40% of the predicted HERVKs given by the pipeline mapped to previously reported HERVKs. When applied to a simulated genome, 9 out of 11 predictions given by this pipeline were true positives. We applied this pipeline to 84 control and 196 ALS case genomes. It predicted 317 HERVK insertions in total, 101 of these insertions mapped to HERVKs previously described in the literature.
The case control analysis did not find any HERVKs significantly associated with ALS. The survival modelling did show one HERVK to be a significant predictor of survival ($P = 0.0014$).

Conclusion
We have presented a first attempt to study the role of HERVK in ALS through WGS data. We have identified the Wildschutte pipeline as a reliable method for such an aim and our initial data suggest that HERVK insertions may affect survival duration in ALS. However, a better powered analysis, and a replication study are required to establish if HERVKs are associated with ALS.

Blood lipid levels and the risk of amyotrophic lateral sclerosis: a prospective population-based cohort analysis

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Introduction:
Metabolic factors including body mass index and physical activity have been associated with altered risk of developing ALS. Genetic studies show evidence of shared genetic risk between ALS and markers of lipid metabolism.

Objectives:
To analyse metabolic factors in a large prospective population cohort study in relation to subsequent risk of ALS.

Methods:
Associations of baseline levels of high and low density lipoprotein (HDL and LDL) cholesterol, total cholesterol, HDL:total cholesterol ratio, apolipoproteins A and B, glycated haemoglobin A1c (HbA1c), self-reported exercise, body mass index and creatinine with risk of subsequent ALS diagnosis were examined using Cox proportional hazards modelling of the UK Biobank cohort (denominator n=502,409).

Results:
In models of individual metabolic factors, controlling for age, sex and socioeconomic status, higher total cholesterol-HDL ratio was associated with increased risk of developing ALS (Hazard ratio (HR) 1.16, 95% confidence interval (CI) 1.05-1.28, p=0.004), whilst higher HDL (HR 0.63, CI 0.44-0.90, p=0.010) and apolipoprotein A (HR 0.50, CI 0.31-0.82, p=0.006) were associated with reduced risk of subsequent diagnosis of ALS. Excluding those diagnosed with ALS within 5 years of sampling, higher total cholesterol-HDL ratio (HR 1.18, CI 1.03-1.35, p=0.017) was associated with increased risk of ALS and higher HDL (HR 0.58, CI 0.36-0.93, p=0.025), and higher apolipoprotein A (HR 0.52, CI 0.27-1.01, p=0.051) were associated with reduced risk of ALS.

In models incorporating multiple metabolic factors, higher HDL cholesterol (HR 0.49, CI 0.30-0.80, p=0.004) and apolipoprotein A (HR 0.43, CI 0.24-0.79. p=0.007) were associated with lower risk. Excluding those diagnosed within 5 years of first visit, higher age and higher LDL cholesterol (HR 1.38, CI 1.12-1.71, p=0.003) or apolipoprotein B (HR 2.34, CI 1.09-5.04, p=0.030) were associated with increased of ALS, and higher HDL cholesterol (HR 0.31, CI 0.15-0.63, p=0.001) or apolipoprotein A (HR 0.32, CI 0.14-0.76, p=0.009) with reduced risk.

Conclusions:
The associations of HDL and apolipoprotein A, and to a lesser extent LDL and apolipoprotein B levels, with risk of ALS contributes to a growing picture of differences in the premorbid metabolic landscape. The basis for his has important implications for understanding much earlier cellular mechanisms associated with the development of ALS and for the targeting of future preventative therapies.

Brain 18F-FDG-PET signature of SOD1 and TARDBP ALS patients

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Introduction.
Amyotrophic Lateral Sclerosis (ALS) is a genetic disease in about 10% of cases. SOD1 and TARDBP cause respectively 12% and 4% of familial ALS cases and smaller percentages of sporadic cases. Data about the brain 18F-FDG-PET features of ALS patients carrying SOD1 (SOD1-ALS) and TARDBP (TARDBP-ALS) mutations are very limited.

Objectives
To evaluate the brain metabolic changes of SOD1-ALS and TARDBP-ALS as compared to sporadic ALS using 18F-FDG-PET.

Methods
Eighteen SOD1-ALS and 14 TARDBP-ALS (carrying the p.A382T mutation) patients were compared to 46 wild-type sporadic ALS patients (sALS) randomly selected from 665 subjects who underwent brain 18F-FDG-PET at diagnosis between 2008 and 2019 at the ALS Centre of Turin. Forty healthy controls (HC) were enrolled.
We performed two subsets of analyses. SOD1-ALS on one hand and TARDBP-ALS on the other hand were compared to sALS and HC. The Full Factorial design and the two-sample t-test model of SPM12 were used. Age at PET and sex were included as covariates in all the analyses, site of onset (spinal/bulbar) and King's stage were included in comparisons among affected subjects. In all the analyses the height threshold was set at $P<0.001$ ($P<0.05$ FWE-corrected at cluster level).

Results
The full factorial analyses resulted in a significant main effect of groups. As compared to sALS, TARDBP-ALS showed a cluster of relative hypometabolism in right precentral and postcentral gyrus, superior and middle temporal gyrus and insula. As compared to HC, TARDBP-ALS patients showed large clusters of relative hypometabolism in bilateral frontal, parietal, temporal and occipital regions. When compared to SOD1-ALS, sALS patients showed a relative hypometabolism in right precentral and medial frontal gyrus, right paracentral lobule, and bilateral postcentral gyrus. SOD1-ALS showed a cluster of relative hypermetabolism as compared to HC, including right precentral gyrus and paracentral lobule. As compared to HC, sALS patients showed large clusters of relative hypometabolism in frontal, temporal and occipital cortices.

Conclusions
As compared to sALS TARDBP-ALS patients showed a relative hypometabolism in motor and extramotor regions. The relative hypometabolism in the motor cortex in sALS patients as compared to SOD1-ALS subjects might be related to the higher prevalence of upper motor neuron signs in sALS, while the relative hypermetabolism of SOD1-ALS as compared to HC in motor regions might be due microglial activation.

Brain architecture changes across the FTLD spectrum

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Introduction. Motor neuron disease (MND) and the behavioral variant of frontotemporal dementia (bvFTD) lie on the same pathologic/genetic continuum.
Objective. The aim of this study was to unravel distinct and shared structural MRI connectomic features of these syndromes.
Methods. 115 MND (83 amyotrophic lateral sclerosis [ALS] and 32 primary lateral sclerosis patients), 35 bvFTD patients and 61 controls underwent clinical/cognitive and MRI evaluations. According to neuropsychological testing, MND patients were classified in 79 pure-motor (MNDpm) and 36 cognitive and/or behavioral impaired (MNDci/bi – including 8 ALS-FTD). A sub-analysis was performed considering ALS patients only (54 ALSpm, 21 ALSci/bi and 8 ALS-FTD). Graph analysis and connectomics assessed structural and functional global/local topological network properties and regional connectivity.
Results. Globally, bvFTD showed altered structural and functional network properties compared to all other groups. At lobar level, bvFTD showed altered structural and functional network properties within the frontotemporal and basal ganglia areas relative to all groups. Structural alterations in the parietal lobe discriminated bvFTD from controls and MNDpm only. MND groups showed altered structural properties within sensorimotor and basal ganglia areas relative to controls. Focusing on ALS, structural alterations were confirmed within the same areas. Regionally, bvFTD showed widespread structural damage and decreased functional connectivity relative to all groups. MND groups showed disrupted structural architecture and enhanced functional connectivity within sensorimotor, basal ganglia and frontotemporal areas relative to controls. Results were confirmed in the ALS sub-analysis, highlighting that ALSci/bi showed an increased FC relative to ALS-FTD within the same areas.
Conclusions. The disruption of the structural architecture in MND phenotypes worsens in relation with the progression of cognitive deficits. Functional changes are characterized by increased FC in presence of exclusive motor impairment that intensifies with the occurrence of cognitive impairment in MND. The comorbidity of ALS and FTD leads to a decrease in FC.
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Calculating variant penetrance using family history of disease and population data

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Introduction
Genetic penetrance is the probability of a phenotype manifesting given that one harbours a specific variant. For most Mendelian genes, penetrance is high, but not complete, and may be age-dependent. Accurate estimates of penetrance are important in many biomedical fields including genetic counselling, disease research, and for gene therapy. However, the main methods for its estimation are limited in situations where large family pedigrees are not available, the disease is rare, late onset, or complex. These issues are particularly relevant for amyotrophic lateral sclerosis, for which unbiased penetrance estimates have been difficult to generate owing to its complex genetic architecture and the limited availability of data suitable for traditional penetrance estimation techniques.

Objectives
With the advance of high-throughput technologies, population-scale genetic data is available for an increasing range of genetic diseases and population-based penetrance estimation is becoming increasingly viable. Here we present a novel method for penetrance estimation in autosomal dominant phenotypes. We demonstrate the validity of the approach by applying it in several variant-disease case studies, and generate estimates of penetrance for risk variants in major ALS genes.

Methods
The approach uses population-scale data regarding the distribution of a variant among unrelated people affected and unaffected by an associated phenotype and can be restricted to samples of affected people only by considering family disease history. The method avoids kinship-specific penetrance estimates and the ascertainment biases that can arise when sampling rare variants among control populations.

We test the method by estimating penetrance in several variant-disease case studies. Particularly, we assess penetrance for: the LRRK2 p.G2019S variant for Parkinson's Disease; BMRP2 variants for Pulmonary Arterial Hypertension; SOD1 variants for ALS; the C9orf72 repeat expansion for ALS. Input data for each case study is drawn from published articles and public genetic databases.

Results
Across the included case studies, our penetrance estimates align closely with those in past research and with current understanding of each case. In the ALS case studies, we estimate the aggregate penetrance of SOD1 variants harboured by people with familial and sporadic ALS manifestations to be 0.749 (95%CI: 0.629, 0.864) in Asian and 0.660 (95%CI: 0.494, 0.812) in European populations. For the pathogenic C9orf72 repeat expansion, we estimate penetrance as 0.282 (95%CI: 0.023, 0.514) in Asian and 0.449 (95%CI: 0.377, 0.518) in European populations.

Conclusions
We have presented a novel approach to estimate penetrance in autosomal dominant traits. The estimates generated align with extant research and the method is well-suited for use in rare-diseases such as ALS.

Cerebrospinal Fluid Heavy Neurofilaments Discriminate Motor Neuron Diseases with Upper Motor Neuron involvement

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Introduction: Upper motor neuron (UMN) involvement may be the onset manifestation of different diseases widely diverging in prognosis, ranging from ALS to primary lateral sclerosis (PLS) and hereditary spastic paraplegia (hSP). Apart from ALS, in absence of a reliable biomarker, the diagnosis of UMN syndromes still relies mostly on clinical long-term observation and on genetic confirmation.
Objectives: I) to assess whether phosphorylated neurofilament heavy chain (pNfH) may discriminate different UMN syndromes at diagnosis; II) to test pNfH prognostic value in UMN diseases.
Methods: Cerebrospinal fluid (CSF) and serum pNfH were assessed in 143 patients presenting with UMN signs and then diagnosed with classic/bulbar ALS,UMNp-ALS,hSP, and PLS. Diagnosis has been confirmed through long term follow up (median time:85 months).
Results: ALS and UMNp-ALS patients had higher levels of CSF pNfH compared to PLS ($p<0.01$ and $p<0.01$, respectively) and hSP ($p<0.01$ and $p<0.01$, respectively). Higher levels of CSF pNfH were observed in patients presenting bilateral Babinski sign and widespread fasciculations ($p=0.03$ and $p<0.01$, respectively). ROC curves for discriminating ALS from PLS and hSP showed an area under the curve (AUC) of 0.75 and 0.95, respectively, for CSF and 0.66 and 0.86, respectively, for serum. ROC curves for discriminating UMNp-ALS and hSP showed an AUC of 0.97 and 0.93 for CSF and serum. ROC curves for discriminating PLS and hSP showed an AUC of 0.72 and 0.79 for CSF and serum. There was a significant correlation between CSF and serum pNfH and diagnostic delay, progression rate at sampling and at last observation, and ALSFRS-R score at sampling.
In multivariable survival analysis carried out among the totality of patients, CSF pNfH independently predicted survival; among classic/bulbar ALS, CSF pNfH represented the strongest variable predicting survival (HR 7.89, $p<0.01$) together with time to generalization (HR 0.96, $p<0.01$). At multivariable survival analysis among UMNp-ALS patients only progression rate (HR 4.71, $p=0.01$) and presence of abundant fasciculations (HR 15.69, $p=0.02$) resulted to be independent prognostic factors.
Conclusions: Our study suggest that CSF pNfH can discriminate between ALS (including UMNp-ALS), PLS and hSP. CSF pNfH resulted to predict survival in classic and bulbar ALS, but not among UMNp, where clinical signs remained the only independent prognostic factors.

Chitinase Dysregulation in ALS: Novel insights on Expression Dynamics from a Clinical Cohort and Murine Models

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Background: The chitinases are considered biomarkers of glial activation in neurodegenerative conditions, including ALS. However, the implications of chitinase upregulation for disease severity and progression and their cellular origins are not well understood. Although studies have reported CHIT1 and CHI3L1 expression in microglia and astrocytes, respectively, the majority of these results are based on post-mortem patient material, thus limiting information on expression dynamics.
Objectives: We analyzed the diagnostic and prognostic utility of key chitinases (CHIT1, CHI3L1 and CHI3L2) using the D50 disease progression model. Established murine models of ALS were then employed to address: 1) Which cell types are in vivo sources of key chitinases during disease progression 2) How chitinase expression relates to the disease course and 3) Whether expression is influenced by the underlying pathology.
Methods: Using immunoassays, we characterized CHIT1, CHI3L1 and CHI3L2 levels in matched CSF and plasma samples from ALS patients (n = 39), neurodegenerative disease controls (NDEGs, n = 13), and non-neurodegenerative disease controls (NDCs, n = 11). SOD1-G93A, C9orf72 GA-CFP, and rNLS8-hTDP43 mice were selected for further study at pre-symptomatic, disease-onset, and late-disease stages. Immunostaining on spinal cord and brain sections was performed to identify CHIT1 and CHI3L1 expressing cell types (astrocytes: GFAP, microglia: Iba1, oligodendrocytes: Olig2, neurons: NeuN).
Results: We confirmed substantial elevations of all three chitinases in the CSF of the ALS cohort relative to NDCs. CHIT1 and CHI3L2, but not CHI3L1, were significantly elevated in the ALS cohort relative to NDEGs. No differences were noted in plasma. CSF chitinase elevation was a feature of highly aggressive disease in ALS and correlated robustly with neurofilaments, a neuron-specific cytoskeletal component and an established prognostic biomarker.
Surprisingly, we observed in wild-type mice that neurons (NeuN+), including lower motor neurons (ChAT+), express both CHIT1 and CHI3L1. Preliminary results in the SOD1-G93A model further suggest that advancing pathology skews chitinase expression towards the glia. Initial results from the C9orf72 mouse model indicate CHIT1 and CHI3L1 expression in neurons that contain pathogenic poly-GA aggregates.
Discussion and Outlook: These results are an important translational bridge between observations in clinical cohorts and the corresponding animal models. We confirm that in human patients, chitinase upregulation is a feature of the CNS rather than the periphery. Importantly, our preliminary results suggest that chitinase-mediated gliosis may actually originate in neurons i.e. the most vulnerable cell population in ALS. The shift to glial expression reiterates the duality of the neuroinflammatory response across neurodegenerative diseases.

Circulating miR-181 is a prognostic biomarker for amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a relentless neurodegenerative syndrome of the human motor neuron system, for which no effective treatment exists. Variability in the rate of disease progression has limited the efficacy of ALS clinical trials. Thus, better biomarkers of progression are desperately needed in order to achieve therapeutic progress. Here, we applied unbiased next-generation sequencing to investigate the potential of plasma cell-free microRNAs as biomarkers of ALS prognosis in 252 patients with detailed clinical-phenotyping. First, in a longitudinal cohort of 22 patients, we identified miRNAs, whose plasma levels remain stable over the course of disease and tested them further in a discovery cohort of 126 patients. We demonstrated that high levels of miR-181, a miRNA enriched in neurons of the brain and spinal cord, predicts a >2 fold risk of death and validated these findings in an independent replication cohort. Finally, miR-181 performance is comparable with the established neurofilament light chain biomarker and their combination results in superior prediction of ALS prognosis. Together, plasma miR-181 predicts ALS disease course, enhances precision of current biomarkers for patient stratification and suggests that a novel protein-miRNA biomarker approach may greatly enhance the power of clinical trials.

Clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion

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Introduction: Amyotrophic lateral sclerosis is a progressive neurodegenerative disease causing muscle atrophy, which by spreading to respiratory muscles causes death. The average disease duration is 3-5 years. Hexanucleotide GGGGCC repeat expansion in non-coding region of the C9orf72 gene is the most common cause of familial ALS (30-50%). The same expansion is present in patients with sporadic ALS, FTD and ALS/FTD phenotype. Also, this mutation is detected in other neurodegenerative disorders with variable frequencies.
Objectives: Review of clinical characteristics of ALS patients carriers of this expansion, a correlation between clinical and genetic data, and survival rate.
Methods: Study performed at the Neurology Clinic of the UCCS included 383 patients with definitive and probable ALS (El Escorial criteria), from 2011 to 2020. Patients underwent genetic testing by using two-step PCR protocol and for all the samples showing expansion Southern blot was performed. The presence of expansion was demonstrated in 31 patients. Clinical data was gathered by searching our ALS patient database.
Results: The GGGGCC expansion was found in 31 patients (8.1%) where 51.6% were male and 48.4% were female. Median age at onset was 63 years, the diagnostic delay was 1 year and disease duration was 25 months. Positive family history for ALS and/or dementia was present in 25.8% of the expansion carriers. Bulbar onset ALS was observed in 22.6%, spinal onset in 77.4% of patients. The mean of ALS FRS-r at the moment of diagnosis was 33.7±7.8. FTD/ALS was discovered in 5 patients (16.1%). Correlation between disease duration and age of onset, and between disease duration and ALS FRS-r had no significance ($p=0.753$; $p=0.601$). A negative relationship was detected between disease duration and ALS FRS-r slope ($p=0.004$; $R=-0.503$). A significant difference in survival rates according to a gender ($p=0.969$), site ($p=0.696$), neuropsychological tests ($p=0.107$), age of onset (groups older or younger than 55 years) was not proved ($p=0.080$). A significant difference in survival rates was proved for those treated with riluzole in relation to those who were not ($p=0.024$).
Conclusion: Examination of patients carrying GGGGCC expansion in C9orf72 gene in Serbia showed variability of the disease onset and clinical manifestation with a progressive disease course and positive therapeutic response to riluzole.
Keywords: C9orf72, survival, frontotemporal dementia, ALS FRS-r slope, therapy

Clinical features at onset and longitudinal trajectories of decline in MND patients with cognitive-behavioral impairment.

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Introduction. Cognitive and/or behavioral disturbances are frequently observed in motor neuron diseases (MNDs). However, it is still unclear whether MND patients with cognitive and/or behavioral impairment (MND-CBI) exhibit unique clinical profiles compared to MND patients with a pure motor syndrome (MND-motor).
Objectives. To explore clinical differences between MND-CBI and MND-motor patients both at disease onset and over disease course.
Method. 51 MND patients were followed longitudinally with cognitive/behavioral, mood and motor examinations approximately every 6 months for up to 1 year. Cognitive and behavioral alterations were assessed using the Edinburgh Cognitive and Behavioural ALS Screen (ECAS), raw scores were age and education corrected using corresponding normative values. Mood disturbances were evaluated using the Hospital Anxiety and Depression Scale (HADS) and motor impairment was graded using the ALS Functional Rating Scale Revised (ALSFRS-r) and the Medical Research Council (MRC) scale. Individual slopes of decline for each clinical measure were generated and Chi-squared and Mann-Whitney U tests were then used to compare baseline and longitudinal features between MND-CBI and MND-motor patients.
Results. According to current criteria, 37.25% of patients (N=19) were classified as MND-CBI, while the remaining 62.75% of cases (N=32) as MND-motor. At baseline, no significant differences were observed between the two groups in terms of demographic, motor and mood features. MND-CBI patients performed poorer than MND-motor cases also in ALS non-specific measures (ALS non-specific total score p=0.05, memory p=0.04 and visuospatial abilities p= 0.01). Longitudinally, no significant differences were observed in the rates of motor decline. 9.37% of MND-motor patients converted to MND-CBI and 5.26% of MND-CBI cases developed additional behavioral symptoms. Both groups declined in ALS-specific functions, however, a significantly more severe worsening was selectively observed for the executive domain in MND-CBI (p=0.01). MND-motor patients remained stable in ALS non-specific functions, while MND-CBI patients exhibited only a mild decline. Mood disturbances ameliorated in MND-CBI and worsened in MND-motor cases (p=0.04).
Conclusions. This study provides novel insights into the clinical profile of MND-CBI. Our results suggest similar onset features and paths of progressive motor decline but also distinct trajectories of extra-motor disturbances in this MND phenotype compared to pure motor cases.

Clinical features of speech and respiration accompanying decision-making for PEG insertion in bulbar ALS: Hellenic experience from ENCLAS center - Patras University Hospital

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Introduction: To date, clinical and behavioral measures are utilized in protocols to guide the decision-making for alternative feeding route in patients with ALS. Historically these included a bulbar onset diagnosis, decline in respiratory function, change in body weight (1). New evidence for the changes in clinical features of speech and non-speech movements has surfaced (2) which could potentially inform the timely management of ALS patients, if clinically significant.
Objectives: Our aim was to prospectively track changes in speech, swallowing and respiration-speech coupling as an add-on neurological assessment in bulbar and nonbulbar onset patients.
Method: Seven ALS patients (5 female, 61± 8 mean age±SD) participated in this preliminary prospective study. Four were diagnosed with bulbar onset according to the El Escorial revised and Awaji criteria (3,4). All patients were examined twice (6 months apart) and performed assessments were neurological clinical scales, spirometry and cough reflex testing and assessments of speech and non-speech oral mechanism, voice and swallowing.
Results: Results demonstrated that the patients with bulbar onset showed initially lower scores in several measures of oromotor mechanism repetition tasks compared to the normative data and the nonbulbar patients. Changes between the two visits were evident for spirometry results (PEF, PVC, MVV), oral repetition tasks, reading speech (words/min) and coupling of phonation and respiratory volume (s/z ratio) for the bulbar onset group. Interestingly, bulbar onset ALS patients had PEG inserted before the second visit due to rapid change in dysphagia on liquid boluses, increased residue post swallow and weight loss.
Conclusions: Our study presents preliminary evidence of the extended protocol utilised in our Clinic as a means to review additional clinical features to guide decision making for alternative feeding in bulbar as well as nonbulbar onset ALS. Only descriptive data is presented at this stage. Yet data are in keeping with the recent literature regarding the changes in jaw movements, oral diadochokinesis, and respiratory-speech coupling clinical features (5).
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Clinical Utility of Whole-Genome Sequencing in a large ALS cohort

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Introduction:
The complex genetic landscape in ALS presents a significant challenge in the clinical setting. Despite the increasing number of pathogenic/risk mutations reported in ALS, the true proportion of cases that can be attributed to genetic factors remains unclear and the actual role of genome-wide screening in clinical practice is still undetermined.

Objectives:
To provide a comprehensive analysis of frequency and spectrum of known genetic risk in ALS patients and assess the clinical utility of performing next-generation sequencing.

Methods:
Whole-genome sequencing was performed on 1043 ALS cases and 775 control samples from an Italian population-based ALS cohort. We screened for coding single-nucleotide variants, indels and expansions in 45 ALS-related genes. Variants were classified by likelihood of pathogenicity according to a pipeline that accounted for minor allele frequency, absence from the control cohort and integrated pathogenicity predictions. Samples were also screened for known repeated expansions.

Results:
We identified 44 known ALS-related variants, 18 pathogenic and likely pathogenic variants, 32 loss of function variants and 61 missense variants classified as deleterious according to our pipeline. These variants were observed in 190 patients (18.2% of our cohort). 79 patients (7.6%) carried the C9ORF72 hexanucleotide expansion.
Overall, 25.8% of our cohort carried a pathogenic mutation and 3% of cases were found to carry multiple pathogenic variants. Furthermore, we observed the ATXN2 intermediate repeat in 36 patients (3.5%). Age at onset ($p=0.0252$) and family history ($p< 0.00001$) predicted the likelihood of finding a genetic factor, while gender, site of onset and clinical picture did not.

Conclusions:
We conclusively show that genetic mutations and risk factors in both familial and sporadic ALS patients are more common than previously thought, thus highlighting that genetics play a central role in ALS pathogenesis. Next-generation genetic testing should be offered to all patients diagnosed with ALS for both diagnostic and prognostic purposes.

Clustering for clinical stratification of ALS patients:

Unearthing the potential of an understudied set of techniques and tools.

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Introduction:
Amyotrophic lateral sclerosis (ALS) heterogeneity is still remaining an intriguing question among neurologists.
Coming to an agreed upon consensus on the different clinical subtypes of the disease is far from being a solved problem.
Machine Learning (ML) literature offers a set of tools to detect possible groups of a given dataset namely Clustering tools.
According to [1], clustering algorithms for ALS patient stratification are used as off-the-shelf tools.
Authors in [1] state that the use of the above-mentioned tools in ALS patient stratification papers is far from being efficient.
The improper employment of such techniques when designing pipelines of data mining of ALS datasets could lead to misleading results about the validity of obtained ALS subtypes (clusters).

Objectives:
The aims of the current study are twofold: showing the positive impact of a careful design of a clustering process on ALS disease progression (as a regression task) and introducing the ALS research community to the various aspects to be meticulously studied before performing any clustering task.
The 2015 ALS Stratification challenge (ALS challenge) resulted in several models studying the effect of the PRO-ACT dataset clustering on two different predictive tasks.[2]
A top-ranking ALS challenge model was chosen as a reference model (benchmark) to be studied.

Method:
The current study analyzed the influence of a better choice of a specific clustering parameter on the disease progression model performance.
The performance of the disease progression model was gauged by the Root Mean Squared Error (RMSE).
Using a majority vote of 7 clustering validation indices (CVIs) on the best number of clusters, the original Random Forest regression model was run on a dataset comprised of PRO-ACT data on 2187 patients.
The performance of the solution proposed in the challenge was compared to the model with “a more natural” number of clusters as well as to the regression model run on the dataset without clustering.

Results:
The majority vote of the CVIs indicated that fragmenting the studied population into 4 clusters was the closest possible to a geometrically “natural” clustering.
Testing the Random Forest model with the parameters set by the original solution led to a decrease of the RMSE from 0.5309 (for the model without data clustering) to 0.5169 (after clustering the data into 4 separate groups).
Performance of the benchmark model was less than the one with 4 clusters.

Conclusions:
Preliminary results studying an already evaluated ALS progression model have shown caveats pertaining to testing ML-based stratification techniques.
Adhering to [1]’s recommendations, a thoroughly conducted empirical assessment of ALS stratification methods is a work-in-progress.

[1] Grollemund et al., Frontiers in Neuroscience, 2019
[2] Kueffner et al., Scientific Reports, 2019

Cognitive deficits reported in the M323K mouse, a TDP-43 ALS model

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron disease which is characterised by progressive muscle weakness and subsequent motor defects, due to degenerative changes of upper and lower motor neurons in the brain and spinal cord. Traditionally, ALS was believed to spare cognitive functions. However, ~50% of ALS patients develop cognitive and behavioural impairments, whilst ~15% of patients develop frontotemporal dementia (FTD). ALS and FTD are now considered parts of the same spectrum disorder.
TDP-43 is an RNA-binding protein that is implicated in both ALS and FTD pathology. This protein is normally localised in the nucleus and is involved in a number of RNA metabolism processes, including splicing. Cytoplasmic aggregates of TDP-43 protein are found in >90% of ALS cases and in 45% of FTD cases. Currently, there is no physiological TDP-43 model that exhibits both the motor and cognitive defects seen in ALS-FTD. The M323K mouse contains a point mutation in the endogenous TDP-43 gene (Tardbp). Homozygous mice display a mid-to-late life onset neurodegenerative phenotype, mainly motor symptoms (Fratta et al, 2018). However, cognitive testing on these mice has not yet been explored.

Objectives: This study focused on identifying progressive phenotypic changes in homozygous M323K mice; heterozygous mice were omitted since they show minimal neurodegenerative changes.

Method: A longitudinal study, consisting of a comprehensive phenotyping pipeline, was conducted on a cohort of M323K female mice (n=9 wild-types and n=9 homozygotes). A range of motor, cognitive and metabolic tests were conducted at an early (3-months) and late (1-year) time point.

Results: Preliminary data show that homozygous mice display cognitive deficits from 3-months of age. General well-being tests, such as marble burying and nesting, revealed impairments in normal rodent behaviours, indicating non-specific hippocampal dysfunction. Further in-depth cognitive tests, spontaneous alternation and fear conditioning, revealed learning and memory problems in the homozygous mice at 1-year.

Conclusions: The M323K mouse is an excellent physiological model of ALS-FTD. Its ability to display motor and cognitive phenotypes, which mimic clinical observations, make it a unique and invaluable tool to further understand ALS-FTD TDP-43 pathophysiology.

Cognitive Reserve in ALS: A longitudinal study

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Introduction
In the past year, cognitive reserve has become a hot topic in ALS research. Two cross-sectional imaging studies have shown that higher education and IQ can protect cognition from the effects of brain atrophy and glucose hypometabolism. One cross-sectional study showed that lifestyle factors may moderate clinical presentation. Finally, a longitudinal study has shown that lifestyle factors help retain cognitive functions longer.
Objectives
Our present work investigates the effect of cognitive reserve against brain atrophy longitudinally.
Method
We recruited 109 ALS patients, 45 of whom were available at 16 months follow up. We calculated cognitive reserve based on verbal intelligence, educational length and attainment. We investigated its interaction with regional atrophy and onset type, and their combined effects on progression speed (ALSFRS-R slope) and cognitive performance. Data analysis was conducted in a Bayesian probability framework.
Results
These are limited preliminary results. The interaction between cognitive reserve and volume loss in the primary motor cortex explained 45% of the variance in progression speed over 16 months (BF=4). Of the two, cognitive reserve was more influential (b=0.13). The effects of cognitive reserve differed between onset types: low reserve limb-onset patients progressed faster, and high reserve bulbar-onset progressed faster.
Conclusions
Longitudinally, lifestyle factors exhibit a stronger influence on disease progression than motor cortex atrophy. This supports the cognitive reserve hypothesis in ALS further.

Common and rare variant association analyses in Amyotrophic Lateral Sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology

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Introduction
ALS is a complex trait with heritability estimated at 50%. Nevertheless genome-wide association studies GWAS have found few genome-wide significant risk loci.

Objectives
To find new genetic risk factors for ALS and reveal biological mechanisms that determine ALS susceptibility.

Methods
We conducted a new GWAS combining individual level genotype data from all previous ALS GWAS in European ancestries, newly genotyped ALS patients and controls as well as summary statistics of the Asian ancestries GWAS in ALS. Variants were imputed from the Haplotype Reference Consortium down to an allele-frequency of 0.001 followed by logistic mixed model regression. After the cross-ancestry meta-analysis, we prioritized genes in GWAS loci based on rare variant association analyses including screens for repeat expansions, and brain-specific eQTL and mQTL analyses. Genetic correlations were calculated and we ran colocalization analyses across the spectrum of neurodegenerative diseases (AD, PD, FTD, PSP, CBD). Subsequently we performed tissue and cell-type enrichment analyses, as well as biological pathway analyses of ALS "core genes" obtained by combining our GWAS with the largest, new, brain-specific transcriptome dataset (MetaBrain). Finally we made causal inferences for putative environmental/life-style risk factors through Mendelian randomization.

Results
In this GWAS included 29,612 ALS patients and 122,656 controls that identified 15 risk loci in ALS. When combined with 8,953 whole-genome sequenced individuals (6,538 ALS patients, 2,415 controls) and the largest cortex-derived eQTL dataset (MetaBrain) and mQTL datases we prioritized genes in 14 loci. This revealed locus-specific genetic architectures where we find evidence for ALS genes harboring rare variants, repeat expansions or regulatory effects. ALS associated risk loci were shared with multiple traits within the neurodegenerative spectrum and colocalization analyses identified two addicational loci associated with ALS, shared with AD and PD. In ALS we find strong evidence for neuron-specific enrichment, particularly in glutamatergic neurons, but not in immune-mediated tissues/cells such as in Alzheimer's disease. Brain-specific "core genes" in ALS play a role in vesicle mediated transport and autophagy. Of the environmental and life-style risk factors obtained from literature, Mendelian randomization analyses indicated a causal role for high cholesterol levels.

Conclusion
We find new genetic risk loci and risk genes for ALS that together provide evidence for cell-autonomous disease initiation in glutamatergic neurons and implicate vesicle-mediated transport, autophagy and cholesterol metabolism as important processes in ALS susceptiblity.

Comparative study of CSF chitinases in ALS: correlation with clinical features

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Introduction

Amyotrophic lateral sclerosis (ALS) is a complex disease characterized by several pathophysiological features including neuroinflammation. Neuroinflammation is characterized by immune cell activation causing specific chitinases induction. ALS has no cure at present and the identification of biomarkers is a topic of intensive research with implications in therapeutic trials and the understanding of disease mechanisms.

Objective

In this work we aim at investigating the biomarker potential of the chitinases chitotriosidase (CHIT1), chitinase-3-like protein 1 (CHI3L1), and chitinase-3-like protein 2 (CHI3L2) from the cerebrospinal fluid (CSF) of ALS patients.

Methods

The study population consisted of 34 ALS patients and a control group of 24 patients with other neurological diseases. CSF was collected by lumbar puncture
into polypropylene tubes without additives and immediately stored at −80 °C. CSF CHIT1, CHI3L1 and CHI3L2 were quantified by ELISA. pNFH was used as benchmark and was quantified by ELISA. CSF of ALS patients was analysed by UHPLC-mass spectrometry (UHPLC-MS).

Results

CHIT1 was significantly higher in ALS than in controls and ROC curve analysis showed a high CHIT1 diagnostic performance (AUC 0.80, p=0.0001) only slightly lower than that of pNFH (AUC 0.84, p<0.0001).
The three chitinases correlated with disease progression and CHIT1 (r=0.56, p=0.0007) had better performance. CHIT1 correlated with forced vital capacity (FVC) (r=-0.45, p=0.020) but not the other chitinases nor pNFH. Strong to moderate correlations were detected between all chitinases studied and pNFH. CHI3L2 correlated with CHIT1 and CHI3L1. Cox model showed that low CHI3L2 level was an independent predictor for survival, in addition to age and disease duration.
UHPLC-MS of CSF oligosaccharides indicated the presence of di-N-acetylchitobiose, which could constitute a product of chitin hydrolysis catalysed by CHIT1.

Conclusions

Our results supported the value of CHIT1 as a diagnostic and progression rate biomarker, suggesting a potential as a biomarker for respiratory function. CHI3L2 was most promising as a prognostic biomarker for survival. The relevance of di-N-acetylchitobiose in the CSF needs to be further investigated. The results should be confirmed in a larger population of patients.

Comparison between Amyotrophic Lateral Sclerosis Electromyography abnormalities SCORE and diagnostic certainty of ALS

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There are a few surveys that explain quantitative methods for electromyography abnormalities. (Tai et al., 2018).
Objective. The aim of the study is to design a method to quantify the EMG abnormalities score (SCORE) in key muscles and to give a score on patients with ALS.
Methods. A cross-sectional study with prospective data collection was performed between 2019-2021. Patients with ALS at Instituto Roosevelt in Bogotá-Colombia were included. The ALSFRS-R was applied and muscle strength was measured in the biceps brachii, first dorsal interosseous (FDI), quadriceps and anterior tibialis (AT) with the MRC scale (0-5). If the principal muscle (PM) did not meet criteria for active denervation (AD) and chronic reinnervation (CR) replacement muscle (RM) was examined. When AD and CR were found, 1 point was assigned to each. Points were assigned only to the PM or to the RM. Bilateral evaluation is required, PM and RM were: FDI/extensor indicis, biceps brachii/deltoid, AT/medial gastrocnemius, vastus medialis/adductor longus. If AD was found in FDI, and in EI assigned score was 1. If AD was only found in FDI, but AD and CR were found in EI the assigned score was 2. Thoracic paraspinal (TP) and tongue muscles (TM) were examined (each can score 2 points maximum if AD and CR were found). The result is the sum of the score of the 4 limbs (PM or RM), TP, and TM, with a maximum total score of 20 points. Total score was compared with each of the different levels of diagnostic certainty of ALS considering El Escorial Criteria—R (definite, probable, possible) and applying the Wilcoxon test. A Spearman's rank correlation was performed between EMG score and muscle strength, and between EMG score and ALSFR-R scale.
Results. 81 patients were tested in a period of 24 months, 46 (56,8%) were male. The median age in the symptom onset was 57 years (IQ range 47,5-63,5) and the median duration of the disease was 11 months (IQ range 6.5 – 23). Minimum age 30 years and maximum 77 years. Comparison between ALS electromyography abnormalities score and each degree of diagnosis certainty $p< 0,0001$; $p<0,01$.
Discussion. The results indicate that the SCORE correlates with the diagnosis certainty, ALSFR-R scale and muscle strength. Babu et al., publication, an EMG study was systematically performed including PM and RM. The SCORE offers an EMG and complementary view of the severity of the disease. According to the different ALS diagnostic classifications, EMG abnormalities in 2 muscles of a different root and nerve of one extremity, have the same value if are present in both sides in the same region. Tai proposed a method to quantify EMG abnormalities, with the presence of fibrillation potentials and positive sharp waves (rating 0 to 4 per muscle), and estimation of the mean rating of all the tested muscles. Conclusions. The SCORE may be useful for disease follow-up, to communicate EMG findings in a more standardized way, and to define different prognostic groups

Conditioned Medium from Cells Overexpressing TDP-43 Alters the Metabolome of Recipient Cells

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Background
TDP-43 pathology has been associated with multiple pathways in ALS, such as metabolic dysfunction found in patients and in in vivo models. Currently, it has been described as a "prion-like" protein, as studies have shown its propagation in cell culture from ALS brain extract or overexpressed TDP-43 in co-culture and conditioned medium, resulting in cytotoxicity. However, the cellular alterations that are associated with this cytotoxicity require further investigation.
Objectives
Here, we investigated the effects of conditioned medium from HEK-293T cells overexpressing TDP-43 on cellular morphology, proliferation, death, and metabolism.
Methods
We overexpressed His-tagged, wild-type TDP-43 (wtTDP-43-6×His) in HEK-293T cells for 72 h. The presence of TDP-43 in the medium was measured by ELISA. Naïve recipient HEK-293T cells were incubated with this conditioned medium for 24 h. Propagation of wtTDP-43-6×His was investigated by Western blot of recpient cell lysates. Changes in cell death, proliferation, morphology, energy metabolism, and the metabolome were investigated in recipient cells.
Results
Cells overexpressing wtTDP-43-6×His (TDP-43 hereafter) released the protein into the conditioned medium within 72 h ($p = 0.0159$, $N = 3$). The protein was not detected in lysates of naive recipient cells incubated in conditioned medium. Cells in TDP-43-conditioned medium showed increased uptake of propidium iodide ($p = 0.1$, $N = 3$), higher proliferation ($p = 0.0286$, $N = 3$), and decreased structural integrity ($p = 0.127$, $N = 3$). Metabolomics analysis revealed alterations in amino acids, spermine/spermidine ratio, t4-OH-Pro, and glutamate in TDP-43-overexpressing cells. Biogenic amines, glycerophospholipids, sphingomyelins, and acylcarnitine were specifically modified in naive cells in TDP-43-conditioned medium. Essential amino acids, proline, glycine, threonine, asparagine, and serine were modified in both conditions.
Discussion
The findings here suggest that the toxicity induced by TDP-43-conditioned medium is associated with changes in the metabolome. Importantly, the metabolic alterations mentioned above have been associated with ALS. We observed a metabolism alteration associated with TDP-43 overexpression and TDP-43 conditioned medium with common and distinct discriminant metabolites, suggesting that the putative mechanisms of toxicity are not completely the same. It will be necessary to show the relationship between these metabolism alterations and cell toxicity and to focus on these alterations, regardless of the specificity to TDP-43. Since we did not observe TDP-43 propagation, the results shed light on the hypothesis that TDP-43 propagation could not be the only threatening factor in the medium surrounding cells.

Continued Intravenous (IV) Edaravone Treatment of ALS Patients Increases Overall Survival Compared With No IV Edaravone Treatment in a US Administrative Claims Database

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Introduction: Intravenous (IV) edaravone received FDA approval in May 2017 based on a statistically significant (P=0.0013) 33% slowing of functional loss as measured by the ALS Functional Rating Scale-Revised (ALSFRS-R) compared with placebo treatment at 24 weeks. Previous cross-sectional and longitudinal studies have shown that increased function and slower loss of function predict longer survival. Additionally, in short-term randomized trials of IV edaravone, no survival effect was confirmed over the 6-month double-blind period of the clinical trials or during the follow-up 6-month open-label extension.

Objective: To assess overall survival in a large cohort of patients with ALS receiving IV edaravone compared with ALS patients not receiving IV edaravone enrolled in an administrative claims database.

Methods: The current analysis includes commercially insured patients with ALS who initiated treatment with IV edaravone between August 8, 2017, and June 30, 2019, using Optum's de-identified Clinformatics® Data Mart database. We applied 1:2 matching on propensity scores to find non–edaravone-treated control patients for IV edaravone–treated case patients based on the nearest-neighbor method. Covariates include age, race, geographic region (grouped as West, South, Midwest, and Northeast), sex, pre-index disease duration (defined as the period between the date of first claim for ALS diagnosis and the first claim for IV edaravone), insurance (Medicare Advantage vs commercial), history of cardiovascular disease, and history of riluzole prescription. For cases, the index date was the date of the first claim for IV edaravone. For non–edaravone-treated controls, the index date was the date IV edaravone was available on the market (ie, August 2017). Shared frailty Cox regression analysis was performed on matched data to estimate the benefit of IV edaravone.

Results: A total of 319 cases were matched to 635 controls. Of these, 252 IV edaravone–treated cases (78.9%) had a history of riluzole prescription vs 498 non–edaravone-treated controls (78.4%). There were 117 reported deaths (36.7%) among the cases vs 403 among the controls (63.5%). Median overall survival post initiation of edaravone was 29.5 months in cases and 17.0 months in controls (hazard ratio, 0.57; 95% CI, 0.460-0.71; P<0.001). The estimated probability of survival at 12 months was 80.5% (95% CI, 77.8%-82.9%) in cases and 68.5% (95% CI, 64.6%-72.1%) in controls.

Conclusions: This real-world analysis demonstrated that continued IV edaravone treatment may improve overall survival compared with not using IV edaravone in a predominantly riluzole-treated cohort.

Cortical and subcortical damage in C9orf72 ALS patients compared to matched wild-type ALS patients.

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Introduction

C9orf72 mutation carrier with different neurological phenotypes, also in pre-symptomatic phases, shows cortical atrophy in multiple different brain regions, suggesting that it could be considered as a neurodevelopmental disorder. Besides cortical thinning, also subcortical structures, like thalamus, basal ganglia and amygdala have been found to be altered, both in C9orf72 carriers and in wild-type patients.

Objectives

The aims of this study is to assess cortical and subcortical alterations in a more large sample of mutation carriers and matched wild-type ALS patients.

Methods

We paired 24 C9orf72 positive (C9orf72+) ALS patients with 24 C9orf72 negative (C9orf72-) ALS patients, matched for age, sex, ALSFRS-R total score, and disease duration. They underwent 1.5T MRI assessment, performing 3D T1-weighted image scans. Vertex-wise between-group analyses for cortical thickness (Freesurfer) and subcortical region shape (FSL-FIRST) was performed to assess significant differences between C9orf72+ and C9orf72- groups. Total intracranial volume, disease progression, and number of body regions involved were used as nuisance regressors in both analyses. Significant cortical clusters were anatomically labeled according to the Desikan Cortical Atlas. Results were corrected for multiple comparisons using $p<0.05$ by method of Monte Carlo simulation in Freesurfer and $p<0.05$ FWE in FSL-FIRST.

Results

The cortical vertex-wise between-group analysis showed a reduced cortical thickness in C9orf72+ patients, in comparison to C9orf72- patients, in extended regions of the brain and in particular in: bilateral precentral and postcentral cortex, superior frontal gyrus, and precuneus, in left inferior temporal cortex, fusiform and parahippocampal gyri, lateral and medial orbital frontal cortex, posterior and rostral anterior cingulate, in right superior parietal and supramarginal cortex. The subcortical vertex-wise between-group analysis showed an extensive reduced volume in C9orf72+ patients, in comparison to C9orf72- patients, in bilateral thalamus, more prominent in the dorsal portion, in bilateral caudate, and left putamen. On the other side, C9orf72- patients, in comparison to C9orf72+ patients, showed a selective reduction of volume in the anterior portion of left amygdala.

Conclusions

Extensive brain atrophy is present in C9orf72+ patients when compared to wild-type ALS patients with similar disease burden. Different extra-motor cortical regions and subcortical structures, such as the amygdala, could play a different role in ALS patients, according to the genetic status.

Corticomuscular Coherence Patterns in Primary Lateral Sclerosis

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Background:
Neurodegenerative conditions such as PLS are associated with widespread changes in the motor networks. There is preliminary evidence from (f)MRI studies that show these changes occur beyond the primary motor cortex. Changes are evident in frontal and parietal cortices which may reflect direct impairment or compensatory overactivity of cortical and spinal networks.
Recordings of joint multi-channel electroencephalogram (EEG) and electromyogram (EMG) for time-series analyses can reflect the communication between cortical brain regions by quantifying the oscillatory motor drives to muscles during specified motor tasks; hence, providing direct neuro-electric signatures of network disruption.
We hypothesize that in PLS, there is an adaptive cortical network change that reflects the progressive degeneration of the corticospinal pathways, and that this can be harnessed as a quantitative measure of network change in PLS .

We hypothesize that cortico-muscular coherence (CMC) between EEG-EMG can interrogate disease-specific alterations in the brain's motor networks within and beyond the primary motor cortex in PLS.

Objective:
To determine whether Cortico-muscular Coherence (CMC) can identify changes in the cortical reorganisation in PLS during functional isometric motor tasks.

Methods:
16 patients with PLS, comprising 40% of the Irish PLS population, and 19 healthy controls were recruited and studied during the performance of isometric precision grip tasks. Simultaneous recordings of high-density 128-channel EEG and 8 bipolar surface EMG recordings from extrinsic and intrinsic hand muscles were taken. Coherence analyses quantified the neural communication between cortical brain regions and the muscles, that represent the oscillatory motor drives to muscles during the adapted pincer grip motor tasks.

Results:
Analysis of the PLS patient group showed pathological presence of CMC patterns between EMG and EEG signals recorded from non-primary motor areas in abnormal frequency bands. This included gamma-band increases over the parietal (Pz) region which extend to other frequency bands in the central (Cz) and contralateral motor (C3) regions.

Conclusions:
EEG-EMG coherence during functional motor tasks shows pathological changes in the central-peripheral communication in PLS patients. The presence of pathological CMC patterns seen, provide evidence of alternate compensatory patterns in the PLS group compared to controls. This study provides a proof of concept demonstrating that interrogation of CMC patterns could be developed as a marker of therapeutic efficacy at a network level in UMN conditions such PLS, where current quantitative clinical outcome measurements are limited.

COVID-19 pandemic impact on the respiratory function of ALS patients at first visit

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Introduction:
Phrenic nerve study assesses non-invasively the respiratory function in amyotrophic lateral sclerosis. We investigated if a worsening of phrenic nerve evaluations at first visit was present in 2020, during the COVID-19 pandemic, as compared to the previous 10 years.
Methodology:
All ALS patients followed in our ALS unit with recordings of phrenic studies at first observation were included. Patients were divided into 2 groups – G1 included patients investigated first in 2020; G2 included those investigated for the first time between 2010-2019. In both groups, we further considered differences between patients with subgroups of patients considering dichotomic phrenic nerve amplitudes (PhrenAmpl <0.4/>=0.4mV; <0.2/>=0.2mV) and the respiratory subscore of the ALS functional rating scale (RofALSFRS-R <12/ =12). Comparisons between groups were done using one-way ANOVA with Bonferroni correction. P<0.05 was considered as significant.
Results:
We included 753 patients (74 in 2020). No significant differences between groups were found regarding onset age, disease duration to 1st visit or to 1st phrenic, body mass index at 1st visit, mean ALSFRS, mean/ dichotomic (<12/12) RofALSFRS-R, mean/ dichotomic (<0.4mV/=>0.4mV) PhrenAmpl. The number of ALS patients with a PhrenAmpl <0.2mV was significantly higher (p=0.006) in G1 (G1 24.7% vs G2 13.97%) but not when considering the 0.4mV cut-off. Moreover, the number of patients with abnormal RofALSFRS-R (<12), was higher in G1 (p=0.02). In G1, the number of patients with respiratory function tests at entry strikingly decreased due to the fear of viral transmission.
Conclusion: COVID19 pandemic did not impact negatively on the diagnostic delay of ALS patients. However, a subset of patients with faster respiratory decline were probably observed later, with poor ventilatory function. This finding could derive from the technical difficulties in performing respiratory tests or because respiratory distress was associated with a possible viral infection.

Creatinine Kinase-MB as a complementary biomarker in the evaluation of patients with Amyotrophic Lateral Sclerosis.

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Introduction: Creatinine Kinase (CK) is mild to moderately elevated in 30 to 40% of Amyotrophic Lateral Sclerosis (ALS) patients, correlates with lower motor neuron loss, and is proposed to be an independent prognostic factor for survival. Similarly, cardiac isozyme CK-Myocardial Band (CM-MB), a marker used to evaluate patients with acute coronary syndromes, is elevated in neuromuscular diseases. In recent years, it has been replaced by more cardiac specific biomarkers such as troponins.

Objective: We asked whether CK-MB in serum could provide similar or superior information to CK in the evaluation and follow-up of patients with MND.

Methods: Over six months, we systematically evaluated CK and CK-MB levels in our workup of patients with suspected or confirmed motor neuron disease at our clinic. Our analysis of 124 consecutive cases comprised 111 patients with ALS, 8 patients with Primary Lateral Sclerosis (PLS), and 5 patients with benign fasciculation syndrome.

Results: Approximately 50% of the patients presented with CK levels above the 99th percentile cut-off. 68% of the patients with ALS had CM-MB levels above the 99th percentile cut-off. CK levels are signifi¬cantly elevated only in female patients. CK-MB levels are signi¬ficantly elevated in ALS patients, particularly among male patients. We performed subgroup analyses and clinical correlations. Only patients with spinal-onset ALS showed significantly elevated CK serum levels. Patients with spinal and bulbar-onset ALS showed significantly elevated CK-MB serum levels. Patients with PLS and benign fasciculation syndrome always showed normal CK-MB serum levels. In contrast to cTnT, CK-MB levels in ALS patients stabilize over time. The CM-MB level in serum was positively correlated with CK and cTnT serum levels and slightly correlated with the bulbar sub-score in the ALSFRS-R and disease duration.

Conclusions: We propose that CK-MB elevation in ALS is of non-cardiac origin and may serve as a marker of lower motoneuron or skeletal muscle involvement. CK-MB levels may thus be helpful in defining restricted phenotypes of ALS such as PLS and may also have value as a prognostic marker. Further research is necessary to determine the biological origin of the CK-MB elevation and to confirm its validity as a diagnostic marker.

Cryptic splicing and loss of synaptic gene UNC13A is induced upon TDP-43 pathology and exacerbated by ALS/FTD risk variants

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Introduction:
Genome-wide association studies have detected intronic UNC13A polymorphisms that worsen risk/severity of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), but the molecular mechanism remains unknown.

Methods:
We have used transcriptomics and proteomics data from iPSC-derived neurons and neuronal lines, in combination with RNA-seq from over 1,300 post mortem brain samples, to characterise UNC13A splicing. We then applied specific approaches to dissect the link between UNC13A splicing and TDP-43.

Results:
Here, we report a novel cryptic exon (CE) within UNC13A, which is induced by loss of nuclear TDP-43 and is abundant in affected post-mortem tissues.
The CE promotes nonsense mediated decay, inhibiting expression of this critical synaptic protein.
Strikingly, we find that two risk polymorphisms, identified in GWAS, alter TDP-43 pre-mRNA binding, increasing this cryptic event in vitro and in patients.

Conclusions:
We thus reveal both a novel disease mechanism by which TDP-43 pathology induces loss of UNC13A in the majority of ALS/FTD cases, and the specific mechanism by which UNC13A variants exacerbate this process to worsen disease risk/severity.

Cytoskeletal analysis in sensory nerve fibre endings as a new “pathological window” for axonopathies in ALS

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Introduction: Amyotrophic lateral sclerosis (ALS) is a multisystemic and multifactorial fatal disease characterized by axon degeneration and motor neuron loss. The underlying etiology causing these degenerative processes remains unknown. Increased neurofilaments, a component of the neuronal cytoskeleton, is a hallmark of pathological lesion and a well-established biomarker in serum and CSF of ALS patients.

Objectives: While neurofilaments are essential for caliber maintenance and transmission of electrical signals, microtubules provide the basis for axonal transport as molecular motors walk along them. Neurofilaments and microtubules, together with their posttranslational modifications, likely are a critical component in ALS pathology. We analyzed the cytoskeleton of sensory nerve fiber endings of ALS patients.

Method: Skin biopsies of the proximal leg in seven ALS and four healthy controls were immunostained for microtubular mass (ßIII-tubulin) and neurofilaments (phosphorylated/non-phosphorylated neurofilaments – pNfH/npNfH). Individual fibres were analyzed for mean intensity grey values and the axonal caliber in confocal images of the dermis. Using the D50 progression model, we included the individual disease course and aggressiveness of ALS patients.

Results: ALS patients with high disease progression showed a significant loss of the immunostained cytoskeletal components compared to low progressive ALS (ßIII-tubulin/pNfH $p<0.05$; npNfH $p=0.054$). Also, spinal onset-ALS showed a significant decrease in ßIII-tubulin and pNfH levels compared to bulbar onset ($p<0.05$). We also detected an age dependent increase of cytoskeletal compounds with age.

Conclusions: Light and electron microscopy of skin biopsies provide a simple tool to study axonal pathology in ALS. Known and new biomarkers can be analyzed and extended to other motor neuron diseases, for example spinal muscular atrophy. Skin biopsies could devise a method to monitor new therapeutic approaches and their success, as sampling several times is unproblematic.
As our preliminary results point to a strong correlation of disease aggressiveness and cytoskeletal loss, we want to compare different motor neuron diseases and healthy controls in a greater cohort. Additionally, results of the immunostainings will be correlated with clinical data and biomarkers (neurofilaments in serum/CSF).

Decreased expression of macrophage migration inhibitory factor (MIF) in human post mortem tissue and in motor neurons from mutant SOD1-ALS patient-derived iPSCs

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Introduction & Objectives:
Macrophage migration inhibitory factor (MIF) is a cytokine which plays an essential role in the immune system, but has also been implicated in intracellular protein chaperone activity. In recent years it could be shown that it is capable to prevent accumulation of misfolded superoxide dismutase 1 (SOD1) in the degenerative motor neuron disease amyotrophic lateral sclerosis (ALS). Increasing expression of MIF in motor neurons (MN) in mutant SOD1 ALS mouse models has been shown to inhibit the accumulation of misfolded SOD1 and its association with intracellular organelles and to delay the onset and the progression of the disease.

Method:
To investigate the expression level of MIF in ALS and healthy MNs we differentiated MNs starting from the expandable population of human induced pluripotent patient and control stem cell lines (iPSCs) - derived smNPC. Two cell lines of ALS patients carrying a homogenous D90A mutation and one with a heterozygous R115G mutation in the SOD1 gene and three cell lines of healthy controls were used. A detailed characterization regarding expression of neuronal and motor neuronal markers as well as MIF using immunocytochemistry was performed.
In addition, protein expression of MIF was analyzed in human post mortem tissue (motor cortex and spinal cord specimens) of patients with a diagnosis of ALS according to the El Escorial criteria by Western Blot analysis and compared to post mortem tissue of patients with no evidence of neurologic disease (after informed consent by the patient or his/ her relatives as approved by the ethics committee of Hannover Medical School).

Results:
First results show that protein expression levels of MIF in mutant SOD1 patient cell lines are significantly lower than in controls. A similar reduction of MIF was observed in human ALS post mortem tissue. mRNA expression analyses are currently ongoing.

Conclusions:
These findings support the conclusion that elevation of MIF levels in motor neurons might be a novel therapeutic approach to counteract neurodegeneration in ALS.

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Delayed diagnosis and diagnostic pathway of ALS patients: where can we improve?

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Introduction:
Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease with unsatisfactory treatment options. Early diagnosis is important in order to provide patients best management and opportunity for enrolment in clinical trials. However, ALS diagnosis can be challenging, with resultant significant diagnostic delay.

Objectives:
Analysis of diagnostic pathway and identification of predictors of diagnostic delay.

Method: A cohort of 580 ALS patients followed in our ALS clinic in Lisbon was studied. Demographic, disease and sociocultural factors were collected. Time from first symptom onset to diagnosis, time from first symptoms to first consultation, specialist's assessment and investigation requested were analyzed. Predictors of diagnostic delay were evaluated by multivariate linear regression, adjusting for potential confounders.

Results:
We included 580 ALS patients (mean age of 65±12 years). Most patients were classified as probable ALS according to the revised El Escorial criteria; 22% of patients were diagnosed with progressive muscular atrophy. One fifth of patients had a bulbar onset. The median diagnostic delay from first symptom onset was 10 months (IQR=5-18 months). Bulbar onset and faster disease progression were associated with a lower probability of diagnostic delay (bulbar onset: coef. -10.54, $p < 0.001$; fast progression: coef. -6.50, $p < 0.001$, respectively). Lower annual income was associated with longer diagnostic delay (coef. 5.25, p=0.003). The majority of patients were first assessed by non-Neurologists (80%), namely General Practitioners. The median time from first medical observation to diagnosis was 6 months (IQR=2-11). The majority (70%) of patients had 2 or 3 medical evaluations before diagnosis was achieved, and almost 15% consulted 4 or more specialists. Patients who were evaluated by a Neurologist had an increased likelihood of being correctly diagnosed, decreasing time to diagnosis. Almost all specialists who made the diagnosis (95%) requested the Electromyography (EMG).

Conclusions:
Late referral from non-Neurologists to a Neurologist is a potentially modifiable factor contributing to significant diagnostic delay. Increased awareness of ALS and, consequently earlier referral to a Neurologist at a specialized tertiary center, could reduce diagnostic delay.

Deregulation of TBK1-Mediated Autophagy by ALS-Associated MicroRNA-340

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Background

Growing evidence implicates microRNA (miRNA) deregulation as a hallmark of amyotrophic lateral sclerosis (ALS) and many studies have evaluated the potential of circulating miRNAs as disease biomarkers. However, whether miRNA expression changes in ALS patients are simply an indication of cellular dysfunction and degeneration, or in fact promote functional changes in target gene expression relevant to disease pathogenesis, is unclear. Our recent review of candidate miRNA biomarkers in ALS patients (1) highlighted that many are predicted to target components of the autophagy pathway, altered function of which is a potential pathomechanism in ALS.

Objectives

To select a candidate miRNA previously reported to be dysregulated in ALS patients, with implications in autophagy regulation. We aim to investigate whether this miRNA can impact cellular autophagic flux and determine a potential target through which it may exert its effects.

Methods

To select a candidate miRNA, we employed miRNA-target prediction software to identify miRNAs implicated in the autophagy pathway and cross-referenced these with hundreds of miRNAs previously shown to be dysregulated in ALS patients. Selection of a candidate miRNA was made from those which were additionally predicted to target TBK1, a critical regulator of autophagy and ALS gene product. We subsequently utilised a live cell autophagic flux assay (2) to investigate whether the candidate miRNA impacts autophagy in a live cell model, and utilised the dual luciferase assay to determine its potential to target TBK1.

Results

Our systematic bioinformatic selection process has identified miR-340-5p as a suitable candidate miRNA. Here, using a live-cell autophagy assay, we show that miR-340 expression is associated with reduced incorporation of a SQSTM1/p62 reporter protein into acidic autophagic vesicles, consistent with an impairment of autophagy. Luciferase reporter assays indicate miR-340 can directly target a predicted TBK1 3'UTR site and western blotting provides further evidence miR-340 targets endogenous TBK1 to reduce its expression.

Discussion and Conclusions

Investigating the functional relevance of ALS dysregulated miRNAs develops knowledge of the pathomechanisms contributing to ALS. Combined, our observations suggest that patient changes in miR-340 levels may be associated with ALS-relevant deregulation of autophagy, by directly targeting TBK1. This supports the notion that specific miRNAs could be used as clinical biomarkers and therapeutic targets in the future.

Acknowledgements

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Design of an adaptive Phase 1b/2a randomized controlled trial of WVE-004 in patients with C9orf72-ALS/FTD

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Hexanucleotide (G4C2)-repeat expansions found in the C9orf72 gene are one of the most common genetic causes of the sporadic and inherited forms of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). The discovery that these diseases share a common cause has led to the realization that C9orf72-ALS/FTD is a single genetic disorder that manifests across a clinical spectrum. WVE-004 is an investigational stereopure oligonucleotide that is designed to target C9orf72 transcripts containing the hexanucleotide-repeat expansion and has potential as a disease-modifying therapy for patients with C9orf72-ALS/FTD. This adaptive, Phase 1b/2a study of WVE-004 is a global, multicenter, randomized, double-blind, placebo-controlled trial that is planned to enroll up to 50 patients who have a documented hexanucleotide-repeat expansion in C9orf72 and is inclusive of ALS and FTD. We will assess the safety and tolerability of single- and multiple-ascending doses of WVE-004 administered intrathecally (IT) by lumbar puncture. Secondary objectives include studying pharmacokinetics (PK) in plasma and cerebrospinal fluid (CSF) and poly glycine-proline (polyGP) in CSF, a biomarker of pharmacodynamic (PD) effect. Exploratory objectives will consist of biomarkers of neurodegeneration, such as neurofilament light chain (NfL) in the CSF, as well as functional measures, such as ALSFRS-R (Revised ALS functional rating scale), FVC (Forced vital capacity) and CDR-FTLD (Clinical dementia scale-frontotemporal lobar degeneration). The trial is designed to be adaptive, so that PK, PD, and safety and tolerability results from each cohort will inform the dose and dosing frequency for subsequent cohorts in the single- and multiple-ascending dose phases of the trial. This first-in-human study will provide proof of concept of the safety, tolerability and PD effects of WVE-004 in C9orf72-ALS/FTD.

Design of the International, Randomized, Placebo-Controlled Phase 3 PHOENIX Trial of AMX0035 in Amyotrophic Lateral Sclerosis

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Introduction

AMX0035 is an oral, fixed-dose coformulation of sodium phenylbutyrate (PB) and taurursodiol (also known as ursodoxicoltaurine). AMX0035 was shown to significantly slow functional decline in adults with definite ALS (revised El Escorial criteria) and slow vital capacity (SVC) >60% who were ≤18 months from symptom onset in the 24-week US multicenter, randomized, placebo-controlled phase 2 CENTAUR trial. Similar adverse event rates were observed with AMX0035 and placebo. Overall survival (OS) was significantly longer in those originally randomized to AMX0035 vs placebo at nearly 3 years after randomization.

Objective

To describe the nearly finalized design of a phase 3 trial assessing AMX0035 safety and efficacy in an international population of people with ALS.

Methods

PHOENIX will be conducted in approximately 55 Treatment Research Initiative to Cure ALS (TRICALS) and Northeast ALS Consortium (NEALS) sites in Europe and the US and include up to 600 participants (EU, n≈400; US, n≈200). Inclusion criteria expand on those in CENTAUR to enroll a broader population: definite or clinically probable ALS (revised El Escorial criteria), SVC >55%, and ≤24 months from symptom onset. Participants will be randomized 3:2 to receive AMX0035 (3 g PB/1 g taurursodiol per sachet) or placebo by mouth or feeding tube, 1 sachet per day for approximately 14–21 days and then, if tolerated, 1 sachet twice a day for the remainder of the 48-week study.

Results

Safety and tolerability of AMX0035 vs placebo will be assessed. The primary efficacy outcome will assess disease progression by evaluating the change from baseline in ALS Functional Rating Scale–Revised total score at 48 weeks with a joint assessment of function and survival. Secondary efficacy outcomes include change in SVC, measured both at home using a self-administered spirometer and at clinic sites; serial assessments of patient-reported outcomes (ALSAQ-40, EQ-5D, and EQ VAS); time to transition through King's and MiToS stages; and ventilation-free survival rates (defined as death, tracheostomy for respiratory distress, or permanent noninvasive ventilation [>22 hours/day for 7 consecutive days]). Exploratory outcomes include measurement of plasma biomarkers of neuron damage and neuroinflammation. Long-term OS (all-cause mortality) of all participants will be assessed beyond the planned 48-week follow-up. Post-trial AMX0035 will be made available to participants completing the 48-week study period in accordance with each region's regulatory guidance.

Conclusions

The phase 3 PHOENIX trial of AMX0035 in ALS will build on findings of the phase 2 CENTAUR trial by incorporating a larger, global population of people with ALS followed for a longer duration. As the first phase 3 ALS trial to employ at-home spirometry, PHOENIX will inform feasibility of this technique in future trials. Enrollment is projected to begin in Q3 2021

Development and validation of the remote administration of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS): Work in progress

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Introduction: The COVID-19 pandemic has created unprecedented challenges to MND clinical care and research, with the inability to offer face-to-face visits. This has accelerated the need to remotely continue to provide the best care to patients and pursue research. A fundamental component in the provision of appropriate care for people with MND (pwMND), and which 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 MND is the Edinburgh Cognitive and Behavioural ALS Screen (ECAS). However, the ECAS has been developed and validated for face-to-face administration only.

Objectives: To develop and validate a remote administration method of the ECAS, and explore the experiences of pwMND, clinicians and researchers who have completed or administered the ECAS remotely.

Methods: Documents and materials suitable for the remote administration of the ECAS via teleconferencing have been developed. This includes: guidance notes for the clinician/researcher, an instruction sheet for pwMND, visual stimuli for ECAS versions A-B-C, and written response booklets. The validation process will consist of three components. (1) Two versions of the ECAS (A and B) will be randomised and administered to 35 healthy controls (HC), completing one in-person and the other remotely. (2) The ECAS will be administered remotely to 35 pwMND, with a second rater independently scoring the ECAS. (3) pwMND, clinicians, and researchers will be invited to take part in an online survey to explore their experiences of completing/administering the ECAS remotely.

Results: Over 100 clinicians and researchers have been given access to the documents and materials for the remote administration of the ECAS, and feedback to date has been positive. The study is still in progress, but preliminary data will be available for presentation. Proposed analyses will include: exploring the equivalence of in-person and remote administration using between group comparisons, and Bayesian statistics to directly test the null hypothesis; intra-class correlations (inter-rater reliability); and thematic analysis of feedback obtained.

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

Development of Circulating miRNA Diagnostic Prediction Model for ALS and FTD

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Introduction:
Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) reside on a neurodegenerative continuum and a substantial number of patients manifest with clinical comorbidity.
The diagnosis of both FTD and ALS is often delayed due to symptom heterogeneity and since it requires observed progressive deterioration.

Objectives:
To show the fascinating potential that circulating miRNAs hold a as diagnostic biomarkers in ALS and FTD. Such miRNA biomarkers may allow early-stage detection of high-risk individuals and contribute to subtyping of patients, as well as a cost effective screening approach for clinical trials that can facilitate drug development.

Method:
We prospectively enrolled, from different clinical centers, a large cohort of 370 patients diagnosed with ALS (n=202) and FTD (n=168) with different clinical phenotypes and pathological forms, as well as healthy controls (n=125).
We have performed a miRNA profiling in biofluids and profiled plasma miRNAs in these individuals in an unbiased manner. Then, novel diagnostic prediction models are developed and validated by implementing advanced machine learning algorithms under K-fold stratified cross validation and additional held-out dataset.

Results:
The diagnostic prediction models are able to distinguish between ALS, FTD and non-degeneration controls with an average ROC AUC of 0.91 in a held-out cohort. Furthermore, shared and disease-specific microRNA were identified. Intriguingly, our classifier is capable of predicting ALS-FTD comorbidity.

Conclusions:
Circulating miRNAs hold a fascinating potential as diagnostic biomarkers, which may allow early detection of high-risk individuals, clinical subtyping of patients on the ALS-FTD continuum and cost-effective screening that can facilitate diagnosis and clinical trials for patients with ALS-FTD.

Differences in impairment classifications between cognitive screening tools

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Introduction

Around 50% of people with ALS experience changes in their thinking and behaviour. Screening tools, designed to identify impairment in cognition (ALSci) and which can be used in research and NHS settings, have been developed. Whether or not there is a difference in their classifications of ALSci is unknown.

Objectives

This study aimed to assess differences in classifications of impairment by two screening tools of ALSci and classifications of ALSci based on the revised frontotemporal spectrum disorder diagnostic criteria for people with ALS.

Method

The Edinburgh Cognitive and Behavioural ALS Screen (ECAS), ALS Cognitive Behavioural Screen (ALS-CBS) and a neuropsychological test battery used to assess the revised frontotemporal spectrum disorder diagnostic criteria for people with ALS were administered to 39 people with ALS. Classifications of impairment on the ECAS and ALS-CBS were determined using pre-established cut-off scores. Impairment on the test battery was classified according to the revised diagnostic criteria. Differences in classification of ALSci between screening tools and between the screening tools and the test battery were assessed using the McNemar test. The false discovery rate was controlled using the Benjamini–Hochberg procedure.

Results

While there was no significant difference in the ALSci classification between the ECAS and the neuropsychological test battery, there was a significant difference between the ALS-CBS and the test battery ($p = .001$), and between the ECAS and ALS-CBS ($p < .001$).

Conclusions

In conclusion, the ECAS and ALS-CBS should not be used interchangeably in research and clinical applications as their classifications of ALSci differ. This could lead to inaccurate comparisons of research findings in systematic reviews and meta-analyses, and inappropriate healthcare provision. The ECAS performs better than the ALS-CBS in reflecting the revised frontotemporal spectrum disorder diagnostic criteria. This is likely to be due to cognitive domain content.

Different roles for laughter and crying in emotional lability: a neuropsychological study on a large cohort of incident ALS cases

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Introduction
Recurrent episodes of involuntary or exaggerated laugher and crying are recognized symptoms reflecting damage to corticobulbar motor neurons and prefrontal and limbic areas in Amyotrophic Lateral Sclerosis (ALS). These symptoms, termed emotional lability (EL), have been considered a disorder of emotional expression, but overlaps between depression and EL-associated crying have been described. There is little information on the interplay of crying and laughter and social cognition deficits, behavioural anomalies, emotional dysregulation and social isolation. To accomplish this, we examined crying and laughter as separate outcomes adjusting for mood disorder.
Methods
One hundred and fifty-five incident ALS patients completed the CNS Emotional Lability Scale (CNS-LS). Partial correlation analysis (controlled for mood and age) were performed between scores of crying (CNS-LS-C) and laughter (CNS-LS-L) with measures of motor functional status, cognition, behaviour, and psychological measures including mood, emotional regulation, quality of life (QoL), loneliness, ($p \leq 0.001$). We used CNS-LS-C, CNS-LS-L and depression scores of the Hospital Anxiety and Depression Scale (HADS-D) for hierarchical cluster analysis (HCA) to identify subgroups with different presentations of EL and mood disorder.
Results
Fifty-two patients had pathological EL, showing behavioural changes, depressive symptoms and anxious personality traits. They revealed lack of emotional clarity and difficulties in using emotional regulation strategies. CNS-LS-C scores were related to depression, anxiety and emotional dysregulation. CNS-LS-L scores were associated with bulbar symptoms and dysexecutive behaviours. HCA identified 5 groups. The largest (N=67) did not have EL or mood disorder. HADS-D scores were related to CNS-LS-C scores only in the group (N=23) with moderate mood disorder. The group with the highest CNS-LS-C scores (N=27) had anxious personality traits. The group with high CNS-LS-L scores had normal mood (N=30). Finally, 7 patients had higher CNS-LS-L, CNS-LS-C and HADS-D than other groups.
Discussion
In a large incident cohort of ALS patients, one-third present pathological EL that is accompanied by affective and emotional disorders and behavioural impairment, without social cognition deficits. Laughter and crying have differing associations. Laugher is related to dysexecutive behaviours. Crying is associated with mood disorder and emotional dysregulation, possibly leading to poor Qol and loneliness. However, crying in EL overlaps with the depressive mood only in 15% of patients, suggesting that EL is a distinct condition from mood disturbance.
Conclusion
Laugher and crying can be expressed differently in ALS. Pathological laugher can be a mayor determinant for behavioural change, pathological crying can reflect a disorder of emotion regulation.

Differentiation between MND phenotypes: the role of clinical features at the time of diagnosis.

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Introduction. Motor neuron diseases (MNDs) can affect the upper motor neuron (UMN) and/or the lower motor neuron (LMN), and it is now widely accepted that pure/predominant UMN (pUMN) and pure/predominant LMN (pLMN) phenotypes have significantly better prognosis compared to classic amyotrophic lateral sclerosis (ALS). Despite this consideration, the heterogeneity of the initial manifestations often challenges an accurate differentiation between these MND phenotypes, with important consequences in terms of prognosis estimation.
Objectives. To determine which clinical features at the time of diagnosis may help differentiating pUMN and pLMN phenotypes from classic ALS.
Method. 50 MND patients were included in this retrospective study (22 classic ALS, 11 pUMN and 17 pLMN). At the time of diagnosis patients underwent a detailed clinical characterization, including: site (bulbar, proximal spinal, distal spinal) and side of disease onset, disease duration, overall degree of functional impairment (assessed using the ALS Functional Rating Scale-revised [ALSFRS-r]), regional UMN involvement (graded using the UMN score) and muscle strength (evaluated using the Medical Research Council [MRC] scale). Mann-Whitney and Chi-squared tests were used to identify significant differences between pUMN and classic ALS as well as between pLMN and classic ALS. Logistic regression analyses were then applied to isolate significant predictors of diagnoses.
Results. Compared to classic ALS, UMN phenotypes presented with longer diagnostic delay ($p=0.01$) and disease duration ($p=0.01$) as well as greater lower limbs UMN damage ($p=0.01$), but only the latter 2 variables were significant predictors of a pUMN diagnosis ($p=0.03$ and $p=0.03$ respectively). LMN phenotypes were characterized by longer disease duration ($p=0.02$), more severe fine ($p=0.05$) and gross ($p=0.03$) motor functional impairment and more preserved bulbar function ($p=0.02$) as well as lower muscle strength in the right lower limbs ($p=0.04$) and more preserved UMN involvement in the upper limbs ($p<0.001$). Significant predictors of a pLMN diagnosis were longer disease duration ($p=0.01$), bulbar preservation ($p=0.05$), gross motor impairment ($p=0.03$), reduced muscle strength in the right lower limbs ($p=0.03$) and preserved UMN involvement in the upper limbs ($p=0.02$).
Conclusions. Our findings suggest that specific clinical features at the time of diagnosis may help differentiating between more benign and more aggressive MND phenotypes. These findings have potential to facilitate appropriate stratification for clinical trials enrollment, clinical management and prognosis estimation.

Disease progression and survival in ALS patients diagnosed according to El Escorial and Gold Coast criteria

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Introduction
The recently proposed Gold Coast criteria aim to simplify diagnosis and allow more patients with motor neuron diseases to enroll in trials, possibly improving generalizability of trial results. The main difference between the Gold Coast criteria and the rEEC is that patients without upper motor neuron dysfunction who exhibit lower motor neuron dysfunction in >=2 body regions, previously diagnosed as progressive muscular atrophy (PMA), will now fulfil the criteria for ALS.
Objective
To define disease progression of the Gold Coast and revised El Escorial (rEEC) criteria for amyotrophic lateral sclerosis (ALS), and assess variability in progression rates per diagnostic category.
Methods
Data from population-based ALS registries from the Netherlands and Belgium were analyzed. Patients that did not fulfill the rEEC were classified as Gold Coast ALS if >=2 body regions had lower motor neuron dysfunction. Between-patient variability in progression rates and proportion eligible for the MAGNET trial was estimated, using the European Network for the Cure of ALS (ENCALS) risk profile, per rEEC and Gold Coast diagnostic category.
Results
In total, 6,060 patients were enrolled, 5,460 (90.1%) fulfilled the rEEC, while 5,862 (96.7%) had Gold Coast ALS. There were 198 (3.3%) rEEC patients with upper motor neuron signs in two body regions that did not fulfill the Gold Coast criteria. Likewise, 600 (9.9%) patients only fulfilled the Gold Coast criteria. These 600 patients were more often male (69% vs. 57%), almost exclusively had spinal onset (95%) and average decline was relatively slow with -0.46 points/month on the ALS functional rating scale. Similar to the rEEC categories, there was considerable heterogeneity among patients that only fulfilled the Gold Coast criteria, with progression rates ranging from -1.72 to +0.29 points/month and 60.2% would be eligible for the MAGNET trial based on their ENCALS risk profile.
Conclusions
Gold Coast ALS patients, that do not fulfill the rEEC, progress, on average, more slowly than patients fulfilling the rEEC. Within each diagnostic category, there is, however, a wide variation between patients. Excluding categories from trials may unnecessarily exclude patients, underscoring the need for individualized strategies to enhance patient selection for future studies.

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 (polyQ) that generally consist of 22 repeats in healthy individuals. Intermediate polyQ 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 double mutant mice using a previously described ALS mouse model harbouring human mutant TDP-43. Our double mutant mice carry this ALS TDP-43 background in addition the human ATXN2 gene with either healthy (CAG22) or ALS-associated (CAG33) repeat expansions. We have performed an extensive characterization of the different mutant mouse lines including behavioural tests and histological, cellular and molecular analysis. Double mutant mice carrying intermediate ATXN2 repeat expansions (TDP43-CAG33) show a progressive reduction of motor function accompanied by Purkinje cell degeneration in the cerebellum. However, no spinal cord motor neuron loss or neuromuscular junction defects are observed. Although TDP43 does not aggregate or mislocalize in spinal cord motor neurons, double mutant animals (both CAG22 and CAG33 repeats) show a modest increase in TDP43 insolubilization in the spinal cord. RNA sequencing of whole spinal cords revealed that TDP43-CAG33 mice have a distinct transcriptome consisting of more than 3200 DEGs. Pathway analysis shows a significant downregulation of the inflammatory response and upregulation of oxidative phosphorylation, amongst other dysregulated pathways. Our dataset includes differentially expressed homeostatic and disease-associated microglia genes, and, interestingly, we observe a change in microglia morphology. Microglia display a more ameboid shape, in the absence of microgliosis or astrogliosis. In order to further study the role of microglia in TDP43-CAG33 mice, we have performed RNA sequencing on isolated spinal cord microglia, which shows differential gene expression related to neurotoxicity and neuroinflammation. In summary, our study aids in understanding how ATXN2 intermediate repeat expansions influence ALS pathogenesis, with a focus on microglial dysfunction. In future work, we are aiming to integrate our mouse model data with data from human iPSC-based in vitro models derived from ALS patients carrying ATXN2 repeat expansions.

Dissecting the role of microglia in C9ORF72 ALS using cerebral 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 in 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 an 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. Initial quantitative PCR analysis showed decreased homeostatic microglia markers. Additionally, organoid-isolated C9ORF72-ALS microglia display increased expression of proinflammatory cytokines after stimulation with lipopolysaccharide (LPS). Moreover, to detect changes in microglia morphology we are performing morphological analysis including number of ramifications, branch length, complexity of junction points. Additionally, transcriptome profiling of the microglia using (sc)RNA sequencing will provide insight into molecular pathways that are affected and help identify distinct populations of microglia. 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.

Disturbed Task-Related Cortical Oscillations As Potential ALS Biomarkers

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Introduction: Communication within cortical and subcortical networks associated with sensory, motor or cognitive function can be quantified by task-related changes to ongoing cortical oscillations at characteristic frequencies, recorded with EEG. This may be observed as an increase (event-related synchronisation, ERS) or decrease (event-related desynchronisation, ERD) in oscillation intensities during task performance or following stimulation [1]. Disruptions to ERD/ERS can inform of disturbances to intracortical and corticothalamic communication and have been found to provide better diagnostic utility than traditional event-related potential measures in mild cognitive impairment and Alzheimer's disease [2]. Such measures may therefore enable detection of early motor and non-motor ALS pathophysiology in the absence of detectable clinical symptoms.

Objectives: To investigate if cortical oscillations associated with sensation, cognition and motor performance are disrupted in ALS.

Method: A randomised sustained attention to response task (SART) was undertaken by 24 ALS patients and 33 controls, and an auditory oddball paradigm was undertaken by 94 ALS patients and 62 controls during 128-channel EEG. Complex Morlet wavelet transform was used to quantify ERD/ERS. Electrical source imaging was used to identify the sources of these oscillations. The relationships between these perturbations and task performance, motor and cognitive changes in ALS were investigated.

Results: ALS patients performed similarly to controls in the SART; however, prefrontal and parietal beta-band ERD was significantly lower (AUROC>0.8). ALS patients with higher ECAS ALS-specific scores demonstrated greater ERS in beta and theta rhythms upon successful withholding (rho>0.7). ALS patients also showed auditory sensory-associated alpha oscillation hypersynchrony predominant in the medial and lateral temporal cortex, including the hippocampus as well as the right insula, but also present in the thalamus and basal ganglia.

Conclusions: EEG-captured cortical oscillations detect cortical and subcortical network pathophysiology in the absence of task performance decline, which may facilitate development of sensitive, early ALS biomarkers.

1. Pfurtscheller et al. Clin Neurophysiol 1999
2. Fraga et al. Comput Methods Programs Biomed 2018

Do ecological factors influence the clinical presentation of amyotrophic lateral sclerosis?

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INTRODUCTION. ALS is pleiotropic and its phenotype could vary depending on several factors: age of onset, prevalent damage to the upper (UMN) or the lower motor neuron, body region mostly affected, progression rate and presence of non-motor symptoms, the most frequent being cognitive impairment. It is unclear if different etiologies could justify this variability. Spatial epidemiology could give some hints on the etiology of diseases by identifying clusters and looking for their genetic or environmental causes.
OBJECTIVES. To perform a spatial analysis of ALS cases stratified by their clinical presentation.
METHODS. All patients included in the Piemonte and Valle d'Aosta ALS Register (PARALS) who received an ALS diagnosis between 2007 and 2014 and who were resident in Piemonte at the time of diagnosis were considered. The residence municipality at the time of diagnosis were considered for each patient. Cluster analysis was performed stratifying patients by sex, age at diagnosis, onset site and phenotype (classified as classic, flail arm, flail leg, predominant UMN, bulbar and respiratory). All analyses were performed both including and excluding genetic cases to consider a possible different susceptibility to environmental exposure. Cluster analyses were assessed using the Kulldorff spatial scan statistic and the SaTScan software.
RESULTS. A total of 943 patients were included. No significant clusters were revealed for any of the subgroups considered with the only exception of a small low-incidence cluster of patients with flail arm located in Northern Piedmont (over 201 municipalities with 655537 residents, 7.86 cases were expected and none was observed, relative risk=0.0, p=0.024). Analyses excluding genetic cases did not modify the results.
CONCLUSIONS. Although several hypotheses have been made to justify the heterogeneity of ALS clinical presentation, to date the biology underlying ALS phenotypes is unclear. Our data, based on the geographical distribution of cases, do not indicate that different ecological causative factors underlie ALS clinical presentations. We believe that clinical presentation should not be the only factor to consider when looking for ALS causative elements. Since ALS is thought to be caused by the interplay of genetics, epigenetics and environmental factors, a stratification based on genetics could be considered when looking for susceptibility to different environmental factors. A meaningful subgrouping could ideally lead to the development of efficient personalised therapies.

Dual role of lysophosphatidic acid receptor 2 (LPA2) in amyotrophic lateral sclerosis

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Lysophosphatidic acid (LPA) is a pleiotropic extracellular lipid mediator with many physiological functions that signals through 6 known G protein-coupled receptors (LPA_{1-6}). In the central nervous system (CNS), LPA mediates a wide range of effects including neural progenitor cell physiology, neuronal cell death, axonal retraction and inflammation. Since inflammation is a hallmark of most neurological conditions, we hypothesized that LPA could be involved in the physiopathology of amyotrophic lateral sclerosis (ALS). We found that LPA_2 RNA was upregulated in post-mortem spinal cord samples of ALS patients and in sciatic nerve and skeletal muscle of SOD1G93A mouse, the most widely used ALS mouse model. To assess the contribution of LPA_2 to ALS, we generated a SOD1G93A mouse that was deficient in Lpar2. This animal revealed that LPA_2 signaling accelerates disease onset and neurological decline but, unexpectedly, extended lifespan. To gain insights into the early harmful actions of LPA_2 in ALS, we studied the effects of this receptor in the spinal cord, peripheral nerve and skeletal muscle of ALS mice. We found that LPA_2 gene deletion increased microglial activation but did not contribute to motoneuron death, astrogliosis, degeneration and demyelination of motor axons. However, we observed that Lpar2 deficiency protected against muscle atrophy. Moreover, we also found the deletion of Lpar2 reduced the invasion of macrophages into the skeletal muscle of SOD1G93A mice, linking LPA_2 signaling with muscle inflammation and atrophy in ALS. Overall, these results suggest for the first time that LPA_2 contributes to ALS, and its genetic deletion results in protective actions at early states of the disease, but shortens survival thereafter.

Dynamic Bayesian networks for stratification of disease progression in ALS

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Introduction

Considering that progression rate is quite variable in ALS, implying different times for medical interventions, new tools for profiling disease progression can be useful for promoting quality of life and prolonging survival.

Objective:

To apply Dynamic Bayesian networks (DBNs) to determine the influence of clinical and demographic variables on the disease progression rate.

Methods:

We included 1214 patients from our data base, who were stratified in 3 groups, according to ALSFRS-R rate of decay during their first 2 years of follow-up: slow, average, and fast progressors (SP, AP, FP). We analyzed both static [gender, age at onset, onset region, body mass index (BMI) at entry, disease duration at entry, familial history, diagnostic category on the revised-El Escorial criteria and C9orf72] and dynamic variables [ALSFRS-R scores and subscores, forced vital capacity (FVC), maximum inspiratory pressure (MIP), maximum expiratory pressure (MEP) and phrenic nerve response amplitude]. We used a machine learning model for graphically represent the conditional dependencies among variables: the sdtDBN framework, which learns optimal dynamic Bayesian networks (DBNs) with both static and dynamic variables.

Results:

Disease duration and BMI have higher influence than other static variables, but different among groups: BMI has less influence on SL and disease duration has residual influence on FP. Disease duration is the variable that better differentiates the 3 groups. Gender, onset form and family history have diminished influence. Age of onset has medium influence but only on FP. MEP is the respiratory test with the highest influence on all groups, suggesting it is a better prognostic indicator of patient´s decline than the FVC and the phrenic nerve amplitude, both with little influence. Regarding the functional scale, the ALSFRS score has high influence on FP, but minor or medium influence on AP or SP, respectively. The bulbar sub-score has high influence on FP, but almost none on SP. The sub-scores that evaluate limb function have higher influence on AP and SP. The respiratory sub-score has scarce influence on all groups. Considering the ALSFRS-R scale questions separately, question 1 is the most important in FP, while in AP and SP is question 9. Questions 5 and 6 have intermediate influence on all groups.

Conclusions:

Disease duration is a critical marker for distinguishing groups defined by the progression rate. BMI and bulbar function are the most influential markers for fast progressors, and lower limb function for slow progressors. MEP is the most important respiratory marker for all groups. DBNs is a promising predictive and descriptive tool. This insightful information can lead clinicians to pay particular attention to specific variables when evaluating the patients, thus helping to improve prognosis and care.

Effect of familial clustering in the genetic screening of 235 French ALS families

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Objectives: To determine whether the familial clustering of amyotrophic lateral sclerosis (ALS) cases and the phenotype of the disease may help identify the pathogenic genes involved.

Methods: We conducted a targeted next-generation sequencing analysis on 235 French familial ALS (FALS), unrelated probands to identify mutations in 30 genes linked to the disease. The genealogy, that is, number of cases and generations with ALS, gender, age, site of onset and the duration of the disease were analysed.

Results: Regarding the number of generations, 49 pedigrees had only one affected generation, 152 had two affected generations and 34 had at least three affected generations. Among the 149 pedigrees (63.4%) for which a deleterious variant was found, an abnormal G4C2 expansion in C9orf72 was found in 98 cases as well as SOD1, TARBP or FUS mutations in 30, 9 and 7 cases, respectively. Considering pedigrees from the number of generations, abnormal G4C2 expansion in C9orf72 was more frequent in pedigrees with pairs of affected ALS cases, which represented 65.2% of our cohort. SOD1 mutation involved all types of pedigrees. No TARDBP nor FUS mutation was present in monogenerational pedigrees. TARDBP mutation predominated in bigenerational pedigrees with at least three cases and FUS mutation in multigenerational pedigrees with more than seven cases, on average, and with an age of onset younger than 45 years.

Conclusion: Our results suggest that familial clustering, phenotypes and genotypes are interconnected in FALS, and thus it might be possible to target the genetic screening from the familial architecture and the phenotype of ALS cases.

Effect of genotype on cerebral 18F FDG uptake in patients with Amyotrophic lateral sclerosis

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Introduction:
Amyotrophic lateral sclerosis (ALS) is the most common form of motor neuron disease (MND), characterized by progressive loss of upper and lower motor neurons. The majority of patients know a sporadic form of the disease, however in 10% of patients ALS and/or frontotemporal dementia runs in the family.

Objectives:
The aim of this study was to study the impact of disease-causing gene mutations on cerebral 18F-FDG uptake in patients with ALS.

Methods:
A large cohort of 447 MND patients, consecutively seen at the University Hospital of Leuven between January 2010 and August 2020, underwent both genetic testing for mutations with a strong evidence of causality for ALS and 18F-2-fluoro-2-deoxy-D-glucose-PET (FDG-PET), within one year from diagnosis. Of these 447 patients, we identified 39 C9orf72-ALS patients and 17 SOD1-ALS patients. Both groups were compared to a subset of 50 matched sporadic ALS patients and a historic cohort of 20 healthy controls. All FDG-PET images were assessed using a voxel-based and volume-of-interest approach, to study the association between genotype and regional glucose metabolism.

Results:
The degree of relative glucose metabolism in SOD1-ALS in motor and extra-motor regions did not differ significantly from sporadic ALS. In C9orf72-ALS we observed relative excess hypometabolism in the peri-rolandic region and relative excess hypermetabolism in the brainstem and posterior cerebellum, in comparison to sporadic and SOD1-ALS.

Conclusion:
Cerebral glucose metabolism in SOD1-associated ALS is strikingly similar to that observed in sporadic ALS, unlike C9orf72-associated ALS.

Effect of NEK1-C9ORF72 double mutation on DNA damage response in patient-derived neural stem cells

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Emerging evidence suggest that DNA damage and impairment of DNA damage response (DDR) are implicated in the pathogenesis of ALS. C9ORF72 repeat expansion associates to DDR defects in motoneurons from human induced pluripotent stem cells (iPSC) and mutations in NEK1 gene, involved in both DDR and maintenance of chromosomal stability, have been identified in ALS patients. Moreover, increased DNA damage and impaired DDR have been recently described in iPSC-motoneurons from an ALS patient carrying a loss-of-function mutation in NEK1 gene. By mutational analysis we identified an Italian ALS patient carrying a concomitant repeat expansion in C9ORF72 gene and a loss-of-function mutation in NEK1 gene (p.Ser1036Ter).

In order to study the effect of the double mutation on DNA damage repair, we obtained primary fibroblasts from the patient then reprogrammed into iPSC.

When we characterized the generated double mutant iPSC line, FISH analysis revealed a significant higher number of C9ORF72 RNA foci compared to three different mutant C9ORF72 iPSC lines already available in our laboratory. Since a previous study described no difference in DDR in iPSC from mutant C9ORF72 and healthy controls, we differentiated iPSC into neural stem cells (NSC) to obtain a neural committed cell model in which the DDR could be better investigated. We induced DNA damage with the radiomimetic agent Neocarzinostatin, that causes DNA double-stranded breaks, and compared the DDR in NSC from the double mutant C9ORF72-NEK1, the three different C9ORF72 and two healthy control lines. We quantified γH2A.X histone- and BP53-positive nuclear foci as markers of DNA damage and further divided NSC in four arbitrary categories according to the γH2A.X foci number per cell: (I) 2-5 foci, (II) 5-20 foci, (III) 20-30 foci and (IV) >30 foci.

Our results showed that all NSC displayed low and comparable levels of DNA damage in basal condition without significant differences among the experimental groups. After DNA damage induction, we observed a similar increase of both γH2A.X- and BP53-positive nuclear foci in all the analyzed cell lines. DNA damage was rescued in a time-frame between 4 and 8 hours after Neocarzinostatin removal, returning to the basal values at 24 hours, with no significant differences among all the analyzed NSC lines.

In conclusion, our preliminary results indicate that, although the C9ORF72-NEK1 iPSC showed increased pathological RNA foci, the induced DNA damage could be efficiently repaired in the highly-mitotic NSC, independently from the presence of C9ORF72 or C9ORF72-NEK1 double mutation. This suggests to further investigate DDR in a more differentiated and post-mitotic neuronal model, such as iPSC-motoneurons, to better understand the possible interplay between NEK1 and C9ORF72 genes. Our study aims to assess the relevance of DNA damage and DDR as novel and druggable pathomechanisms in ALS.

Effects of presymptomatic lifestyle on ALS stratified by C9orf72 genotype: a longitudinal population-based study

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Introduction

Amyotrophic lateral sclerosis (ALS) is considered to be caused by both genetic and environmental factors. The causal cascade is, however, not known.

Objectives

To assess presymptomatic lifestyle, stratified by C9orf72 mutation, and examine evidence supporting causality for ALS.

Method

In this longitudinal, population-based, case-control study, 143 patients with (C9+) and 1322 patients without (C9-) C9orf72 mutation were recruited between January 2006 and January 2016. Patients fulfilled the revised El Escorial criteria. 1322 population-based controls, matched for age and sex, were enrolled via the patients' general practitioners. We studied the relationship between ALS risk and smoking (cigarette pack-years), alcohol (units), physical activity (PA; metabolic equivalent of task), body-mass index (BMI; kg/m2), and energy intake (kJ) by use of structured questionnaires. Smoking, PA and BMI were longitudinally assessed up to 50 years before onset (defined as the period before onset of muscle weakness or bulbar symptoms for cases, or age at completing the questionnaire for controls). We calculated posterior probabilities ($P(\theta|x)$) for causal effects of smoking, alcohol and BMI, using Bayesian instrumental variable analyses (IVA).

Results

Compared to controls, cigarette pack-years (mean difference 3·15 (0·36-5·93), p=0·027; 3·20 (2·02-4·39), p<0·0001) and energy intake at symptom onset (712 (212-1213), p=0·0053; 497 (295-700), p<0·0001) were higher, and BMI (-2·01 (-2·73 to -1·29), p<0·0001; -1·35 (-1·64 to -1·06), p<0·0001) and alcohol intake (-5388 (-9113 to -1663), p=0·0046; -2185 (-3748 to -622), p=0·0062) lower for C9+ and C9-, respectively. Median presymptomatic BMI for C9+ was lower (-0·69 (-1·24 to -0·13), p=0.015) and PA similar (-348 (-966 to 270), p=0·27) to controls, while both were higher in C9- (0·27 (0·04-0·50), p=0·022; and 585 (291-878), p=0·0001). Longitudinal analyses demonstrated more cigarette pack-years in C9- and C9+ (starting 47 and 24 years pre-onset, respectively), and higher PA over time in C9- (starting >30 years pre-onset). BMI of C9+ increased more slowly and was significantly lower (starting 36 years pre-onset) than in controls, while BMI of C9- was higher 23-49 years pre-onset, becoming lower 10 years pre-onset. IVA supported causal effects of alcohol consumption ($P(\theta|x)$=0·9347) and smoking ($P(\theta|x)$=0·9859) on ALS in C9-. We found evidence supporting a causal effect of increased BMI at younger age (mean: 33·8±11·7 years) in C9- ($P(\theta|x)$=0·9272), but not at older ages.

Conclusions

Presymptomatic lifestyle differs between ALS patients and controls decades before onset, depends on C9-status, and is likely part of the presymptomatic causal cascade. Identification of modifiable disease-causing lifestyle factors offers opportunities to lower risk of developing neurodegenerative disease.

Electromyographic findings in primary lateral sclerosis: a longitudinal study

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Introduction: Primary lateral sclerosis (PLS) is a slowly progressive neurodegenerative disease affecting upper motor neurons (UMN) in adults. Electromyography (EMG) is essential to exclude significant lower motor neuron (LMN) involvement, but minor or transient changes may be present.
Objectives: Our aim was to characterize EMG findings in patients with PLS over time.
Methods: We retrospectively enrolled 20 patients with definitive diagnosis of PLS. Demographic features and functional progression (ALSFRS-R) were registered. We qualitatively scored the presence of spontaneous activity (fibrillation/sharp waves, fasciculation potentials) and motor unit potentials (MUAP) and compared it over time. We grouped patients according to lower (group 1) and higher (group 2) score of EMG abnormalities. A p-value <0.05 was considered significant.
Results: Fasciculation potentials were more common than fibrillation/sharp waves, (n= 16 vs n= 3), mainly in upper limbs. These abnormalities seemed to be stable or transitory. Abnormalities in MUAPs were found in most patients (n=16) with some worsening over time, particularly in lower limbs, but no statistically significant change was found. Compared to group 1 (n=7), patients in group 2 (n=13) were older at disease onset (median= 56 vs 40 years, p=0.023), and have shorter disease duration (median= 10.4 vs 18.8 years, p=0.029).
Conclusions: In conclusion, most PLS patients show minor, stable or transitory EMG abnormalities. Patients with more evident EMG abnormalities are older and might have a faster disease progression. The impact of these findings on predicting progression to ALS is still to elucidate.

Electromyography as a marker of prognosis and a disease stage indicator in Amyotrophic Lateral Sclerosis

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Introduction

ALS is a neurodegenerative disease, involving upper and lower motor neurons. The diagnosis of ALS is based on clinical criteria supported by EMG, which investigates spontaneous activity, motor unit potential and the voluntary maximum recruitment pattern.

Objectives

The first aim of this paper is to assess, at diagnosis, some of the main ALS electromyographic findings and compare them to King's staging system, MiToS and NBRI. The second aim is to stratify patients' prognosis on the basis of electromyographic findings.

Methods

Electromyographic data of 7 muscle districts in 394 patients, belonging to PARALS register (2012-2018) were collected, selecting the EMG examination performed at diagnosis. We compared the main EMG parameters to ALSFRS-r scale, stratifying patients for different MiToS and Kings stages.

Results

The increase of MiToS, King's and NBRI systems stage is correlated with a higher percentage of muscle districts with alterated voluntary maximum recruitment pattern, while fibrillation/positive sharp wave (Fib/PSW) did not clearly correlate with clinical staging, being predominant in distal muscle districts. Fasciculations are more frequently recorded in proximal upper limbs rather than lower limbs (chi-squared test $p<0,001$). Moreover a higher fasciculations activity in the upper limbs was found to be useful to stratify patients belonging to the same clinical stage in different prognostic categories.

Conclusions

Different features of EMG spontaneous activity (Fib/PSW vs fasciculations) have distinct distribution in muscle districts, maybe underlying different neuropathological mechanisms. Fib/PSW are frequently found in distal muscle districts: this may be explained by early distal denervation in ALS onset. Fasciculations are often observed in upper limbs, possibly correlating to wider cortical projection of upper limb muscles (De Carvalho et al, 2017). Fasciculations has been correlated not only to a lower motor neuron disease, but also to an increased cortical excitability (Kleine et al, 2008). The presence of spontaneous activity in the proximal districts of upper limbs could be linked to a faster involvement of the respiratory muscles (Zhang et al., 2016). Needle EMG is a useful tool in motor neuron diseases, as it can be used to highlight different neuropathological characteristics in ALS patients cohorts with different prognosis.

Elevated levels of HDL-cholesterol at diagnosis are associated with shorter survival in patients with amyotrophic lateral sclerosis

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Introduction: Parameters of the lipid profile (e.g. cholesterol and triglycerides) may be of prognostic value in patients with amyotrophic lateral sclerosis (ALS). They are an essential component for mammalian cells and might reflect motor neuron loss in these patients. Dysregulation in lipid metabolism and mitochondrial dysfunction result in oxidative stress, which has been described as hypothesis in the pathogenesis of ALS. Therefore, high plasma cholesterol might indicate oxidative stress and, moreover, metabolic stress resulting in a shorter survival. However, it also has been found that elevated levels of serum cholesterol might be prognostic more favourable due to neuroprotective properties.

Objective: To systematically review previous studies on the prognostic value of the parameters of the lipid profile and to determine the prognostic value of the serum lipid profile for survival time in patients with amyotrophic lateral sclerosis (ALS).

Methods: We conducted a systematic review (SR) to summarize previous literature that determined the prognostic value of the lipid profile in patients with ALS. Subsequently, we evaluated the relationship between the lipid profile and survival time in a large population-based registry. We used Cox proportional hazards models to assess the independent prognostic value of several aspects of the lipid profile.

Results: The SR composed of eight articles; four studies did not find any relationship, while four studies did. These studies found a beneficial effect on survival in patients with high levels of TC, LDL/HDL ratio or TRI. However, only two studies concluded the same parameter from the lipid profile as prognostically favourable. Our prospective cohort study included 1,346 consecutive patients with ALS. We assessed all parameters from the lipid profile. A dose-response relationship between HDL-cholesterol and survival (HR 1.35 (95% CI 1.15 – 1.57, $p < 0.001$)) was found, increasing the risk of death with 35% per every point increase in HDL-cholesterol.

Conclusions: In this study, we demonstrate a variety of studies assessing the prognostic value of the lipid profile in patients with ALS. Due to differences in their results an overall conclusion cannot be easily made. Our population-based study shows that HDL-cholesterol may be an independent predictor of survival. Gaining further insights in cholesterol metabolism, HDL functioning, and metabolic stress, might reveal novel aspects of ALS pathogenesis and potential targets for treatment.

ENGRAILED-1 homeoprotein is a non-cell autonomous neurotrophic factor for spinal alpha-motoneurons

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Introduction
Engrailed-1 (EN1) is a homeoprotein transcription factor expressed in adult oculomotor neurons but not in spinal motor neurons (MNs). The former are resistant in ALS, while the latter degenerate, raising the possibility that EN1 is protective for MNs as shown for mesencephalic dopaminergic neurons. In the spinal cord En1 is expressed in V1 interneurons including Renshaw cells that synapse on the large αMNs and may thus be protective following intercellular transfer.

Objectives
To test EN1 non-cell autonomous activity on αMN survival, we analyzed En1+/- (En1-het) mice and neutralized extracellular EN1 in WT mice by expressing a single chain anti-EN1 antibody (scFvEN1).

Method
En1 expression was followed by in situ hybridization and q-RTPCR. Neuromuscular junction (NMJ) morphology and αMN number were analyzed. Forepaw grip strength, time hanging onto an inverted grid and the hind limb extensor reflex were evaluated. EN1 (1µg/5µl) was injected intrathecally at lumbar 5 level and the scFvEN1 virally expressed in astrocytes.

Results
Reduced En1 expression in V1 interneurons in ventral spinal cord does not modify their survival, nor does it induce degeneration of oculomotor NMJs. En1-het mice show reduced muscle strength and an abnormal extensor reflex at 2 months of age, NMJ denervation at 3 months and significant αMN loss at 4.5 months with an increased expression of the p62 autophagy mark.
Intrathecal administration of EN1 at 3 months, when En1-het mice have muscle weakness and NMJ denervation but before αMN loss, restores muscle strength, normalizes the extensor reflex and prevents αMN loss and p62 accumulation at 4.5 months of age and these beneficial effects last 2 to 3 months.
Neutralizing extracellular spinal cord EN1 by expression of the scFvEN1 phenocopies the En1-het phenotype with reduced muscle strength, abnormal reflex, NMJ denervation, αMN degeneration and increased p62.

Conclusions
These data show that EN1 is an adult αMN neurotrophic factor with non-cell autonomous activity and suggest that EN1 may be useful for treating MN disease.

Note:SEVA and ML contributed equally to this work.

Epidemiological and clinical characteristics of a motoneuron disease retrospective cohort according to different riluzole formulation

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Introduction: Riluzole is the only approved drug to modify the course of amyotrophic lateral sclerosis in Europe and it should be offered to all patients to slow the progression disease. Riluzole is currently available in two different bioequivalent formulations: tablets and oral suspension. Riluzole oral suspension may provide with more accurate dosing, increase compliance and adherence to treatment, improve therapeutic outcomes and enhance quality of life for ALS patients and caregivers. Importantly, riluzole oral suspension has been recently authorized for use in ALS patients with gastrostomy, which is of relevance since up to 63.7% of ALS patients undergo gastrostomy.

Objectives: To collect the most relevant demographic and clinical characteristics, as well as relevant nutritional and neurological assessments of motoneuron disease (MND) patients in the Functional Motor Neuron Unit (FMNU) of Bellvitge University Hospital treated with riluzole tablets or oral suspension.

Methods: Retrospective cohort of patients with defined or probable MND according to the El Escorial-Arlie criteria, with different phenotypes and stage disease, evaluated in the FMNU in the period between January 1, 2011 and December 31, 2020 (n=742). Relevant demographic, clinical, nutritional and neurological data were collected. Descriptive univariate and bivariate analyses, both parametric (Student's t, ANOVA) and no parametric (Chi-squared, Mann-Whitney's U, Kruskall-Wallis test) were carried out.

Results: Analysis of demographic and clinical characteristics of the patients according to the different riluzole formulation administered in the baseline, showed statistically significant differences (at 95% confidence) regarding sex, age group, phenotype at diagnosis, gastrostomy, cognitive assessment, smoking, age at diagnosis, weight, amyotrophic lateral sclerosis functional rating scale (ALSFRS) assessment and premorbid weight (although in this case at 90%). There were also significant differences in terms of last weight and ALSFRS assessments, although differences between last and baseline evaluation were not significant when compared between groups. Riluzole oral suspension was the most prescribed formulation among patients with worst prognosis factors such as older than 65 years, with respiratory ALS and/or progressive muscular atrophy and gastrostomy, as well as patients with dysphagia with liquids and/or with solid food.

Conclusions: We found that there were differences in the demographic, clinical, nutritional and neurological characteristics between patients treated with riluzole tablets or oral suspension. Given the characteristics of MND patients, we believe that a good option would be to offer oral suspension, but always based on clinical criteria and taking into account all relevant factors, including the preference of the patient and/or their caregivers.

Epidemiology and genetic architecture of ALS in the isolated island population of Malta

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Introduction
Population isolates are compelling tools for mapping genes and identifying environmental factors that increase ALS risk. The archipelago of Malta, a sovereign microstate in the south of Europe, is home to a geographically and culturally isolated population.

Objectives
We aimed at investigating the epidemiology and genetic profile of Maltese patients with ALS, identified throughout a two-year window (2017-2018).

Methods
Phenotypic information was gathered via a detailed questionnaire in addition to a clinical examination. The denominator for the calculation of the incident rate was the sum of total population of Malta in 2017 and 2018. The prevalence rate was estimated on 31st December 2018. Whole-genome sequencing allowed us to determine rare DNA variants that change the protein-coding sequence of ALS-associated genes.

Results
Cases (n=24) were largely male (66.7%) with a predominant spinal onset of symptoms (70.8%). Disease onset occurred around mid-age (median age: 64 years, men; 59.5 years, female); 12.5% had familial ALS (fALS). Annual incidence rate was 2.48 (95% CI 1.59–3.68) per 100,000 person-years. Male-to-female incidence ratio was 1.93:1. Prevalence was 3.44 (95% CI 2.01–5.52) cases per 100,000 inhabitants on 31st December 2018. The southeast of mainland Malta had an increased number of ALS cases relative to other regions. One third of the ALS patients recruited had a history of heavy smoking and more than half reported an occupation associated with strenuous activity.

Intriguingly, the Maltese ALS patient cohort was found to be negative for deleterious variants in C9orf72, SOD1, TARDBP or FUS genes, which are the most commonly mutated ALS genes globally. Nonetheless, ALS-associated repeat expansions were identified in ATXN2 and NIPA1. Variants predicted to be damaging were also detected in ALS2, DAO, DCTN1, ERBB4, SETX, SCFD1 and SPG11. A total of 40% of patients with sporadic ALS had a rare and deleterious variant or repeat expansion in an ALS-associated gene, whilst the genetic cause of two thirds of fALS cases could not be pinpointed to known ALS genes or risk loci.

Discussion and conclusions
Population-specific aspects of ALS cases in Malta overlap those reported for other neighbouring European populations, especially those in the Mediterranean including the island of Sicily and the southern region of Puglia in Italy and Cyprus. Incidence and prevalence of ALS in Malta is similar to the European median. Rare deleterious variants in the major ALS were absent in Maltese ALS patients confirming the presence of a North-South gradient in the frequency of mutations within these genes across Europe. Our initial results warrant further studies aimed at elucidating novel genes and/or environmental factors that increase ALS risk in this unique population isolate.

Establishing a novel in vitro model of ALS by co-culturing iPSC-derived motor neurons and microglia

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Introduction: A growing body of evidence supports a role for neuroinflammation in amyotrophic lateral sclerosis (ALS) pathophysiology, with microglia particularly implicated. However, their specific role, harmful or protective, at different stages of the disease course and in the context of different ALS mutations is unresolved. To date, studies on microglia have been largely restricted to animal models, and there remains a need for more authentic disease models using human cells. In our previous work, human induced pluripotent stem cell (iPSC)-derived microglia co-cultured with iPSC-derived cortical neurons expressed relevant microglia markers, displayed dynamic ramifications, and were functionally active with respect to phagocytosis and secretion.

Objectives: This project aims to co-culture iPSC-derived microglia with iPSC-derived spinal motor neurons (MNs) to establish a novel in vitro model of neuroinflammation in ALS.

Method: We differentiated iPSC-derived microglia and MN precursors from three healthy controls and optimised the culture medium in order to establish compatibility between both cell types. We then combined both microglia and MN precursors in co-culture and maintained cultures to allow maturation of both cell types. Co-cultures were analysed for the maturity and identity of both MNs and microglia.

Results: Compared to MNs grown in monoculture, MNs in co-culture with microglia retained neuronal morphology and electrophysiological properties, and showed similar expression of the neuronal and MN markers TUJ1, ISLET-1, and ChAT. Moreover, co-cultured MNs displayed clear spontaneous activity in calcium imaging and were responsive to stimulation with potassium chloride. Microglia in co-culture were positive for IBA1, CD11b, TMEM119, and TREM2, made direct contact with MNs and their neurites, and showed ramifications with highly dynamic remodelling of primary and secondary branches. Furthermore, co-cultured microglia were phagocytically competent, released pro- and anti-inflammatory cytokines upon stimulation, and were responsive to ADP in calcium imaging.

Conclusions: These findings provide preliminary evidence for the functionality of our co-culture model. We are now evaluating the transcriptomic profile of co-cultured microglia. This co-culture model will further our understanding of the functional role of microglia in ALS.

Estimating the minimum important difference in the ALSFRS-R in people with amyotrophic lateral sclerosis

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Introduction
The ALSFRS-R is a 12-item, widely used outcome measure in trials of therapeutics in amyotrophic lateral sclerosis (ALS). It is scored on a 48 to 0 scale, with declining function resulting in a lower total. There is no consensus as to what represents a meaningful change in ALSFRS-R score for patients. Therefore, identifying the smallest relevant change is essential both in standardising the evaluation of drug efficacies and enabling regulatory authorities and patients to weigh the risk/benefit ratios of potential new treatments.

Objectives
The aim of this study was to estimate the minimal important difference (MID) for the ALSFRS-R in a prospective cohort of people with ALS. The MID will represent the smallest difference in ALSFRS-R score at which patients are able to detect a change in their condition and could be used to help assess whether a drug is appropriate for use in the management of ALS.

Method
Anchor and distribution based methods were used to estimate the MID scores for people with ALS in a longitudinal, observational study. ALSFRS-R data were collected at approximately 3 month intervals. For the anchor-based method, participants answered a global rating of change question (GRoC) to rate how their overall health-related quality of life compared to that at the previous visit. The data were grouped according to GRoC outcome, at visit 2 ("about the same", "better" or "worse"), with the change in ALSFRS-R scores between visits 1 and 2 then compared in the provisional analyses.

Results
A total of 114 people with ALSFRS-R data for both visits 1 and 2 (approx 3 months apart) and GRoC data from visit 2 are included in these analyses. According to the GRoC at visit 2, 54 participants reported their overall health as "about the same"; 2 as "better" and 58 as "worse". Initial analyses show that the mean change in ALSFRS-R score between visits 1 and 2 for each group were: "worse", -4.1 (SD 4.9), "about the same", -1.7 (SD 4.1) , "better", +1.5 (SD 0.7), with a positive mean change suggesting an improvement in function. The standardised response means were -0.8, -0.4 and +2.1 for those groups respectively. Note that data for the study is still being uploaded, hence we expect to have a larger dataset analysed by May.

Conclusions
The majority of participants reported their health had stayed the same or got worse over the ~3 month period. Provisional analyses indicate that the MID for the ALSFRS-R is approx 4 points in relation to a deterioration in health. The group of participants who could not detect any difference in their overall health were also associated with a decline in function based on the change in ALSFRS-R scores (mean of -1.7), highlighting the need to be cautious when interpreting ALSFRS-R data.
The results of this study can assist clinicians and researchers in the interpretation of ALSFRS-R scores for comparisons between groups or within groups of people with ALS.

EVALUATION OF PERIPHERIN IN BIOFLUIDS OF PATIENTS WITH MOTOR NEURON DISEASES

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INTRODUCTION: Peripherin (PRPH) is a type III intermediate filament, 58kD protein, which assembles with NFs mostly in neurons of the peripheral nervous system (PNS) and also in neurons of the central nervous system (CNS) with projections to peripheral structures, such as lower motor neurons (LMNs). PRPH function is incompletely understood though a role in neurite growth and stability has been suggested. PRPH seems to be involved in motor neuron vulnerability in motor neuron diseases (MND).

OBJECTIVES: we exploratively evaluated PRPH concentrations in cerebrospinal fluid (CSF) and serum from patients with MNDs and assessed possible correlation with motor neuron degeneration.

METHOD: utilizing commercial ELISA kits, we assessed PRPH and neurofilament light chain (NfL) in cerebrospinal fluid (CSF) and serum of 91 patients with MND (63 definite ALS according to the revised El Escorial criteria14; 20 Spinal Muscular Atrophy, SMA, type 3, and 8 Spinal and Bulbar Muscular Atrophy, SBMA) and 59 controls (26 patients with dementia, 14 with peripheral neuropathy (PN) and 29 healthy subjects)

RESULTS: we found PRPH to be more concentrated in serum than in CSF. Serum PRPH resulted significantly increased in MND patients but it was unrelated to CSF-NfL or survival in the amyotrophic lateral sclerosis (ALS) subset. Among MND patients, S-PRPH levels were higher in the SBMA group.

CONNCLUSIONS: high serum levels of PRPH might be a general marker of LMN axon disorders.

Evidence of a verbal fluency endophenotype in familial ALS.

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Introduction

Relatives of patients with Amyotrophic Lateral Sclerosis (ALS) report a heightened rate of schizophrenia, suicide and other neuropsychiatric conditions (1). It is hypothesized that this is due to the pleiotropic (i.e. multiple) effect of risk genes such as C9orf72 (2).

Objectives

This study aims to build on previous research by characterizing the cognitive profile of non-affected relatives of ALS patients.

Method

Two hundred and eleven first- and second-degree relatives of ALS patients and 134 controls completed an Edinburgh Cognitive and Behavioural ALS Screen (ECAS). Of these, 105 were from known familial ALS kindreds (FALS), and 109 we from kindreds in which only the proband had ALS (SALS). Relatives and controls were compared on ECAS total, ALS specific, ALS non-specific, language, fluency, executive, memory and visuospatial score. Further sub-group comparisons were carried out comparing familial and sporadic kindred to controls; and comparing C9orf72 positive (n=39) and negative kindreds (n=158).

Results

Non-manifesting members of ALS kindreds performed significantly worse than controls on verbal fluency score, $t(324)= 4.14$, $p<.001$. Relatives generated significantly fewer words on both unrestricted (no letter limit), $t(192)=2.64$, $p<.01$; and restricted (4 letter limit) protocols, $t(177)=2.49$, $p<.05$. Bonferroni-Holm post hoc analysis revealed that FALS kindreds performed significantly lower SALS kindreds ($d= -0.55$, $p<.05$) and controls on verbal fluency ($d = -0.74$, $p<.05$). In contrast, no significant difference was observed between SALS kindreds and controls. No significant differences were observed between C9orf72 positive and C9orf72 negative kindreds on any ECAS score.

Conclusions

These findings suggest that verbal fluency deficits occur in non-affected members of ALS kindreds, specific to kindreds with FALS, but not specific to individuals the C9orf72 gene mutation. Deficits in fluency may signify disruption to underlying fronto-striatal networks in these individuals. These finding, if replicated, may improve statistical power in gene discovery studies, and lead to an improved understanding of the extended genetic, neuropsychological and neuropsychiatric profile in ALS.

Examining the effects of the TDP-43 M337V mutation on cellular energy metabolism in embryonic stem cell-derived motor neurons

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Dysregulation of cellular energy metabolism has become increasingly recognised as a hallmark of ALS, with impairment of mitochondrial respiration consistently reported across ALS models. Motor neurons are particularly vulnerable to metabolic deficits, due to the high energy demand required for maintaining cellular processes such as axonal transport. Our group has previously developed a BAC transgenic mouse expressing human TDP-43 (M337V) at low levels. Expression of TDP-43M337V leads to a progressive motor phenotype, in vivo defects in axonal transport, alterations in stress granule characteristics and reduced survival in response to oxidative stress compared to controls1,2. Furthermore, comparative analysis of the TDP-43WT and TDP-43M337V interactome in mouse embryonic stem cell-derived motor neurons (mESC-MNs), revealed loss of interaction of mutant TDP-43 with proteins involved in mitochondrial respiration, glycolysis and vesicle-mediated transport.

This project aims to investigate dysregulation of cellular energy metabolism and axonal transport in TDP-43M337V mESC-MNs compared to NTg and TDP-43WT controls. Subsequently, we aim to examine the effects of a candidate drug on these disease-associated pathways.

Mouse ESCs (NTg, TDP-43WT/- and TDP-43M337V/-) were expanded as embryoid bodies and differentiated to MNs 1. Immunocytochemistry was performed to investigate TDP-43 mislocalisation and stress granule formation. Western blotting and immunocytochemistry were performed to assess the expression of mitochondrial proteins and glycolytic enzymes. Mitochondrial respiration and glycolysis were examined using the Seahorse XF Analyser. To determine the effects of a candidate drug on cellular phenotypes, mESC-MNs were treated at optimised concentrations 24h prior to phenotypic analysis.

Here we perform a longitudinal analysis of mESC-MNs from 3-10 days in vitro, to study the progression of TDP-43 mislocalisation and stress granule formation after oxidative stress. Proteomic analysis of TDP-43M337V interactors revealed dysregulated interaction with transcripts related to the mitochondrial electron transport chain, glycolysis, the TCA cycle, and vesicle trafficking. Preliminary experiments suggest dysregulation of cellular energy metabolism in TDP-43M337V mESC-MNs relative to TDP-43WT. Treatment with a candidate drug applied at 2.5 µM 24 hrs prior to the assay, enhances cellular metabolism in NTg, TDP-43WT and TDP-43M337V mESC-MNs.

This study sheds light on the mechanisms of mutant TDP-43 toxicity in ALS and may highlight mechanisms through which a candidate drug exerts pro-survival effects.

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Functional and -omic analysis of a cellular model of ALS-related neuronal damage

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Introduction. Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disorder affecting upper and lower motor neurons (Hardiman et al., 2017). Multiple evidence suggests that ALS is a dying-back neuropathy characterized by axonal degeneration which occurs earlier than motor neuron loss (Fischer et al., 2004). Several ALS-related mutations affect RNA-binding proteins, including TDP-43, impairing transport of mRNAs along axons and their local translation; thus, altered RNA metabolism seems to be one of the key mechanisms underlying this disease (Butti and Patten, 2019). Mutations in the TARDBP gene, encoding TDP-43, represent 5% of familial ALS patients while 97% of patients, both sporadic and familial, show TDP-43 proteinopathy with nuclear delocalization of TDP-43 and its accumulation in the cytoplasm.
Objective. The aim of our project is to identify differentially translated transcripts in the axon of cortical neurons overexpressing wt-TDP-43 or a mutated form (A315T), a cellular model of ALS neuronal damage, relative to control axons.
Method. We have developed a cellular model of ALS neuronal damage consisting of mouse cortical neurons lentivirally transduced with wt or A315T human (h)TDP-43, N-terminally fused to monomeric tRFP. Cortical neurons expressing only tRFP were used as a control. Transduced neurons were cultured in microfluidic chambers, in order to physically separate axons from cell bodies. In this model, we performed next generation sequencing of free and polysome-engaged mRNAs isolated from cell bodies and axons.
Results. Cortical neurons overexpressing hTDP-43 (wt or A315T) show disease-related features, including the presence of TDP-43 proteolytic fragments, TDP-43 cytoplasmic accumulation, increased oxidative stress, altered calcium homeostasis and reduced exocytosis relative to control cells. By RNA-seq analysis we have identified mRNAs involved in oxidative stress response, synaptic vesicle trafficking and Ca2+ homeostasis whose axonal levels and/or translation efficiency are deregulated in ALS axons compared to controls.
Conclusions. The results of this study point out the importance of axonal mRNA transport and translation in ALS neurons. This study will contribute to the dissection of molecular mechanisms underlying early stages of ALS and may uncover novel potential therapeutic targets.

FUS-ALS mutants alter FMRP phase separation equilibrium and impair protein translation

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Introduction:
FUS is an RNA binding protein (RBP) with multiple nuclear and cytoplasmic functions. FUS mutations cause the neurodegenerative disease amyotrophic lateral sclerosis (ALS), and lead to its cytoplasmic mislocalisation. Here, we use mouse and human models with endogenous ALS-associated mutations to study the early consequences of increased cytoplasmic FUS.
Methods:
We use a combination techniques to investigate in vitro, in primary motor neurons and iPSC-derived motor neurons de novo translation and phase separation or RBPs.
Results:
We show that in axons mutant FUS condensates sequester and promote the phase separation of FMRP, another RBP associated with neurodegeneration and involved in translation regulation. This leads to repression of translation in mouse and human FUS-ALS motor neurons, and is corroborated in vitro, where FUS and FMRP co-partition and repress translation. Finally, we show that translation of FMRP-bound RNAs is reduced in vivo in FUS-ALS motor neurons.
Conclusions:
Our results unravel new pathomechanisms of FUS-ALS and identify a novel paradigm, by which mutations in one RBP favour the formation of condensates sequestering other RBPs, impacting on crucial biological functions, such as protein translation.

Gamma Prime fibrinogen as a predictor of survival in Amyotrophic Lateral Sclerosis

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Introduction
Amyotrophic lateral sclerosis (ALS) is an aggressive neurodegenerative disorder related to neuroinflammation that is associated with increased risk of thrombosis.

Objectives
We aimed to evaluate gamma prime fibrinogen plasma level (an in vivo variant of fibrinogen) as a biomarker in ALS, and to test its role as a predictor of disease progression and survival.

Methods
We studied 67 consecutive ALS patients followed in our center. In the same period and using the same methodology we studied 82 healthy controls.
Plasma gamma prime fibrinogen levels were measured in plasma by ELISA. We performed Cox regressions firstly by grouping ALS patients by gamma prime fibrinogen levels.

Results
Gamma prime fibrinogen levels in plasma were significantly higher in the ALS patients (51.58 ± 24.50 mg/dL) than in controls (38.66 ± 16.65 mg/dL), $p < 0.001$.
The survival analysis (via Cox proportional hazard regressions) provided strong evidence towards the existence of an association between higher gamma prime fibrinogen levels and longer survival in ALS patients.

Conclusion

We disclosed positive associations between gamma prime fibrinogen levels and both motor function (assessed by ALSFRS-R global scores) . We found, for the first time to our knowledge, a link between gamma prime fibrinogen level and survival: patients with higher gamma prime fibrinogen plasma levels survived longer.
Remarkably, we found that increased levels of gamma prime fibrinogen can have a neuroprotective role increasing survival of ALS patients, which is against a possible action promoting neuro-inflammation and causing neuronal degeneration.
Our findings regarding the association between gamma prime fibrinogen and survival suggests that this new avenue should be further investigated in ALS.

GBA variants influence cognitive status in Amyotrophic Lateral Sclerosis patients

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Introduction. The heterogeneity of cognitive function involvement in Amyotrophic Lateral Sclerosis (ALS) is not fully understood, but genetic factors may have a role.
Objectives. To evaluate the impact of GBA variants on cognitive status in a population-based series of ALS patients.
Method. All ALS patients diagnosed in Piemonte and Valle d'Aosta, Italy, between January 1st 2007 and December 31st 2015 were considered eligible. We included 751 ALS patients with full genetic and neuropsychological data and 677 healthy controls (HC).
Patients' cognitive performance was classified as: ALS with normal cognition (ALS-CN); ALS with behavioral impairment (ALS-Bi); ALS with cognitive impairment (ALS-Ci); ALS with cognitive and behavioral impairment (ALS-Cbi); ALS with FTD (ALS-FTD). A single-variant association test was used to compare the frequency of GBA variants between ALS cases and HC. A binomial test was used to assess the prevalence of GBA mutations across cognitive groups. Linear mixed-effects models were used to test for associations between GBA genotype and cognitive functioning. A gene-based rare variants association test was also performed.
Results. We identified three common GBA polymorphisms (p.E365K, p.T408M, p.N409S), which are known risk factors for Dementia with Lewy Bodies (DLB) and cognitive impairment in Parkinson's Disease. These variants were found in 18 ALS patients (2.26%) and 15 HC (2.23%). The single-variant analysis confirmed that GBA variants are not a risk factor for ALS. We detected seven other GBA variants. Among ALS patients carrying GBA variants, 72.2% displayed cognitive impairment, as compared to 47.1% among non-carriers (p=0.0357). A linear mixed-effects model that controlled for sex, age, site of onset, bulbar signs at diagnosis, ALSFRS-R decline and C9orf72 status, confirmed the association (OR=3.74, 95% CI 1.25–12.72, p=0.023). Collapsing tests revealed enrichment of rare disruptive GBA variants in cognitively impaired ALS patients (CMC p-value=0.025).
Conclusions. We found that GBA variants were associated with an increased risk of cognitive impairment in ALS patients. Our results strengthen the role of lysosomal impairment in the neurodegenerative process underlying ALS and highlight that genetic factors can modulate the vulnerability to cognitive impairment.

GENE-BASED ASSOCIATION ANALYSIS OF SURVIVAL ASSOCIATED RARE VARIANTS IN ALS

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Background

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. There is considerable variation in duration of survival; although 50% survive less than three years from first symptoms, up to 10% of people with ALS live more than 8 years. Finding gene variants that influence survival might provide a direct route for therapeutic intervention. While previous studies have largely examined common variants that associate with survival, here we test the hypothesis that rare genetic variation might also impact survival in ALS.

Methods

Samples were from patients attending centres across the UK. DNA was sampled at or soon after diagnosis and processed by standard procedures. Whole genome sequencing was performed on the Illumina HiSeq 2000 platform, and alignment and bioinformatics analysis performed using the Isaac pipeline to the hg19 reference. To identify rare variants influencing survival in sporadic amyotrophic lateral sclerosis we used the variable threshold test, a variation of the rare variation burden test which uses whole gene variant burden as the unit of analysis.

Results

There were 1128 cases. Mean age of onset was 62.9 (SD 11.08) with 38% of the sample being female. Median survival was 3.9 years. Mean sequencing depth was 40x. Using variable threshold test, there was an association between survival and rare variation in the PLCG2 gene ($p = 5.0 \times 10\text{-}7$), with rare variants increasing survival by 6.3 months.

Conclusions

Rare variation in the PLCG2 gene is associated with longer survival in ALS. Validation in a larger cohort is required.

Genome-wide study of DNA methylation in Amyotrophic Lateral Sclerosis identifies differentially methylated loci and implicates metabolic, inflammatory and cholesterol pathways

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with an estimated heritability of around 50%. DNA methylation patterns can serve as biomarkers of (past) exposures and disease progression, as well as providing a potential mechanism that mediates genetic or environmental risk. Here, we present a blood-based epigenome-wide association study (EWAS) meta-analysis in 10,462 samples (7,344 ALS patients and 3,118 controls), representing the largest case-control study of DNA methylation for any disease to date. We identified a total of 45 differentially methylated positions (DMPs) annotated to 42 genes, which are enriched for pathways and traits related to metabolism, cholesterol biosynthesis, and immunity. We show that DNA-methylation-based proxies for HDL-cholesterol, BMI, white blood cell (WBC) proportions and alcohol intake were independently associated with ALS. Integration of these results with our latest GWAS showed that cholesterol biosynthesis was causally related to ALS. Finally, we found that DNA methylation levels at several DMPs and blood cell proportion estimates derived from DNA methylation data, are associated with survival rate in patients, and could represent indicators of underlying disease processes.

Hsp90-mediated regulation of DYRK3 couples stress granule disassembly and growth via mTORC1 signaling: implications for ALS/FTD.

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Introduction: Stress granules (SGs) are dynamic condensates associated with protein misfolding diseases. They sequester stalled mRNAs and signaling factors, such as the mTORC1 subunit raptor, suggesting that SGs coordinate cell growth during and after stress. However, the molecular mechanisms linking SG dynamics and signaling remain undefined. SGs dysfunctionality is emerging as an important pathomechanism in diseases including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal lobar degeneration (FTD). In these diseases, failure to disassemble SGs has been linked to disturbances in RNA metabolism and to the development of aggregates that cause neuronal death.

Objectives: Identifying and characterizing the factors that govern SG dynamics will provide targets with therapeutic potential to treat uncurable diseases such as ALS and FTD.

Method: We use genetics and fluorescence microscopy of fixed and live cells (HeLa cells and iPSC-derived motor neurons) to study the functional effects of Hsp90 and dual-specificity tyrosine-phosphorylation-regulated kinase 3 (DYRK3) on SGs in mammalian cells.

Results: We report that the chaperone Hsp90 is required for SG dissolution. Hsp90 binds and stabilizes DYRK3 in the cytosol. Upon Hsp90 inhibition, DYRK3 dissociates from Hsp90 and becomes inactive. Inactive DYRK3 is subjected to two different fates: it either partitions into SGs, where it is protected from irreversible aggregation, or it is degraded. In the presence of Hsp90, DYRK3 is active and promotes SG disassembly, restoring mTORC1 signaling and translation. Finally, we show that ALS cell models are vulnerable to Hsp90 inhibition and show defective induction of DYRK3 expression in the stress recovery phase. Moreover, surviving α-motor neurons harboring FUS aggregates in lumbar spinal cord of FUS-ALS patients also showed a significant reduction of DYRK3.

Conclusions: Together these data support the notion that 1) Hsp90 links stress adaptation and cell growth by regulating the activity of a key kinase involved in condensate disassembly and translation restoration; 2) alterations of Hsp90 and DYRK3 could lead to selective motor neuron vulnerability in ALS.

Reference: Mediani et al., EMBO Reports 2021, in press.

Identification of distinct pathological motor neuron phenotypes according with the expression of misfolded SOD1 and microgliosis during the progression of disease in the SOD1G93A ALS mice

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Misfolded SOD1 (mfSOD1) accumulation, mitochondrial vacuolization, microgliosis and motor neuron (MN) loss are hallmark events in familial ALS. An important variability in the vulnerability of distinct MN subtypes in ALS was also seen. This fact appears to be correlated with different characteristics of their excitability. C-boutons are cholinergic inputs to α-MNs which are important regulators of its excitability. However, the contribution of C-boutons to ALS pathology is controversial.

To understand the relation between the mentioned pathological aspects which converge in MN degeneration, we performed a quantitative correlative analysis of the neuroinflammatory reaction, mfSOD1 accumulation and C-bouton alteration in the SOD1G93A ALS mice. The effects of a supplementary stress on ALS MNs induced by peripheral nerve injury were also assessed.

SOD1G93A mice were used. Sciatic nerve axotomy were performed according to stablished procedures. Multiple fluorescent immunolabeling was performed on spinal cord cryostat sections and observed under the confocal microscope. Some tissue samples were also processed for electron microscopy.

The immunocytochemical analysis with the C4F6 antibody allowed us to define three MN phenotypes. In phenotype 1, there was no evidence of mfSOD1 accumulation, but the postsynaptic organization of C-boutons was impaired. In phenotype 2, mfSOD1 accumulation was observed in MN neuropile together with important microgliosis; however, no changes in C-boutons were observed. In phenotype 3, mfSOD1 accumulation was extended to MN somata; microglial cells were active and both pre- and post-synaptic C-bouton compartments were reduced. After sciatic nerve axotomy performed in a presymptomatic stage, MNs worsen their phenotype, emulating those predominating in later stages of disease in non-axotomized animals. However, when axotomy was performed in early symptomatic stages, the added stress provoked additional microgliosis, but this did not result in an intensification of the proportion of worse MN phenotypes.

By classifying MNs according to mfSOD1 amount, we dissociate the alterations in MNs from the clinical time-course points of disease. Compensatory mechanisms acting in early disease stages contribute to hide the pathological changes early seen in the vulnerable MNs. Any added stress worsens the MN pathology phenotype in early but not in late stages of ALS.

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Identification of oxidative stress response modifying drugs and their targets in an ESC-derived motor neuron model of amyotrophic lateral sclerosis

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Introduction
Despite considerable research into the basic pathophysiology of ALS, the translational pipeline has produced few successful drugs. The genetic and aetiological complexity of ALS, its clinical heterogeneity, and the lack of reliable biomarkers are all factors contributing to unsuccessful therapeutic drug development. Our previous development of a BAC transgenic mouse model of ALS, based on expression of mutant human TDP-43-M337V at low levels, provides a potentially relevant platform for translational research as it recapitulates several important features relevant to ALS [1,2]. Mouse embryonic stem cell-derived motor neurons (ESC-MNs) expressing the human TDP-43-M337V BAC display impaired stress granule responses, distinct interactome profiles and reduced survival following oxidative insult [1,2]. Stress response deficits in M337V ESC-MNs might therefore provide a druggable readout linking genetic susceptibility and oxidative stress to underlying ALS pathogenesis.
Objectives
Having utilised the rescue of M337V ESC-MN survival after oxidative stress to identify candidate drugs after high throughput screening (HTS), we aim to analyse the effects of these drugs on disease-associated pathways.
Methods
WT and M337V ESC-MNs were treated with the PHARMAKON 1600 FDA approved library for 24h (2 μM of each drug). This was followed by the addition of 0.5mM sodium arsenite for 1 h. Resazurin was added to the culture medium and after a further 24h cell viability was measured by fluorescence (Ex 570nm/Em 584nm). The Oxford Target Discovery Institute's (TDI) plate analysis pipeline was utilised to identify drugs that improved M337V ESC-MN survival by a z-score ≥1.8 over controls.
Results
Our HTS pipeline identified 16 primary candidates from 1600 compounds tested. Six candidate drugs continued to improve TDP-43-M337V ESC-MN survival during secondary hit validation and dose-response testing, compared to non-drug treated controls. The presence of TDP-43-M337V in ESC-MNs reduces the number of stress granules/ESC-MN following sodium arsenite insult; however, 24h treatment of M337V ESC-MNs with the six candidate drugs prior to oxidative stress rescued the number of stress granules/ESC-MN to control levels.
Conclusions
Our data suggest that impaired stress responses may underlie the link between mutant TDP-43 and selective MN loss in our mouse model of ALS. With the Oxford TDI, we performed a screen of FDA approved drugs and identified those that restored survival and the stress granule response in M337V-expressing ESC-MNs. Drugs will be further validated through analysis of phenotypic and transcriptional changes in primary mouse MNs, and iPSC-derived motor and cortical neurons from ALS patients carrying TDP-43 mutations.
References
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Identifying pathomechanisms in iPSC-derived cortical neurons from C9orf72-associated ALS/FTD and FTD patients

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Background

A hexanucleotide repeat expansion in the C9orf72 gene is the most frequent cause of both Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). While mutations in C9orf72 cause degeneration of motor neurons in the spinal cord and motor cortex in ALS (C9-ALS), they lead to degeneration of cortical neurons of the frontal and/or temporal lobe in FTD (C9-FTD). About 10-15% of patients from both groups suffer from both C9-ALS and C9-FTD (C9-ALS/FTD).
Apart from a haploinsufficiency of the C9orf72 protein, the GGGGCC hexanucleotide repeat expansion leads to the expression of dipeptide repeat proteins (DPRs), of which poly-GR, poly-PR, and poly-GA are believed to be toxic. Several phenotypes associated with C9orf72 mutations have been identified, such as impaired autophagy, excitotoxicity, ER stress, apoptosis, stress granule formation, and mitochondrial dysfunction, all of which were demonstrated in C9-ALS and C9-ALS/FTD induced pluripotent stem cell (iPSC)-derived motor neurons. However, few studies have tested these mechanisms in iPSC-derived cortical neurons and in neurons from C9-FTD patients.

Objective

The aim of this study is to identify disease-associated deficits that are driven by GGGGCC hexanucleotide repeats in iPSC-derived cortical neurons from C9-ALS/FTD, C9-ALS, and C9-FTD patients.

Methods

In this study, we differentiated cortical neurons derived from iPSCs from C9-ALS/FTD, C9-ALS, and C9-FTD patients. We phenotyped day 55 cortical neurons using western blot, immunocytochemistry, functional assays, and calcium imaging, to study autophagy impairment, ER stress, stress granules, mitochondrial dysfunction, cell death, and altered excitability.

Results

We found a significant reduction in mitochondrial proteins, a reduction in mitochondrial membrane potential, and an increase in apoptotic markers in C9-ALS/FTD and C9-FTD cortical neurons, which might be caused by poly-GR associated oxidative stress. Furthermore, we identified a significantly increased number of stress granules in C9-ALS/FTD, but not in C9-FTD cortical neurons treated with sodium arsenite. This altered stress response might be caused by a poly-PR and poly-GR-associated impairment of translation. We also identified small changes in ER stress and AMPA receptor expression in C9-FTD.

Conclusion

This study identifies C9orf72 mutation-induced pathomechanisms in cortical neurons derived from ALS, ALS/FTD, and FTD patients, such as an impaired mitochondrial function and an increased stress granule formation in C9-ALS/FTD cortical neurons. This provides a better understanding of disrupted mechanisms in an early disease stage of frontotemporal dementia.

Immune profiling of plasma-derived extracellular vesicles in Amyotrophic Lateral Sclerosis patients.

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Introduction

Extracellular vesicles (EVs) have a central role in inflammatory processes and they could be plausible targets in Amyotrophic Lateral Sclerosis (ALS), which is characterized by an immunological reaction to motor neuron death. We have previously demonstrated that leukocyte derived EVs are upregulated in ALS patients and they can be considered possible markers of disease progression.

Objectives.

The aim of this study was to investigate more specific immunological surface markers on large and small EVs (LEVs and SEVs) from plasma of ALS patients and healthy donors.

Methods

LEVs and SEVs were isolated from plasma of 40 sporadic ALS patients (sALS), 28 healthy controls (CTRLs) and 19 Frontotemporal Dementia (FTD) patients by differential centrifugation and characterized by Nanoparticle Tracking Analysis (NTA), Atomic force microscopy (AFM), Colorimetric NANoplasmonic method (CONAN). In this study, we used a multiplex bead-based flow cytometric assay for the detection of 37 surface protein markers in one sample simultaneously (MACSPlex Exosome Kit).

Results

Endosome-specific tetraspanins (CD9 and CD63), endothelial marker (CD31), T-cell and Natural Killer markers (CD2, CD45 and CD69), HLA Class 1 group (HLA-ABC) and homing cell adhesion molecule (CD44) were more expressed in SEVs derived from CTRLs than in sALS patients ($P<0,05$).
LEVs derived from CTRLs were enriched in cell adhesion molecule of endothelial cells and platelets (CD31, CD42a, CD41b and CD62P), CD63, CD9 and HLA-ABC ($P<0,05$) compared to sALS patients.
On the other hand, we demonstrated that the expression of the tetraspanin CD81, an MHC class II cell surface receptor (HLA-DR) and the glycosphingolipid SSEA4 were higher in the SEVs derived from sALS patient compared to CTRLs.

Conclusion

Endosome tetraspanins CD9 and CD63 decrease in both LEVs and SEVs in accordance with the autophagy-endolysosomal system dysregulation previously described in ALS. On the other hand, we identified higher expression of exosome surface markers such as CD81 and HLA-DR, molecules presenting the antigens to T cells on SEVs from ALS patients. LEVs from sALS patients have less cell adhesion markers of platelets cells and this is in line with the literature as suggested by the platelet variation in blood of ALS patients. These

data suggest that LEVs and SEVs carry different surface markers which might discriminate their role in ALS pathogenesis.

Impact of COVID-19 respiratory infection among non-invasive ventilated ALS patients

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Introduction: The novel positive-stranded RNA coronavirus-19 (COVID-19) is causing a global pandemic, with a high mortality rate in susceptible people. This infection poses clinical challenges to many neuromuscular disorders, including amyotrophic lateral sclerosis (ALS) due to the risk of respiratory decompensation.
Objectives: We aim to describe the clinical outcome of ALS patients using non-invasive ventilation (NIV) and infected with COVID-19 virus.
Method: We analyzed retrospectively our patients followed regularly at our ALS clinic, from the beginning of the COVID-19 pandemic (March 2020) to March 2021. We included patients on NIV (overnight or during full day) with a documented COVID-19 infection. We recorded clinical data, including gender, onset age, onset region, disease duration, functional status (ALSFRS-R), number of hours on NIV and comorbidities.
Results: Four ALS patients were included. All of them were men and with spinal -onset, the mean age of onset was 63±16.7 (45-85) and the mean disease duration was 17.5±15.9 months (6.1-41). The mean ALSFRS-R score was 16±1 (15-19) and all of them were wheelchair-bounded. One patient was dependent on NIV for 24 hours daily. None of them had particular comorbidities, including arterial hypertension, diabetes mellitus, dyslipidemia, smoking habits and heart disease. Two patients were in skilled nursing facilities, while the remaining were in their own homes. Only one patient required admission in the hospital for 2 days. Supplemental oxygen was needed in three patients and one required full-day NIV for some days. All of them recovered from the infection without additional complications, returning to their baseline condition.
Conclusions: In this small cohort, it is noteworthy that all patients survived their COVID-19 respiratory infection despite being in an advanced stage of the ALS disease, with marked respiratory involvement. This supports that prompt medical treatment is recommended in patients with severe ALS disease and infected by COVID-19.

Implications for the lncRNA ZEB1-AS1 in sporadic ALS: deregulation in neuronal differentiation and characterization of a novel disease pathway

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Introduction: Alterations in the expression levels of RNAs in the pathogenesis of sporadic ALS (sALS) are becoming increasingly relevant, with RNA-seq data highlighting numerous deregulated long non-coding RNAs (lncRNAs) in tissues derived from sALS patients. The oncogenic lncRNA ZEB1-AS1 emerged as strongly downregulated in peripheral blood mononuclear cells (PBMCs) of sALS patients. In cancer-derived cell lines, ZEB1-AS1 has been shown to act in a feedback negative loop with mir200c, acting as a molecular sponge for this miRNA. Furthermore, ZEB1-AS1's interaction with mir200c results in the upregulation of the downstream molecule BMI1.
Objective: The aim of our work was thus to characterize the role of the lncRNA ZEB1-AS1 in ALS pathogenesis identifying a possible disease-modifying target.
Methods: Total RNA was extracted using TRIZOL reagent and the genes' expression levels were determined by Real Time PCR. Nuclear and cytoplasmic RNA was extracted using the Cytoplasmic & Nuclear RNA Purification Kit and genes' expression levels were measured by ddPCR. Western Blot analysis and immunofluorescence were also performed to assess protein expression levels. Live&Dead Assay and MTT assay were performed to assess cell viability.
Results: In PBMCs, undifferentiated SH-SY5Y cells and differentiated SH-SY5Y cells silenced for ZEB1-AS1 we validated the downregulation of ZEB1-AS1's expression but we did not see a concordant downregulation of its sense gene ZEB1. We observed an increase of mir200c and a decrease of BMI1, in an opposite pattern to what is observed in cancer, suggesting a specific pathway in sALS. Furthermore, we observed an upregulation of BMI1's downstream mediator GSK3b, which results inactivated. The silencing of ZEB1-AS1 in SH-SY5Y did not impact on cellular viability. Moreover, we found that ZEB1 and ZEB1-AS1's levels change during neuronal differentiation, suggesting an implication for the lncRNA in this process. Indeed, ZEB1-AS1's silencing results in an alteration in neurite length. We demonstrated that ZEB1-AS1 can bind the ALS-implicated RNA binding protein FUS, both in SH-SY5Y cells and in PBMCs, and in this last tissue we found a reduction in the amount of ZEB1-AS1 bound to FUS in sALS patients.
Conclusions: Our results show an implication for ZEB1-AS1's pathway in sALS and specifically in neuronal differentiation. We also report an impaired interaction of ZEB1-AS1 with FUS in sALS patients, suggesting this is the mechanism connecting ZEB1-AS1 to sALS pathology.

Improving the diagnostic utility of EMG measures as biomarkers of Motor Neuron Disease using multivariate discriminant analysis

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Introduction: Novel biomarkers are urgently needed to allow early diagnosis and accurate tracking of disease progression in MND/ALS patients.

Objectives: To determine whether multivariate data analytics, e.g. Fisher's Linear Discriminant Analysis (FLDA), could be used to enhance the utility of existing biomarkers by providing improved discrimination between patients and controls (1).

Methods: Data used in this study consisted of Compound Muscle Action Potential (CMAP), Motor Unit Number Index (MUNIX), and Motor Unit Size Index (MUSIX) taken from 6 muscles, abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), tibialis anterior (TA), extensor digitorum brevis (EDB), abductor hallucis (AH), in a cohort of 43 MND patients and 40 healthy controls (2). Informative measurements were selected through an automated procedure and combined into a single score using FLDA.

Results: The new combined score provided cross-validated Area Under the Curve (AUC) values as high as 0.965(±0.01), while the maximum cross-validated AUC for any individual measure was 0.887(±0.095). Similarly, by combining measures, cross-validated classification accuracies of 87.8±6.4% could be reached, while the maximum accuracy for any single muscle was 81.9±9.9%. Inspection of the most informative contributing measures showed that APB CMAP, TA CMAP, EDB CMAP were among the most important variables in achieving this discrimination.

Conclusions: A combination of measures using multivariate methods such as FLDA can exploit discriminating information which could not be found in any one of the original univariate measures in isolation. This can shed light on the underlying pathophysiology by identifying the maximally-contributing measurements and muscles for diagnosis and prognosis. Such multivariate approaches could be useful in clinical settings by improving diagnostic accuracy.

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In search of ALS biomarkers: results from Raman Spectroscopy and lipidomics profiling of extracellular vesicles.

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Introduction
There is no validated blood-based biomarker for sporadic Amyotrophic Lateral Sclerosis (ALS). Extracellular vesicles (EVs) have the potential to solve this unmet clinical need, as they can be involved in the pathogenesis/progression of neurodegenerative diseases (Selmaj et al., 2017). Lipids are essential molecular components of EVs, but at the moment the knowledge about their distribution and function is limited.
Objectives
The aim of this work was to find biomarkers of ALS by investigating biochemical composition of plasma-derived EVs with Raman Spectroscopy (RS) and HPLC-MS (High Performance Liquid Chromatography-Mass Spectrometry).
Methods
We isolated small and large extracellular vesicles (sEVs and lEVs), from blood plasma of 20 sporadic ALS patients and a matched group of healthy controls, by differential centrifugation/ultracentrifugation. We characterized sEVs, lEVs and blood plasma firstly by RS and subsequently by HPLC-MS, targeting a panel of around 200 lipids. Statistical analysis included univariate and multivariate analysis techniques such as PCA (Principal Component Analysis) and PLS-DA (Partial Least Squares- Determinant Analysis).
Results
Raman spectroscopy highlighted lEVs as a particularly promising biomarker for ALS. Raman spectra showed in fact that sporadic ALS patients have a different lipid content and less intense bands relative to the aromatic amino acid phenylalanine. HPLC-MS revealed some lipid species discriminating between ALS and healthy subjects. They were mainly phospholipids, belonging to the subclasses of phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylinositols (PI), and sphingolipids, belonging to the subclasses of ceramides (Cer), mono/di-hexosyl-ceramides (M/DHC). In particular the increase of PC(34:1), MHC(24:1) and Cer(24:1) was observed in either plasma, lEVs and sEVs from ALS patients. The species PI(36:3) was up-regulated in both large and small vesicles of ALS patients.
Conclusion
Interestingly, some species significantly altered in our analysis of plasma lipidome, overlap with the ones highlighted by Blasco et al. in their work on cerebrospinal fluid (CSF) (Blasco et al. 2017), namely PC(38:2), MHC(24:1) and the plasmalogen PCO(34 :1). This supports the idea of plasma and plasma-derived EVs as easily available source of robust biomarkers. Among the other results, the perturbed sphingolipids are particularly relevant as they are involved in key pathways for ALS patogenesis, such as autophagy, energy metabolism and neuroinflammation.

Incidence rates of amyotrophic lateral sclerosis across European disease registers.

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Introduction:
European population-based disease registers of amyotrophic lateral sclerosis (ALS) are an invaluable source of annual disease incidence and prevalence data. Collaboration and collation of data across different registers can enable us to investigate variation in incidence rate and lifetime risk of disease. This variation can be linked with differences in potential environmental risk factors or heritable variation that populations in different regions are exposed to in order to better understand the contributions these factors make to ALS susceptibility and disease development.

Objectives:
The current project aims to analyse age- and gender-stratified incidence rates and compare lifetime risk of disease development across European ALS population-based registers.

Methods:
A systematic search of the literature was carried out to identify unique European ALS population-based registers that had published data between 2010 and 2020. Each register was then contacted to provide data on age- and gender-stratified case counts and regional population between 2014 and 2019. Crude and adjusted male and female incidence rates as well as age-stratified incidence rates were then calculated and compared for each region using the EU standard population.

Results:
Of the 22 unique population-based registers identified through literature review, 12 provided data for this analysis. Combined, these registers cover a population of 59,540,649 across Cyprus, Denmark, Ferrara, Ireland, Limousin, Malta, Netherlands, Northern Ireland, Piedmont, Rhineland-Palatinate, Salento, Swabia and Sweden. The overall case count was 4,549 for males and 3,457 for women. The overall crude incidence rate was 2.05 per 100,000 for women and 2.76 per 100,000 for men. The overall adjusted incidence rate was 2.02 per 100,000 for women and 2.93 for men across all ages. Regional female adjusted incidence rates per 100,000 varied from 1.07 in Sweden to 3.03 in Ireland, and regional male adjusted incidence rates varied from 1.21 in Cyprus to 4.67 in Ireland.

Discussion:
These results are consistent with previous estimates of European ALS incidence, and previously known gender differences, with higher incidence rates in men compared to women. This work demonstrates the ongoing importance of European ALS registers in enabling transnational epidemiological research.

Increased risk of ALS in Italian professional soccer leagues

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Introduction: An association between ALS and soccer has been postulated starting from the observation of several deaths from amyotrophic lateral sclerosis (ALS) among Italian professional soccer players (Chiò 2005; Belli 2005).
Soccer players are exposed to repeated injuries, strenuous physical activity, potentially noxious drugs, and possibly environmental toxins. An increased risk of ALS may not be related only to soccer players but also to other professional sports.
Objective: To determine whether players practicing in Italian professional leagues in the First and Second division are at increased risk of ALS.
Methods: All professional soccer players practicing in Italian First and Second division from 1959 to 2000 were identified through the archives of the major Italian football cards publisher. For each player, date and place of birth, playing role and team history were recorded. Each player was followed from the age of 15 years to 31 December 2018. Incident ALS cases were soccer players firstly diagnosed with ALS during the period 1959-2018. The number of expected cases was calculated using incidence rates of a well-defined Italian population for reference. Standardized incidence ratio (SIR) was the ratio between observed and expected incidence rate.
Results: 4,556 players were identified (1,974 in First division and 2,582 in Second division), followed for a total of 207,094 person-years. 17 ALS cases were identified among former professional soccer players. The number of expected cases was 4.3. The SIR was 4.0 (95% CI 2.3-6.4) in the entire sample, 10.5 (95% CI 4.2-21.7) in the <45 years age group, and 4.6 (95% CI 1.9-9.5) in midfielders. The risk was higher for players that spent the majority of seasons during the study period in First division (SIR 5.7; 95% CI 2.7-10.5) than in Second division (SIR 2.8; 95% CI 1.1-5.7). Mean age at diagnosis was 48.4 years, compared to 62.5 years (range 56.0-72.2) in the general population ($p<0.0001$). The disease in former soccer players occurred 14.1 years earlier than in the general population.
Conclusions: This is the first epidemiological study evaluating the relationship between soccer and ALS in a large cohort and after a long follow-up period. Soccer players showed an increased risk of developing ALS. Midfielders and players in the First division were at highest risk. The disease develops at an earlier than expected age.

Inflammasome genes expression as biomarkers in ALS and FTD patients

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Introduction

There is a growing need to identify specific biomarkers that facilitate the diagnosis and prognosis of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Neuroinflammation plays an important role in the pathogenesis of these pathologies.

Objectives

The aim of this study is to determinate the diagnostic capacity of these inflammatory genes as potential biomarkers both on ALS and FTD, and their clinical predictors of prognosis.

Method

cDNA serial samples from lymphocytes of 55 patients with ALS (32 males and 23 females) and 42 patients with FTD (24 males and 18 females) were subjected to quantitative PCR in order to study expression levels of LGALS1 and NLRP3. These levels were related to the main clinical parameters like days since symptoms onset, ALSFRS-r, ALSFRS-r slope, diagnostic delay, age of onset of symptoms, and others. Statistical analysis was performed using SPSS Statistics version 22 (IBM, Spain).

Results

We found a significant down expression of LGALS1 in FTD patients on respect to control group. Significant differences were found in gene expression ratios of both NLRP3 and LGALS1 genes. Additionally, NLRP3 expression correlated significantly with diagnostic delay in FTD patients.

Conclusions

The expression of LGALS1 was found to be altered in FTD patients, but it could not be considered as a diagnostic biomarker due to the low sensitivity and specificity values shown in the present study. Regarding the functioning of the inflammasome, we have not been able to see an alteration in an important component, NLRP3, on ALS patients. Any case, it would be necessary to introduce new components related to inflammasome to reach more significant and conclusive results.

Keywords: ALS, FTD, biomarkers, inflammation, NLRP3, LGALS1

Acknowledgements

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Insights for the development of a miRNA-based therapeutic strategy in ALS exploiting iPSC-derived motor neurons and exosomes.

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder characterized by a progressive degeneration of motor neurons (MNs). Most of the cases are sporadic (sALS) while familial ALS (fALS) occurs only in 10% of all subjects. A dysregulation of microRNA (miRNA) expression in ALS has been already described, although downstream pathological events associated with MN degeneration have not been clarified yet. miRNAs are highly expressed in central nervous system thus they may play important roles in the etiology or progression of neurodegenerative diseases such as ALS. In this study, we aimed at investigating whether alteration of miRNA expression patterns in ALS-MNs may represent a common molecular feature among the different forms of the disease. We performed differential expression profile analysis of miRNAs isolated from iPSC-derived MNs of ALS patients (SOD1, TARDBP and C9ORF72) and healthy subjects. We identified a small group of downregulated miRNAs in ALS-MNs. Interestingly, a dysregulation of the same subset of miRNAs has been detected in exosomes released from the same ALS-MN cultures. Since bioinformatic analysis showed that these miRNAs regulate several pathways related to MN degeneration, we investigated their potential as disease biomarkers assessing their expression level in cerebrospinal fluid (CSF) of ALS patients. We confirmed a different expression pattern of these miRNAs in CSF isolated from ALS subjects suggesting their potential clinical relevance. Taken together our results demonstrate that the neurodegenerative phenotype in ALS can be associated with a dysregulation of miRNAs involved in the control of disease-relevant molecular pathways. The possibility of tuning entire gene networks with a specific subset of miRNAs may provide significant insights on the development of effective new miRNA-based therapies and could be useful as disease biomarkers.

Investigating Fuzzy transcriptional downregulation in C9orf72-related amyotrophic lateral sclerosis

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Introduction:
The C9orf72 hexanucleotide GGGGCC repeat expansion is the most common genetic cause of amyotrophic lateral sclerosis (ALS). Three mechanisms have been implicated in its pathogenicity: repeat-associated non-ATG translation over the hexanucleotide repeat expansion producing dipeptide repeat proteins, accumulation of toxic RNA foci, and reduced transcription of the C9orf72 gene itself.[1] Many of the downstream genetic effects of this mutation are currently unknown. Understanding the effects of the C9orf72 mutation on downstream genes, and the molecular pathways regulated by these, is important to fully understand the pathogenesis of C9orf72-related ALS. The downstream gene of interest in this project is Fuzzy (fuzzy planar cell polarity protein). This gene has previously been shown to function in triggering apoptosis in neurons, associating with pathogenesis in multiple neurodegenerative disease models.[2]

Objectives:
We have found that Fuzzy is downregulated at the RNA level in induced pluripotent stem cell derived cortical and spinal motor neurons from ALS patients carrying the C9orf72 mutation. This project aims to delineate how this downregulation of Fuzzy is achieved.

Method:
We model C9orf72-related ALS using a neuroblastoma cell line transfected with plasmids containing varying lengths of the GGGGCC repeat. The dual luciferase assay was performed to determine the sequence responsive to the GGGGCC expansion in the Fuzzy promotor and to dissect the role of dipeptide repeat proteins and expanded GGGGCC RNA in Fuzzy downregulation. The latter was performed using constructs that solely produce specific dipeptide repeat proteins, and in separate assays, constructs that result in the expression of expanded GGGGCC RNA only.

Results:
Fuzzy expression was downregulated in C9orf72-related ALS patient iPSC-derived spinal motor and cortical neurons. We narrowed down the sequence within the Fuzzy promoter that was responsive to the C9orf72 mutation, resulting in the Fuzzy downregulation, to a 250 base-pair region. The downregulation mediated at this sequence was reproduced in response to the presence of expanded GGGGCC RNA. It was also reproduced independently in response to the toxic dipeptide repeat protein poly(Proline-Arginine)$_{50}$. We identified transcription factors that are potentially mechanistically involved.

Conclusions:
Understanding the mechanism for the downregulation of Fuzzy and the effects of this on molecular pathways in the cell is of interest in understanding the pathology in C9orf72-related ALS and in identifying amenable targets that may be of therapeutic potential.

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Investigating neuroinflammatory dysregulation and its implications for disease phenotype and progression in C9orf72 post-mortem tissue

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Introduction: Recent studies have shown that protein products of the ALS/FTD-associated genes C9orf72, TBK1 and PGRN are involved in inflammatory pathways. Indeed, dysregulation of these pathways is noted to correlate well with clinical phenotype. Unfortunately, despite these links, clinical trials exploring the use of anti-inflammatory drugs for ALS therapy have thus far been unsuccessful. This may be attributed, at least in part, to non-specific mechanisms of drug action, lack of appropriate patient stratification and trial endpoints, or the bias of preclinical studies toward SOD1 mouse models, while SOD1 mutations account for only 2% of ALS cases and are not at all associated with FTD. Thus, further characterisation of neuroinflammation along the ALS-FTD spectrum is warranted to understand how these pathways can be more specifically targeted to harness their therapeutic potential.

Objectives: We aimed to characterise neuroinflammatory profiles in a clinically heterogeneous cohort of post-mortem tissue from ALS patients with a C9orf72 mutation. We then explored the relationship between neuroinflammatory elements, pathological features and clinical symptoms.

Methods: We used a combination of NanoString molecular barcoding and high-throughput immunohistochemistry staining to characterise neuroinflammatory profiles in our C9-ALS cohort across four brain regions, grey and white matter, and vascular-adjacent and non-vascular-adjacent regions. We then analysed correlations between immunohistochemistry, NanoString sequencing and clinical data. Finally, we trained a random forest classifier to reveal which features from the above data were the strongest predictors of disease.

Results: Here we show elevated microglial activation in C9-ALS post-mortem tissue compared with controls via immunohistochemistry, as well as demonstrate a correlation between microglia activation level and TDP-43 burden. Our NanoString analysis reveals key genes that are differentially expressed in C9-ALS that correlate with microglial activation level, TDP-43 burden, or clinical features such as cognitive impairment and disease duration. Lastly, our random forest classifier revealed microglia and microglial activation markers to be the strongest predictors of disease.

Discussion: This study underlines the importance of microglial activation in C9-ALS and reveals dysregulation of relevant neuroinflammation-related genes, some of which correlate with clinical phenotypes. The next step for this work is to perform single-cell RNA sequencing to determine cell-type-specific gene expression changes. Our data serve as a basis for future research to better our understanding of neuroinflammatory dynamics along the ALS-FTD spectrum and highlight the potential to target dysregulated pathways based on individual inflammatory profiles.

Investigating the role of adenosine deaminase in amyotrophic lateral sclerosis

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Introduction:
Previously published research in C9orf72 and sporadic induced neuronal progenitor cell astrocytes (iAstrocytes) demonstrated adenosine metabolism dysfunction, caused by adenosine deaminase (ADA) loss. Loss of this key purine metabolism enzyme lead to several negative consequences for ALS iAstrocytes including increased sensitivity to adenosine-specific toxicity. Higher ADA expression in ALS iAstrocytes protected against adenosine-mediated toxicity. Inosine supplementation to bypass the defect was beneficial bioenergetically in iAstrocytes and decreased iAstrocyte-mediated motor neuron toxicity in co-culture, with higher levels of ADA in iAstrocytes correlating with increased motor neuron survival in the presence of inosine. Our data led us to the hypothesis that restoring ADA levels will be beneficial for ALS iAstrocytes and decrease iAstrocyte-mediated motor neuron toxicity in co-culture.

Objectives:
To investigate the effect of increasing ADA expression in iAstrocytes by lentiviral gene therapy on adenosine-specific toxicity, iAstrocyte bioenergetics, purine metabolism and oxidative capacity.

Methods:
Lentiviral gene therapy was used to increase ADA levels in iAstrocytes derived from C9orf72 ALS cases. ADA levels plus a panel of cellular targets were used to characterise the effect of increased ADA. Adenosine-mediated toxicity and ATP assays were performed on lentivirally treated cells. The effect of gene therapy on ADA activity, inosine levels, uric acid levels and the effect on key intermediaries of purine metabolism are currently underway.

Results:
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.

Conclusions:
Our current data suggest that increasing ADA levels restores inosine output and ADA activity in iAstrocytes but does not ameliorate adenosine-mediated toxicity or improve ATP output. However, further study is required to allow us to more robustly characterise the effect of ADA gene therapy on ALS iAstrocytes including its effect purine metabolism, antioxidant capacity, DNA repair and motor neuron survival in iAstrocyte co-cultures.

Investigation of serum HSP70 as a biomarker for Amyotrophic Lateral Sclerosis

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Introduction:
Chaperone proteins such as the heat shock protein (HSP) family play an important role in protein folding and have been implicated in the pathophysiology of ALS. Previously, serum HSP70 was reported to be higher in Japanese ALS patients (n = 58) compared to controls (n = 85)[1]. We aimed to replicate this finding.

Methods:
Incident ALS cases and matched controls were recruited over 4 years in Ireland and Italy as part of the EuroMOTOR project. On recruitment serum samples were collected from willing participants and stored at -80ºC. Serum HSP70 was measured via ELISA (StressMarq SMQ-SKT-108-96). Logistic regression models adjusting for age and gender were used to determine the association between HSP70 and ALS risk.

Results:
89 patients and 111 controls were included. HSP70 concentrations were similar between patients (median: 18.5 ng/ml) and controls (median: 18.9 ng/ml) overall compared to controls which led to an odds ratio (OR) of OR 0.98 (95% CI: 0.95 – 1.00) per ng/ml HSP70. Stratifying by country revealed that Italian participants had higher HSP70 concentrations (median: 25.6 ng/ml) compared to Irish participants (median 16.1 ng/ml), leading to an OR of 0.95 (95% CI: 0.92 – 0.98) per ng/ml HSP70 in Italian participants, and 0.99 (95%CI: 0.94 – 1.03) per ng/ml HSP70 in Irish participants.

Discussion:
Our findings provide new evidence on the possible role of HSP70 as a biomarker for ALS in European populations. Our results oppose those of the previous Japanese study in that we found lower serum HSP70 in patients compared to controls. However, we also found heterogeneity in HSP70 concentrations between Irish and Italian cohorts, with the Italian cohort having higher concentrations in general and a larger difference between cases and controls.

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Investigation of the Genetic Causes of Neurodegeneration in a Family affected by a wide Range of Neurodegenerative Diseases

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Introduction

There is increasing recognition of overlapping genetic causes between clinically defined neurodegenerative diseases. We discovered a Spanish family pedigree with cases of Parkinson disease, multiple sclerosis, amyotrophic lateral sclerosis, dementia, progressive supranuclear palsy, and dystonia, as well as unaffected members.

Objectives

We hypothesized that there was either one rare pleiotropic gene variant, or a set of rare gene variants responsible for the family's clinical phenotypes. Our objective was to identify such shared genetic factor(s) of neurodegeneration by sequencing the genomes of the family members.

Method

We used next-generation whole-genome sequencing to investigate the hypothesis with both a candidate gene approach, focused on a set of 309 genes commonly used for genetic screening in the family phenotypes, and an unbiased genome-wide approach. Using standard bioinformatics the genomes were screened for single nucleotide variants, small insertions and deletions, and the C9orf72, ATXN1, ATXN2, and NIPA1 repeat expansions. Variants were filtered on the basis of their frequencies, functional consequence and in-silico predicted pathogenicity. Filtered variants in the candidate gene set harboured by at least one affected family member, and variants in the other genes harboured by all affected members were selected. Gene-set enrichment analysis was used on the resulting gene mutations to favour their interpretation.

Results

Candidate gene analysis identified eight potentially pathogenic gene mutations for which literature supporting their involvement in neurodegeneration exists. This set of genes was not significantly enriched for any specific biological function. Whole-genome sequencing identified six gene variants found in all cases and twelve variants found in all patients excluding the one affected by multiple sclerosis.

Conclusion

Our study supports the existence of common genetic factors that contribute to the risk of developing a wide range of neurodegenerative diseases. We identified a number of gene mutations known to be associated with specific clinical phenotypes, that could contribute to the risk of a wider range of neurodegenerative diseases. Moreover, we also identified a new set of candidate genes. Large scale studies and molecular experiments are required in order to confirm our candidate gene-disease associations and provide causative evidence.

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journALS: a comprehensive, uniform analysis of three decades of genetics research in amyotrophic lateral sclerosis and frontotemporal dementia

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Introduction: In the 28 years since the identification of segregating variants in SOD1, many publications have documented potential ALS-associated genes and variants, with varying degrees of supporting evidence. These studies have explained, at most, 70% of familial cases and 15% of sporadic cases; however many of these "explanatory" variants are likely to represent non-pathogenic rare variation. The difficulty of interpreting the clinical significance of rare variants is exacerbated in ALS and FTD due to genetic heterogeneity, late age of onset, incomplete penetrance and a high proportion of sporadic cases.

Objectives: Here we present journALS, a soon-to-be launched web application, designed to assess the clinical significance of all previously reported ALS- and FTD-associated genetic variants.

Methods: 2,914 primary research articles were screened, of which 1,028 were found to be relevant ALS or FTD genetic studies. 3,111 reported variants were identified in 363 genes. Detailed phenotype data including sex, age of onset and family status were gathered, in addition to variant information such as zygosity and de novo status. 479 pedigrees exhibiting segregation were documented. Variants in the 363 identified genes were extracted from ALS-specific and general genomics datasets, creating a final database of 1.5 million variants. By leveraging these datasets in conjunction with the American College of Medical Genetics variant classification guidelines, we uniformly assessed all variants for pathogenicity, penetrance, prevalence, and phenotypic and geographic heterogeneity.

Results: 111 pathogenic and likely pathogenic variants are confirmed in 23 genes, with 10% classified as benign or likely benign; and greater than 89% classified as variants of uncertain significance. 10% of pathogenic or likely pathogenic variants exhibit geographic heterogeneity. We find that due to the high lifetime risk of ALS and low frequency of pathogenic alleles, even the current largest genomics projects will struggle to confidently identify intermediate penetrance rare variants in ALS.

Conclusion: As precision treatments targeting specific ALS-causing mutations in specific patients are becoming an increasingly important therapeutic paradigm , distinguishing truly pathogenic ALS and FTD variants from benign genetic variation is now essential. Our results support a reorientated view of several ALS genes and genetic variants for which the published evidence depends heavily on only a single domain. Supporting evidence and analyses are provided in an interactive user-friendly format for clinicians and researchers.

LncRNAs associated with neuronal development are deregulated in SOD1-G93A murine model of Amyotrophic Lateral Sclerosis

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Introduction: Studies in familial Amyothrophical Lateral Sclerosis (fALS), are implicating more and more neuronal proliferation and differentiation. It has been shown that in the familial preclinical SOD1-G93A ALS murine model, ependymal stem progenitor cells show a higher proliferation and differentiation towards the neuronal lineage. LncRNAs play a role in neuronal cell's development, and the ablation of a panel of 8 lncRNAs (lincENC1, lincBRN1a, lincBRN1b, TUG1, FENDRR, lincp21, HOTTIP and ELDR) causes strong modifications in mouse brain's development. We decided to study the potential involvement of these lncRNAs in ALS pathology.

Materials and Methods: The lncRNAs were investigated in three different spinal cord (SC) areas (cervical, thoracic and lumbar) and in four brain areas (prefrontal cortex, motor cortex, hippocampus, striatum) of pre-symptomatic (8 Weeks) and symptomatic 18 weeks of age SOD1-G93A mice. Furthermore, 6 human homologues (lincBRN1a, TUG1, lincp21, FENDRR, HOTTIP and ELDR) were identified and their expression was investigated in an in vitro model of the disease (SH-SY5Y cells transfected with the SOD1-G93A gene).

Results: The lncRNAs expression profile was deregulated in all analyzed areas. Linc-p21 presented an alteration in its expression levels in all analyzed areas at 18 Weeks. We also found and upregulation for p53 and in symptomatic SOD1-G93A mice, indicating a deregulation in the p53/linc-p21/p21 pathway. Tug1, which was found upregulated in the lumbar spinal cord of SOD1-G93A mice at both 8 and 18 weeks of age and relating to this we found an increased expression of its down-stream targets Tril/Tlr4, indicating an on-going inflammatory phenomenon. For linc-Brn1a and linc-Brn1b we found a predominant deregulation in the pre-symptomatic mice, suggesting they could be relevant in early phases of CNS development of G93A disease model. A reduced, although present, deregulation was found for Hottip, Eldrr, Linc-Enc1, and Fendrr. In SH-SY5Y-SOD1G93A, the 6 human homologues all resulted deregulated versus the wild-type cell line.

Conclusions: In conclusion, our study is the first characterization of a panel of 8 lncRNAs in ALS, indicating a deregulation in all of their expression in specific ALS affected areas or at different stages of the pathogenesis. The analysis in an in vitro human cellule model of the disease indicates a possible translation of these results the human fALS pathology.

Localisation of HNRNPH to nuclear G4C2 foci and cytoplasmic stress granules in C9orf72 Amyotrophic lateral sclerosis

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INRTRODUCTION:
Amyotrophic lateral sclerosis (ALS) is an incurable fatal motor neuron disease whose most common known cause is hexanucleotide (G4C2) repeat expansion from normal 20 repeats to more than 1000 in the first intron of gene C9orf72. This mutation leads to formation of potentional pathogenic G4C2 foci in the nucleus. One of the interaction partners of G4C2 expansion in nuclear G4C2 foci is heterogeneous nuclear ribonucleoprotein H (HNRNPH1), found in ALS and frontotemporal dementia (FTD) patient brains [1]. The binding of HNRNPH1 in these sense G4C2 foci, which are paraspeckle like bodies, was previously demonstrated by our group [2].

OBJECTIVES:
In the presented study we wanted to define the role of individual HNRNPH1 domains on its localisation into G4C2 nuclear foci and cytoplasmic stress granules (SG).

METHODS:
We constructed a series of protein fragments based on HNRNPH1 domain structure including mutated quasi RNA-recognition motif (qRRM) domains. For this study fluorescence in situ hybridisation (FISH) and immunocytochemistry were used. SG were induced by sodium arsenite treatment and visualised with SG protein marker PABP. The RNA-binding domain map (RBDmap) of HNRNPH1 was constructed and a model of the HNRNPH1 binding to RNA generated with Coot and visualized with Pymol.

RESULTS:
We showed that all three individual qRRM's are sufficient for sequestration of HNRNPH1 into G_4C_2 foci. Endogenous HNRNPH1 exhibits nucleocytoplasmic shuttling and abundantly localizes to SGs upon arsenite treatment. Two out of three qRRM domains are required for colocalization with SG markers. The RBDmap showed insight into native HNRNPH1-RNA interactions in living cells and the binding model the possible mode of HNRNPH1 binding to specific RNAs.

CONCLUSION:
The results are interesting since they imply different specificities or binding activities of otherwise related domains. The nuclear foci share a group of interacting proteins with SGs and their simultaneous presence in neurons might have further pathological effects in ALS and FTD.

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Locomotor deficits in a mouse model of ALS are paralleled by loss of V1-interneuron connections onto fast motor neurons

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ALS is characterized by the preferential degeneration of motor neurons innervating fast-twitch fatigable muscle fibers. This work shows that fast fatigable motor neurons receive 2 folds more glycinergic inputs than the slow-twitch fatigable resistant ones, which are more resistant to the disease. Quantifications of soma-near synapses in a SOD1G93AGlyT2GFP mouse model, revealed 50% loss of glycinergic synapses restricted to fast fatigable motor neurons by postnatal day 45, a time point in which both neuromuscular junctions and motor neurons are still spared. When focusing on the V1 Engrailed-1 positive inhibitory interneurons, that control the speed of locomotion in mammals and are known to express glycine, we found the same connectivity pattern in physiological conditions and disease. Importantly, at postnatal day 63, SOD1G93A mice had a reduction to 13% of the V1 soma-near synapses as well as a loss of V1 interneurons. The V1 interneuron degeneration appears before motor neuron death and is paralleled by the development of a specific locomotor deficit affecting speed and limb coordination. The appearing of this group of symptoms was carefully assessed by DeepLabCut deed learning analysis and defined as 'Onset of locomotor phenotype'. To elucidate if the synaptic loss onto fast fatigable motor neurons could explain the changes in the locomotor phenotype, we performed selective silencing of V1 interneurons in the spinal cord utilizing inhibitory DREADDs. An intersectional mouse was generated to allow for dual-recombinase and iDREADDs expression in spinal V1 interneurons. Importantly, the reversible dampening of V1 interneurons phenocopied the ALS-induced reduction in speed in the control mice but did not have any effects in the SOD1G93A animals after the appearing of 'Onset of locomotor phenotype', demonstrating that the iDREADDs effect was occluded in the ALS mice. Overall, this study shows that spinal vulnerable and resistant motor neurons receive different amount of inhibitory inputs, which are selectively lost only on the vulnerable ones during early stages of the disease, leading to locomotor deficits. Strikingly, loss of connectivity with V1 interneurons leads to reduction of speed and stride length, symptoms which were previously observed in ALS patients. . Our study identifies a potential source of non-autonomous motor neuronal vulnerability in ALS and links ALS-induced changes in locomotor phenotype to inhibitory V1-interneurons.

LOSS OF CYCLOPHILIN A FUNCTION INDUCES TDP-43 PROTEINOPATHY: IMPLICATIONS FOR ALS AND FTD

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Introduction

Cyclophilin A (PPIA) is a multifunctional protein that has been associated with different human diseases, but its role in pathogenesis is still unknown. We first associated PPIA with nervous system degeneration, identifying it as a translational biomarker of ALS, with a protective role in ALS pathology. We found that PPIA levels in peripheral blood mononuclear cells (PBMCs) could distinguish ALS patients from controls, suggesting a diagnostic potential. We observed that absence of PPIA in an animal model of ALS exacerbated disease progression and low level of PPIA correlated with a worse disease phenotype in ALS patients, suggesting a prognostic potential. We demonstrated that PPIA, once lysine acetylated (acetyl-PPIA), interacts with TDP-43 and regulates some of its functions. Interestingly, we detected low acetyl-PPIA in PBMCs of sporadic ALS patients.

Objectives

TDP-43 is a key player in ALS pathogenesis. TDP-43 cytoplasmic mislocalization, fragmentation, hyperphosphorylation and aggregation are pathological hallmark in ALS and FTD patients. The molecular mechanisms at the basis of TDP-43 pathology have not been elucidated yet. Here we investigated PPIA function as a player of this process.

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 an FTD-like phenotype with marked TDP-43 pathology and late-onset motor dysfunction. In mice, absence of PPIA induces impairment in nucleocytoplasmic trafficking and synaptic plasticity. We found that PPIA was downregulated in ALS patients and identified a PPIA loss-of-function mutation in a sporadic ALS patient. MD analyses revealed a prominent structural variation between wild-type and mutant PPIA. In vitro we observed that PPIA mutation accelerates PPIA degradation and impairs its interaction with TDP-43.

Conclusions

PPIA is worthy of further investigation since its absence in mice promotes a neurodegenerative disease with key features of ALS-FTD, and a loss-of-function mutation in PPIA gene was found in an ALS patient. We confirmed the importance of PPIA for the stability and function of TDP-43.

Low-dose interleukin-2 as an immune-modulatory therapeutic strategy for ALS.

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Background: Neuroinflammation is a well characterised pathophysiological mechanism involved in the onset and progression of amyotrophic lateral sclerosis (ALS). Regulatory T cells (Tregs) are immune modulators which suppress inflammation and prevent the onset of autoimmune disorders. These cells dramatically and progressively decreased in ALS patients, with lower levels associated with shorted survival. Interleukin2 (IL2) is a crucial cytokine for Treg differentiation and functions and the administration of low dose IL2 (ld-IL2) has been proposed as an immune regulatory strategy for ALS to reduce neuroinflammation through the expansion of protective Tregs. Moreover, evidence of possible direct and pro-survival effects of IL2 on neurons and glial cells has been reported in the literature.

Aims: To evaluate the effect of ld-IL-2 on the blood transcriptome of ALS patients included in the IMODALS clinical trial and to investigate gene expression changes associated with IL2 treatment in patient-derived astrocytes.

Methods: 36 patients were recruited and randomly assigned to three treatment arms: 1MIU, 2MIU-IL2 or placebo. They received one injection daily for five days every 28 days for a total of 3 treatment cycles. At selected time points blood was collected, white blood cells isolated and RNA was extracted. Microarray and NanoString data were generated to investigate transcriptional changes associated with the treatment and results were validated through qRT-PCR. Moreover, to investigate any potential effects of IL-2 on CNS cells, patient-derived astrocytes were differentiated from ALS or healthy volunteer fibroblasts and in vitro treated with IL2. Transcriptional differences were investigated using Oxford Nanopore cDNA sequencing.

Findings: Gene expression and pathway analysis revealed longitudinal transcriptional changes throughout the IMODALS trial. In particular, evidence of a broad immune suppression was provided after the first treatment cycle (day8) while an activation of immune suppressive pathways reached a peak only at the end of the third administration (day64). In fact, a time-dependent and dose-dependent activation of Treg markers was reported which suggested a cumulative effect of ld-IL2. However, inter-individual differences were found amongst patients and participants were classified into fully, moderate and low responders. A predictive biomarker analysis was carried out and two genes were identified for which expression at recruitment was able to predict patient responsiveness to the ld-IL2 administration at day64. Finally, to investigate if IL-2 is able to mediate protective effects on CNS cells, patient-derived astrocytes from 3 C9ORF72-ALS patients, 3 sporadic ALS, and 3 healthy controls were generated and treated with IL2 following optimisation. IL2-associated transcriptional differences were evaluated through cDNA sequencing.

Lymphocytes senescence in amyotrophic lateral sclerosis

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Introduction
The immune system has a regulatory effect on disease progression in amyotrophic laterals sclerosis (ALS), a fatal neurodegenerative disorder. We have previously shown activation of cell senescence pathways in the proteome of peripheral blood mononuclear cells (PBMCs) from ALS individuals.

Objectives
To appraise the lymphocyte expression profile, at baseline and longitudinally, in a cohort of ALS patients. To test a defined senescence signature, including the proportion of T regulatory and B memory cells, in phenotypic variants of ALS.

Method
We have undertaken a multi-dimensional 32-marker time-of-flight (CyTOF) analysis in PBMCs from ALS patients (n= 20) stratified according to rate of disease progression and tested a second ALS cohort (n: 40) with ad-hoc flow cytometry panels to identify senescent lymphocytes, with reference to 30 healthy controls (HC).

Results
CyTOF (FlowSOM) clusters and difference heatmaps revealed higher expression of CD8+CD28-CD57+ T cells in faster progressing ALS along with an over-expression of late memory B cells, in keeping with inflammatory-driven senescence. Flow cytometry indicated a reduction of T regulatory cells (CD4+CD25HIGH+CD127LOW+FoxP3+) in fast progressing (p=0.0374) and of memory B cells (CD19+CD27) in slow progressing ALS (p= 0.0476) compared to HC. Tregs (R=-0.5628, p: 0.0123) and B cells (CD19+; R= -0.7493, p= 0.0026) showed a negative correlation with rate of disease progression. Late memory B-cells (CD19+CD27-IgD-) were elevated in ALS compared to HC and high baseline levels were associated with reduced survival (log rank, p=0.0207; above median: 11 months; below median: 33 months). CD3 and CD25HIGH+CD127LOW+FoxP3+45RO- showed a steady increase with disease progression (mixed model ANOVA, repeated measurements, p=0.0023).

Conclusions
Our data support the concept that changes of the immune system impact on the clinical progression of ALS, confirming that lymphocytes undergoing regulation during the course of the disease develop additional features of inflammatory cell senescence also reported in viral infections. Changes of T regulatory cells expression, as previously reported, but also of memory B cells are likely to be critical in the immunological dysregulation reported in ALS.

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Mathematical modeling reveals the correlates of cognitive impairment across the FTLD spectrum

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Introduction. Amyotrophic lateral sclerosis (ALS) can exhibit cognitive and/or behavioral symptoms, lying on a continuum with the behavioral variant of frontotemporal dementia (bvFTD).
Objectives. The aim was to apply mathematical modeling to unravel MRI connectomic signatures of cognitive and/or behavioral impairment in ALS.
Method. 83 ALS, 35 bvFTD and 61 controls underwent clinical, cognitive evaluations and MRI scan. Neuropsychological testing identified 54 ALS with only motor deficits (ALS-cn), 21 ALS cognitively and/or behaviorally impaired (ALS-ci/bi) and 8 ALS with bvFTD (ALS-FTD). The structural/functional intra- and inter-area connectivity was calculated and compared between patients. Using ROC curve, characteristic patterns of damage of ALS-cn and bvFTD were identified. Structural/functional connectivity cut-off value was identified to differentiate the two groups at best. ALS-ci/bi and ALS-FTD's pattern of damage was assessed through frequency analysis.
Results. Compared to ALS-cn, bvFTD showed a greater structural involvement of the frontotemporoparietal network. Conversely, ALS-cn patients showed greater involvement of sensorimotor-basal ganglia areas compared to bvFTD. Both ALS-ci/bi and ALS-FTD showed an ALS-like pattern of damage within sensorimotor-basal ganglia connections. ALS-ci/bi showed an ALS-like pattern within frontal and between frontal, temporal and basal ganglia areas. ALS-FTD differentiated from ALS-cn within frontal areas. ROC curve analysis identified a bvFTD-like pattern characterized by widespread damage, and an ALS-like pattern characterized by a disruption within sensorimotor-basal ganglia areas. Frequency analysis highlighted an ALS-like pattern in sensorimotor-basal ganglia areas for ALS-ci/bi and ALS-FTD. A non-significant trend within frontotemporoparietal areas between ALS-ci/bi, ALS-FTD and ALS-cn was identified suggesting a mild structural damage related to the degree of cognitive deficit. Functionally, bvFTD significantly differentiated from ALS-cn showing decreased functional connectivity in frontotemporal connections and within the sensorimotor-parietal connections. Functional pattern of damage was found in bvFTD and the frequency analysis revealed a bvFTD-like pattern in frontotemporal area in ALS-FTD and ALS-ci/bi.
Conclusions. Alterations of the frontotemporal and parietal networks characterized bvFTD (bvFTD-like pattern), while a more focal damage within sensorimotor-basal ganglia areas characterized ALS-cn (ALS-like pattern). Commonalities and differences relative to the two ends of ALS-FTD spectrum were found in ALS-ci/bi and ALS-FTD.
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MERIDIAN: A phase 2, randomized, double-blind, placebo-controlled, multicenter study to evaluate the efficacy and safety of pegcetacoplan in patients with amyotrophic lateral sclerosis

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Introduction: Inflammation underlies the pathogenesis of numerous neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). In ALS, the complement system has been implicated in the neuropathology of disease and disease progression. Pegcetacoplan, a subcutaneously administered C3 complement inhibitor, is being investigated in hematology, nephrology, and neurology. The current clinical study (NCT04579666) is investigating whether pegcetacoplan can improve survival and function in people diagnosed with apparent sporadic ALS.
Objectives: Evaluate the efficacy and safety of pegcetacoplan compared to placebo among people diagnosed with ALS in a global, multicenter, randomized, double-blind, placebo-controlled, phase 2 study.
Method: Approximately 228 patients diagnosed with apparent sporadic ALS, ≥18 years of age and with an ALS Functional Rating Scale-Revised (ALSFRS-R) score ≥30, slow vital capacity (SVC) ≥60% of the predicted value at screening, and with symptom onset within 72 weeks prior to screening, are eligible for enrolment. Following screening, patients will be randomized 2:1 to treatment groups receiving either subcutaneous pegcetacoplan (1080 mg) or placebo twice weekly for a duration of 52 weeks. The primary efficacy endpoint is the difference in the Combined Assessment of Function and Survival (CAFS) ranked score at 52 weeks after treatment initiation. Additional, secondary functional efficacy (ALSFRS-R, percent SVC, muscle strength, quality of life, and caregiver burden) and safety endpoints will be analyzed at 52 weeks. Following the placebo-controlled period, all patients will have the option to receive pegcetacoplan in an open-label period for an additional 52 weeks.
Results: This ongoing study is currently enrolling participants.
Conclusions: Results of this study will determine the role of complement and C3 inhibition in patients with ALS.

Metabolomics analysis highlight the involvement of muscle energetic metabolism in ALS pathophysiology

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Many pathological actors identified as putative diagnosis biomarkers or involved in neurodegeneration have been identified in muscle of ALS patients, but the link between muscular atrophy and metabolism alterations is not established yet. The central role of muscle in energetic metabolism suggests that this tissue, quite easily available compared to the brain, should be more analyzed.
We investigated the metabolism of the skeletal muscle and evaluated the mitochondrial function in ALS compared to controls.
We collected a plasma sample and a biopsy from the deltoid muscle from 17 ALS patients at diagnosis and 20 healthy controls (HC). We performed metabolomics analysis (LC-MSMS) of muscle and plasma samples, combined with analysis of mitochondria respiration in muscle (OXPHOS). Statistical analysis was performed using GraphPad Prism, Metaboanalyst or JMP, depending on the variables analyzed.
Median age (± SD) was HC: 66.1±19.2 years (y) and ALS: 67.2±9.9y. 35.3% of ALS presented bulbar onset, disease duration was 9.4±6.8 months (mo) and diagnosis delay was 9.0±4.7mo. At diagnosis, ALSFRS-r: 36.0±7.2. For plasma, multivariate analysis found different metabolomes (p-CV-ANOVA<0.035); 8 metabolites had VIP>1: allantoin, palmitate, dopamine, linoleate, nicotinamide, gluconic acid, ethylmalonic acid and elaidic acid, with impact in arginine biosynthesis (p=0.006), alanine, aspartate and glutamate metabolism (p=0.02), biosynthesis of unsaturated fatty acids (p=0.04) and linoleic acid metabolism (p=0.04). Muscle analysis by univariate volcano plot showed 13 metabolites increased in ALS muscle, between them citramalate (p=0.04). Multivariate analysis found different metabolomes (p-CV-ANOVA<0.0012); 5 metabolites had VIP>1: 4-methyl-2-oxovaleric acid, 4-methyl-2-oxo-pentanoic acid, L-alanine, creatine and glycine, with impact in the metabolism of glycine, serine and threonine (p<0.001); biosynthesis (p=0.002) and degradation (p=0.04) of valine, leucine and isoleucine. OXPHOS analysis in muscle samples revealed higher complex II/CS ratio in ALS (0.37±0.09) than in HC (0.32±0.06; p=0.04). LDH activity was lower in ALS (HC 6060±3045 nmol/min/mg protein; ALS: 3705±1458 nmol/min/mg protein; p=0.03). In muscle of ALS patients, LDH activity correlated positively with carnitine, creatinine and lactate levels, while it correlated negatively with alpha-glucose-1-phosphate. At database lock, 6 patients were alive. Multivariate analysis revealed correlation with muscle C10-carnitine (p=0.047) and blood l-glutamic acid (p=0.012) with survival. Metabolomics analysis in muscle showed major alterations in aminoacids metabolism, oxidation of fatty acids and carnitine synthesis, highlighting the mitochondrial dysfunction described in ALS.

Mitochondrial dysfunction links mutations in TDP-43 and C9orf72 iPS-derived motor neurons from ALS patients

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

Objective
The aim of this study is to identify if C9orf72 and TDP-43 mutations affect mitochondrial function using iPS-derived MNs from patients and isogenic controls.

Methods
In this study, we differentiated patient motor neurons derived from induced pluripotent stem cells (iPSCs) carrying hexanucleotide expansions in the C9orf72 gene or mutations in TDP-43 (M337V and I383T). We generated isogenic iPSC lines where the expansions were successfully removed by CRISPR/Cas9 in C9orf72 iPSCs. Seahorse XFe was used to assess mitochondrial respiration, ATP production and spare respiratory capacity and live calcium imaging was used to determine mitochondrial calcium buffering. Neurons were grown on microfluidic chambers for studying axonal transport and MitoTracker movement was quantified during live imaging in the microgrooves.

Results
We found both C9orf72 and TDP-43 (M337V and I383T) MNs show reduced mitochondrial basal respiration at baseline and reduced spare respiratory capacity when ER stress was induced by thapsigargin. Mitochondrial potential was also reduced in C9orf72 MNs, while the TDP-43M337V and TDP-43I383T MNs did not show differences when compared to healthy controls. When stimulated with 100 µM glutamate during live calcium imaging, we found reduced mitochondrial uptake of calcium from the cytosol in C9orf72 and TDP-43 MNs compared to healthy and isogenic controls. Imaging of axonal transport revealed reduced speed of retrograde mitochondrial transport in TDP-43M337V and TDP-43I383T, which correlated with downregulation of the molecular motor adaptor dynactin-1. We also detect significantly reduced mitochondrial length and surface area in patient iPS-MNs, indicating increased fragmentation.

Conclusions
This study shows that ALS iPS-derived MNs with mutations in C9orf72 and TDP-43 have deficiencies in essential mitochondrial functions, such as respiration, calcium buffering and mitochondrial dynamics.

Modelling Cortical Thinning in C9ORF72-ALS Brain Organoids

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Introduction - 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 mechanism underlying this change remain poorly understood.

Objectives & method - To fill this gap, we developed a brain organoid model to analyze human cortex development in C9ALS patients. The brain organoids are derived from induced pluripotent stem cells of C9ALS patients and healthy controls and generated using cerebral and forebrain-specific protocols.

Results - Here, we present several cellular phenotypes and an overview of future experiments. The iPSC lines used showed a 30% reduction of C9ORF72 protein. Using quantitative PCR and immunostaining decreased gene and protein expression of specific markers for intermediate progenitors and early post-mitotic neurons was detected in organoids, like TBR2 and TBR1, whereas expression of neuronal cytoskeleton markers was not affected in C9ALS organoids. To follow-up on these observations, we performed single cell RNA sequencing on 3 C9ALS and 3 healthy control organoid samples at two timepoints: early and late in organoid development. This dataset will provide insight into the presence of different cell populations in patient and control organoids, as well as reveal how these populations contribute to the cortical identity of the organoids.

Conclusions - Our data indicate that changes in cell populations may contribute to cortical thinning observed in C9ALS patients and repeat carriers. In future studies, we will test whether editing of the C9 repeat expansions by CRISPR/Cas9 editing will rescue the cellular phenotypes observed. Finally, we also want to explore whether C9ORF72-targeting antisense oligonucleotides can reverse disease-associated changes in brain organoids.

Modelling of ALS-associated coding and non-coding KANK1 mutations

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Introduction:
Amyotrophic lateral sclerosis (ALS) is an incurable disease with a strong genetic underpinning. Despite heritability estimates of 52%, GWAS studies have discovered only seven genome-wide significant hits, which are relevant to <10% of ALS patients. Recently, a novel pipeline called RefMap was developed to increase the power of gene discovery by integrating motor neuron functional genomics with ALS genetics in a hierarchical Bayesian model. 690 candidate ALS genes were identified using RefMap and KANK1, which is enriched with coding and noncoding, common and rare ALS-associated genetic variation, was selected for functional characterisation.

Aims and objectives:
To develop cell models to characterise the pathogenicity of ALS-associated KANK1 mutations. To identify novel therapeutic targets for the treatment of ALS.

Methods:
ALS-associated coding and non-coding KANK1 mutations were modelled in SH-SY5Y cells using CRISPR/SpCas9 genome editing. Expression of KANK1 mRNA was evaluated via qRT-PCR. Cellular toxicity was measured using MTT assays. Morphological characterisation of neuronally differentiated cells was performed using confocal microscopy.

Results:
In neuronally differentiated SH-SY5Y and iPSC cells we used CRISPR/SpCas9 editing to recapitulate ALS-associated regulatory and coding mutations within KANK1. Coding and non-coding CRISPR/SpCas9 perturbations reduced the expression of KANK1 mRNA and produced neurotoxicity with disruption of the distal axon.

Conclusions:
KANK1 is a new ALS risk gene, which is enriched with common and rare ALS-associated genetic variation across multiple domains and datasets. KANK1 is functionally related to a number of known ALS genes which are important for cytoskeletal function, including PFN1, KIF5A and TUBA4A. We have experimentally verified the link between variants linked by RefMap to ALS, and KANK1 expression. Moreover, we have demonstrated that reduced expression of KANK1 in a human CNS-relevant neuronal model is cytotoxic. KANK1 upregulation is a new therapeutic target which may be broadly relevant for treatment of sporadic ALS.

Monitoring disease progression with electrophysiological markers derived from compound muscle action potential scans in amyotrophic lateral sclerosis

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Introduction:
The compound muscle action potential (CMAP) scan is a promising electrophysiological method to quantify motor unit number estimates (MUNE), motor unit sizes and axonal excitability. The CMAP scan has shown to be able to quantify disease progression in muscles affected by motor neuron disease (MND). The potential of electrophysiological markers derived from the CMAP scan in clinical trials for ALS remains to be established.

Objectives:
To determine which electrophysiological markers derived from the CMAP scan are most sensitive to monitor disease progression in patients with ALS, and whether they hold value for clinical trials.

Method:
A multicenter collaboration was initiated between MND centers in Denmark, Turkey, Australia and the Netherlands to retrospectively assess longitudinal electrophysiological and ALS functional rating scale (ALSFRS-R) patterns. For each patient we determined the change over time in maximum CMAP (CMAPmax), D50 (number of largest discontinuities within CMAP scans), returners (number of increased CMAPs with decreasing stimulus currents), a motor unit number estimate (MUNE) and MU size properties (e.g. mean, largest unit). Linear mixed models were used to estimate variance components and population averages. Results were translated to required sample sizes for trials.

Results:
In total, 225 thenar CMAP scans from 65 patients were obtained. The ALSFRS-R decreased on average by 0.9 points/month (95% CI -1.2 to -0.7). MUNE, D50 and CMAPmax showed the largest decrease over time with -0.09, -0.09, and -0.05 standard deviations per month, respectively. In terms of sample size, the ALSFRS-R required 388 patients for a 6-month trial with visits every two months, whereas MUNE required 314 patients (-19.1%) to detect a 30% reduction in progression rate.

Conclusions:
The electrophysiological markers show considerable variability in their ability to monitor disease progression. MUNE showed to be the most suitable derivate. Further developments and standardization of the CMAP scan could refine its utility for ALS clinical trials.

Morpholino oligomers ameliorates pathological hallmarks in C9orf72 cellular lines and mice

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Introduction: Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by progressive degeneration of motor neurons (MNs). GGGGCC repeat expansions in C9ORF72 gene are the most common identified genetic cause, and even if their pathogenic processes are still unknown, many possible mechanisms have been proposed, including loss of function of the C9Orf72 protein, gain of function from accumulation of RNA foci and sequestration of RNA binding proteins (RBPs), and toxicity caused by dipeptide repeats proteins (DPRs) produced by repeat-associated non-ATG (RAN) translation. One promising and reliable method to understand C9-ALS pathogenesis is represented by patient-specific induced pluripotent stem cells (iPSC)-derived lines and iPSC-derived MNs.
Objectives: Our therapeutic approaches include the use of antisense oligonucleotides (ASOs) designed to bind complementary mRNA and interfere with specific biological processes. In our laboratory, two different ASOs with Morpholino chemistry have been designed: against the C9ORF72 expansion motif and against the whole C9ORF72 gene; our aim is to characterize the pathological phenotype of the C9-ALS iPSC-derived lines and evaluate the therapeutic effect of ASOs administration on specific pathological markers.
Methods and Results: We reprogrammed iPSCs from C9-ALS patients and controls and differentiated them into MNs using a 14-days protocol. We investigated the phenotype of the C9-ALS lines compared to controls, evaluating cells survival, pluripotency and motor neuronal markers, STMN2 expression, defects in axonal elongation and nucleolar disfunctions. Next step was transfecting ALS-MNs with different Morpholinos and evaluating modification of the previously mentioned pathological markers. Interestingly, we identified in C9-ALS iPSC-derived lines pathological features such as accumulation DNA damage, minor axonal elongation, with decreased levels of Nfh, Stmn1 and Sept7 genes and Nefl and Nefh expression. After Morpholino treatments, we observed that ASO therapy could partially rescue the pathological phenotype. Then we translated the treatment with MoB to C9orf72 mice, and analysed brains in qPCR before and after the treatment for Nefh and Nefl expression: both markers seem to be restored by MoB.
Conclusions: Our results suggest that patient specific iPSCs and iPSC-derived MNs, together with animal models, are a valuable tool to deepen the knowledge of C9ORF72 pathogenic mechanisms, and that Morpholino-mediated approaches represent a very promising therapeutic strategy that needs to be further validated.

Motor neuron disease in Costa Rica

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Abstrac
Introduction Costa Rica, a middle-income country in Central America, has a population of 5,075,000 people. The National Center for Pain Control and Palliative Care is a specialized social security center that provides multidisciplinary care throughout the national territory to patients with ALS. Aim to determine the epidemiological characteristics of Amyotrophic Lateral Sclerosis in Costa Rica. Methods. A data review is carried out in the file from January 2020 to January 2021. Results 24 new cases of patients diagnosed with ALS were reported. For an incidence of 0.47 cases / 100,000 habitants. Currently 154 patients with ALS are followed; for a prevalence of 3.03 cases / 100,000 habitants, of which 66% are men (101 cases) and 34% are women (53 cases). Regarding the age of onset, it was documented that it has a peak between the fifth and sixth decade of life: from 30-39 years, 6 cases were found, representing 4%; between 40-49 years 19 patients for 12%; between 50-59 years 43 patients for 28%; between 60-69 years 44 patients for 29%; between 70 and 79 years 4 patients for 3%. The geographical distribution showed that the majority of people with ALS resided, mostly, in urban areas for 81% (110 patients) and in rural areas for 19% for a total of 27 patients. It was evidenced that the largest number of patients with ALS, 56%, are assessed at home, (86 cases) while 44% (68 cases) arrive at the outpatient clinic. Using the ALSFRS-R scale at the time of admission, it was found that stage 1 was reported 15 cases (11%), stage 2, 28 cases (20%); stage 3, 34 cases (25%); stage 4, 30 cases (22%) and stage 5, 30 cases (22%). At the time of diagnosis, 133 (87%) patients presented with spinal ALS and bulbar ALS 13% (21 patients); however, upon entering the Physical Therapy service, it was evidenced that 53% of these patients had mixed ALS. Conclution. Amyotrophic lateral sclerosis is a progressive and fatal disease, in Costa Rica there is a specialized care center that offers patients a multidisciplinary approach that tries to improve the quality of life and supports the clinical evolution of the disease.

Multi-path direct current stimulation reduces motor neuron hyperexcitability and improves survival in the SODA-G93A model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the spinal cord and brain. ALS causes muscle weakness, paralysis and eventual death, typically within 2-5 years of diagnosis. In EU, it is estimated there are approximately 50,000 ALS patients, while in US, it is estimated there are approximately 16,000 ALS patients. There are exceedingly few approved drugs for ALS, and the effectiveness of these drugs for increasing patient survival is limited.

Spinal direct current stimulation (DCS) modulates electrophysiological characteristics of spinal cord activity depending on polarity (anode vs. cathode), direction of current relative to neuronal orientation, current density, duration of stimulation, and electrical or ionic form of current transmitters. We have developed a novel non-invasive approach, multi-path DCS, that utilizes direct current stimulation at multiple sites to drive current across the spinal cord and down the limb. Using a combination of these parameters, multi-path DCS is able to modulate spinal cord neuronal excitability in either direction. This is relevant to ALS as neuronal hyperexcitability is a known factor of ALS pathology.

Using a transgenic mouse model of ALS (SOD1-G93A), we have demonstrated that multi-path anodal DCS (a) reduces neuronal spinal excitability long-term (b) slows the progression of muscle weakness, and (c) increases life span of stimulated animals. Mean survival time in control group was 8.4 days, while mean survival time in stimulated group was 17.3 days using a therapeutic stimulation paradigm. We also investigated molecular changes in stimulated animals. We found that treatment with multi-path anodal DCS: (a) reduces the expression of mutant SOD1 protein, (b) reduces expression of elevated NKCC1 (c) reduces phosphorylated tau (d) increases expression of HSP70, and (e) increases expression of LC3B. Together, these data provide evidence that multi-path anodal DCS enhances clearance of misfolded proteins by modulating the proteolytic systems of autophagy and the proteasome. By reducing spinal excitability and clearing toxic proteins from spinal cells, multi-path anodal DCS could become a disease-modifying therapeutic intervention against ALS.

Mutations in VCP induce lysosomal alterations and autophagy activation in ALS neuronal models

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Valosin Containing Protein (VCP) is an ATPase protein member of the AAA+ protein family. VCP is involved in various pathways that concur in maintaining cellular homeostasis. VCP mutations have been correlated different proteinopathies including neurodegenerative diseases as ALS. VCP-mutants are associated associated with the presence of alteration of the Protein Quality Control System: ubiquitin inclusions, TDP-43 mis-localization and aggregation, and abnormal vacuoles. To date, the mechanisms correlated to VCP-mutants that lead to cell toxicity and death are still not defined.

In this study, we identify VCP-mutants pathological mechanisms in an ALS-model. We overexpress VCP WT, VCP R155H and VCP R191Q in NSC-34, a motor neuron mouse immortalized cell line. In first instance, we found that both VCP mutants from insoluble aggregates and induce lysosomal alteration in size, morphology, activity and membrane breakage.

Lysosomal damage is known to lead to cell toxicity and death, so cells activate different mechanisms to remove damaged lysosome as the activation of autophagy. Therefore, we studied variance in the autophagic flux in presence of VCP-mutants by analysing LC3 conversion and p62 accumulation. We could determine that VCP-mutants are correlated with an activation of the autophagic flux. Moreover, by analysing transcription factors that regulate autophagy we determined that VCP-mutants positively regulates autophagic flux by specifically activating the transcription factor TFE3. Results also determined that TFE3 activation triggered by VCP-mutants presence is mediated by calcineurin, a Ca2+ dependent phosphatase. In parallel, we excluded the involvement of TFEB in this pathway. Overall, these data suggest that lysosomal damage and leakage induced by VCP-mutants activate calcineurin which in turn mediates TFE3 dephosphorylation and nuclear translocation inducing autophagy. In support to this we found that VCP mutants enhances insoluble protein-aggregates with a specific dependency from the autophagic pathway.

NEK1 variants in a cohort of Italian patients with Amyotrophic Lateral Sclerosis

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Introduction: Recently, large-scale whole-exome sequencing studies highlighted a significant enrichment of NEK1 loss of function (LoF) variants in amyotrophic lateral sclerosis (ALS), as well as an additional role for the p.Arg261His missense variant in the disease susceptibility. Several other missense variants have been described so far; however, their pathogenic relevance remains to be established.
Objectives: to further investigate the presence and impact of NEK1 variants and to explore potential genotype-phenotype correlations in a cohort of Italian ALS patients.
Methods: We sequenced a cohort of 356 unrelated Italian ALS patients by NGS using TruSeq Neurodegeneration Panel by Illumina (San Diego, CA, USA). A cohort of 380 non-neurological unrelated Italian patients was selected as the control group. We included in the study only NEK1 variants with MAF<0.01, which were confirmed by Sanger sequencing. Clinical significance was assessed based on the ACMG guidelines. Kaplan–Meier analysis was carried out to determine the effect of NEK1 variants on survival. Fisher's exact test and a binary logistic regression analysis, adjusted for sex and age at onset, were used to explore differences in gene variant frequencies, as well as the association of NEK1 variants with phenotype.
Results: We detected and confirmed 20 different NEK1 rare variants (4 LoF and 16 missense) in 33 unrelated patients with sporadic ALS. The four LoF variants (two frameshift and two splice-site variants) were absent from all public genomic databases and from our in-house controls. The p.Arg261His missense variant, previously reported as a risk factor for ALS, was found in 13 patients and one control ($p<0.001$). The difference in the frequency of other NEK1 missense variants between patients and control was not statistically significant, in line with previous studies. Among the sixteen missense variants we found, nine were classified as variants of uncertain significance (VUS); of these, four were novel. Fifteen NEK1 variant carriers (45.4%) also harbored variants in other ALS-related genes. ALS patients carrying NEK1 variants did not differ for sex distribution, age at onset or survival from the other patients of the cohort. Notably, binary logistic regression analysis revealed a significant higher risk for NEK1 carriers to present with upper limb involvement (OR 3.23, 95% CI 1,09-9,55; $p < 0.05$), consistently with a previous report.
Conclusions: NEK1 variants are not rare in the Italian population. The fact that we found NEK1 variants only in sporadic patients, together with the high frequency of oligogenic carriers, supports the hypothesis that some NEK1 variants confer a significant susceptibility to ALS, even though they might not be sufficient per se for disease development. These variants may however act as a phenotypical modifier.

Neurofilaments as predictors of ALS disease aggressiveness: an application of the D50 disease progression model

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Introduction:
Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder dominated by progressive motor-neuron dysfunction. The large inter-individual heterogeneity of disease phenotypes and progression speeds represent a major constraint for clinical trials. Biomarkers that reliably reflect disease progression are of paramount importance to overcome these challenges.
Objectives:
The D50 disease progression model was applied to investigate the influence of ALS disease aggressiveness on cerebrospinal fluid (CSF) levels of Neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain (pNfH).
Methods:
The D50 model provides distinct parameters to quantify individual disease courses of ALS patients. The value D50 indicates the patient's overall disease aggressiveness (defined as time taken in months to lose 50% of functionality). The relative D50 (rD50) reflects the individual disease covered and can be calculated for any time-point in the disease course. We analyzed clinical data from a well-defined cohort of 156 patients with ALS. The concentration of neurofilaments in CSF samples was measured at three different laboratories. Based on patients' individual D50 values, we defined subgroups with high (< 20), intermediate (20 - 40) or low (> 40) disease aggressiveness. Neurofilament levels were compared between these subgroups via analysis of covariance (ANCOVA), corrected for the following covariates: age, gender, clinical phenotype, frontotemporal dementia, rD50-derived disease Phase and analyzing laboratory.
Results:
We found highly significant differences in neurofilament concentrations between all three D50 subgroups ($p < 0.001$), representing an increase of neurofilament levels with increasing disease aggressiveness. Only the disease aggressiveness had a significant effect on pNfH, independent from any other covariate. For NfL, the ANCOVA confirmed a highly significant correlation with disease aggressiveness, while age, analyzing laboratory and presence of frontotemporal dementia also showed significant effects on NfL concentrations.
Conclusions:
The present study provides strong evidence for the potential of neurofilaments to reflect disease aggressiveness in ALS. Most importantly, this study demonstrated that both neurofilaments remain at stable levels throughout the disease course, as they were independent from the rD50 at the time of sampling. Thus, neurofilaments represent promising biomarkers to track for therapeutic effects in future clinical trials. The D50 model provides a valuable quantifiable framework of the individual ALS disease course, which enables the assessment of correlations between clinical and laboratory measures. It can be highly recommended for future studies on candidate biomarkers.

Neurologists' perception of well-being under mechanical ventilation and gastrostomy: a role in the management of patients with amyotrophic lateral sclerosis

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Objective: To investigate the neurologists' perception of well-being of patients with amyotrophic lateral sclerosis (ALS) using gastrostomy (PEG), non-invasive (NIV), or invasive ventilation (IV) and to analyse its determinants and impact on the management of these medical interventions (MIs).

Methods: The study was based on anonymous questionnaires answered by 465 neurologists (228 from Germany and 237 from Poland) that considered the clinical approach and personal attitude towards the use of PEG, NIV, and IV in ALS patients.

Results: The neurologists from both countries estimated the quality of life (QoL) of ALS patients using PEG and NIV as neutral (0 and 0, respectively), while low (-2) in individuals using IV. Regression models revealed that the palliative care training (PCT) and age were independent factors influencing the attitude of the German group. Significantly higher values of estimated patients' depressiveness on PEG, NIV, and IV were found among the Polish neurologists. Marital status, experience in ALS, and being a parent negatively influenced the perception of patients' depressiveness using MIs in the German group, while the marital status, age, and PCT in the Polish group. We found a positive correlation between the perception of PEG, NIV, and IV as beneficial to patient with ALS and the favourable estimation of QoL on each of these measures in both the German and the Polish group. In addition, there was a significant relationship between the estimated QoL on the use of PEG, NIV, and IV and a favourable attitude towards a hypothetical decision to opt for each of these measures by physicians themselves — both in the German and the Polish group. Among all neurologists, 33.5% discussed the MIs at the diagnosis, 58.2% at time of indication, and 8.3% when asked by patient. In the German group, the odds for later discussing ("when indications occur" vs "at the diagnosis") of MIs increased by 17%, 15% and 23% for each point of a higher estimated depressiveness in patients using PEG, NIV and IV, respectively. In the Polish group, the odds of earlier discussing ("at the diagnosis" vs "when indications occur") of MIs increased by 27% for each point of higher estimated QoL in patients on PEG

Conclusion: The German and Polish neurologists similarly perceived the QoL as low in patients using IV and neutral in those with PEG and NIV. The levels of depressiveness, ranging from moderate (PEG, NIV) to severe degree (IV), were rated of greater intensity by Polish neurologists. These estimations reflected demographic and professional experience of the physicians, particularly the age, marital status, being a parent and PCT. Of further importance, the perceived well-being on the use of MIs played a role in defining each measure as profitable for the patients and the reported management approaches, especially the timing of the MIs discussion.

Neuroprotective effect of fragment C of tetanus toxin modulates inflammation mediated by IL-6 in SOD1G93A mice.

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Introduction: Amyotrophic Lateral Sclerosis (ALS) is a multifactorial disease of unknown etiology in the majority of ALS cases. No effective treatment has been identified for the disease, although some drugs such as Riluzol, and more recently, edaravone and masitinib, are currently used to slow the disease progression. It has been described that inflammatory factors may modulate the disease progression and survival rate in ALS. In this sense, therapeutic strategies designed to ameliorate inflammatory processes in ALS are in the spotlight. Our group has investigated the neuroprotective role of fragment C of tetanus toxin (TTC), demonstrating its neuroprotective effects in vitro and in the SOD1G93A mouse model. Objectives: To decipher the possible anti-inflammatory role of TTC in the SOD1G93A mouse model to modulate some pro-inflammatory cytokines previously described altered in ALS, and on NLRP3 inflammasome proteins. Method: Intramuscular injections with 1 microgram of TTC recombinant protein at P59 till P113 were administered in the treated group. SOD1G93A mice injected with phosphate-buffered saline (PBS) were used as control group. Serial plasma samples were taken from the twenty-four mice at 63, 92 and 113 days of postnatal life (P63, P922 and P1133). Spinal cord and two different skeletal muscles, extensor digitorum longus (EDL) and soleus (SOL), were isolated from SOD1G93A mice after euthanized with CO2 at P113. Levels of eotaxin-1, interleukin (IL)-2, IL-6 and macrophage inflammatory protein (MIP)-1 alpha and galectin-1 were analyzed by immunoassays in plasma samples, while protein expression of caspase-1, IL-1, IL-6 and NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) was measured in the spinal cord, EDL and SOL muscles. Results: Protein levels of the cytokines were not downregulated by TTC in serial plasma samples. Reduced levels of IL-6 and NLRP3 in the spinal cord and SOL decreased in the group of mice under TTC treatment, albeit levels of NLRP3 were not found downregulated in EDL muscle. In addition, the protective effect of TTC modulated caspase-1 levels only in EDL, which is mostly affected by the disease progression with respect to SOL. Conclusions: TTC could have a potential anti-inflammatory effect by reducing IL-6 levels in tissues drastically affected by the disease, and modulating the expression of NLRP3 inflammasome proteins. These findings can be of help to design promising and novel therapeutic strategies based on TTC in ALS.

Neuropsychological dysfunction in asymptomatic ALS relatives: A marker of disease risk?

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Introduction

An increased risk of developing neurodegenerative and neuropsychiatric disorders is seen in relatives of people with ALS, partly but not entirely attributable to pleiotropic effects from the C9orf72 repeat expansion. Studies of ALS relatives to date have been limited to proxy-reports from probands.

Objective

The Irish ALS Endophenotype Study directly assesses unaffected ALS relatives with the aim of identifying neuropsychologial traits which may serve as markers of increased genetic liability, helping to augment the statistical power of genotype-phenotype studies.

Method

Relatives of Irish ALS patients and healthy controls completed a comprehensive neuropsychological battery and neuropsychiatric assessment. In all participants, C9orf72 repeat expansion status was determined using repeat-primed PCR with amplicon length analysis (positive cut-off: ≥ 30 repeats).

Results

176 asymptomatic relatives (C9orf72 positive [37]; negative [139]) and 207 healthy controls were assessed. C9orf72 carriers displayed subtle executive dysfunction (Stroop switching errors, sequential digit span) and verbal fluency deficits compared with familial non-carriers (p=0.002, p=0.039 and p=0.029 respectively). Greater apathy was observed in ALS relatives compared with controls (64% v 46%, p=0.036), with more ADHD seen in C9orf72 negative relatives compared with controls (15% v 8% respectively, p=0.041). Increasing age in ALS relatives was moderately correlated with more extensive neuropsychological impairment (p=0.003).

Conclusions

Subtle age-dependent neuropsychological deficits are detectable in ALS relatives at a greater frequency than seen in healthy controls and may be markers of early non-motor disease manifestation. However, detection of changes in non-C9orf72 relatives also suggests the co-existence of other genetic modifiers with ALS kindreds, consistent with an oligogenic hypothesis of ALS.

Neurovascular dysfunction distinguishes sporadic ALS cases with long and short disease durations

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Introduction
ALS is a highly phenotypically heterogeneous disease, particularly with respect to disease duration, which ranges from months to decades (1). In the absence of identifiable disease associated mutations and/or a family history, the majority of ALS cases are described as sporadic (sALS). TDP-43 protein aggregation has been identified as a pathological hallmark of sALS (2,3). TDP-43 is a ubiquitously expressed regulator of transcription and splicing, therefore understanding transcriptional profiles associated with distinct sALS clinical phenotypes such as differential disease duration, is a critical area for investigation.

Objectives
To investigate the transcriptional profiles of sALS cases of long and short disease duration.

Method
We carried out transcriptional profiling on motor cortex samples from a cohort of 20 sALS cases stratified by disease duration from symptom onset (short median disease duration of 16 months, long median disease duration of 64 months). The genetic status of all samples was assessed by whole genome sequencing or C9orf72 repeat-prime polymerase chain reaction and the application of an extended 49-gene panel. ALS cases were compared with 11 age and gender matched non-neurological control samples. Transcriptional profiling was carried out using NanoString (4), a novel molecular barcoding panel technology comprised of 760 genes associated with neurodegeneration. A series of bioinformatic analyses were carried out following differential gene expression analysis using DESeq2 (5). Candidate gene validation was carried out using BaseScope RNA in-situ hybridisation (6) and immunohistochemistry.

Results
Comparison of the long versus short sALS disease duration groups identified upregulation of pro-inflammatory and innate immune response Gene Ontology (GO) sets. Cell type specific analyses of differentially expressed genes (7) identified upregulation of microglial and endothelial cell associated genes and synapse downregulation. RNA and protein validation studies of selected candidate genes (AQP4, CLDN5 and ICAM2) confirmed significantly increased transcription and aberrant translation of these key neurovascular components in sALS cases of short disease duration.

Conclusions
Our data demonstrate that inflammatory pathway dysregulation and dysregulation of key neurovascular components distinguishes long and short disease duration sALS cases. We are now conducting functional studies to assess the impact of modulation of these candidate genes on the integrity of the neurovascular unit and TDP-43 aggregation dynamics. Understanding the molecular basis of factors which confer relative neuro-resilience will facilitate the development of biomarkers, targeted disease therapies and enable the stratification of future trials to test these candidates.

Non-Coding Genetic Analysis Implicates Interleukin 18 Receptor Accessory Protein 3'UTR in Amyotrophic Lateral Sclerosis

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Introduction

Advancement in genomic sequencing technologies dramatically accelerated the pace of gene discovery in amyotrophic lateral sclerosis (ALS) - a fatal neurodegenerative syndrome with a strong genetic predisposing component. However, while the non-coding genome is substantially larger than the protein-coding genome, lack of appropriate methodologies for identifying functional variants still limits genetic association studies. Thus, non-coding nucleotide variants in ALS have yet to be systematically explored.

Objective

The main objective of our research is to explore the hypothesis that genetic variations in non-coding regulatory regions, such as miRNAs and 3'UTR of mRNAs, are associated with ALS.

Method

For this aim, we developed analytical tools to identify rare qualifying variants in miRNAs and 3'UTR of mRNAs, and performed collapsed genetic analysis of regions of interest using whole-genome sequencing (WGS) data, to test if these regulatory RNAs are associated with ALS. Next, we validated the functional impact of the identified ALS-associated genetic variants, by utilizing molecular cell biology of human iPSC-derived motor neurons and lymphoblastoid cell lines from different individuals, carrying the putative variants of interest versus others carrying the canonical form.

Results

Region-based burden analysis of >23,000 variants in 6,139 ALS whole-genomes and 70,403 non-ALS controls identified Interleukin-18 Receptor Accessory Protein (IL18RAP) 3'UTR variants significantly enriched in non-ALS genomes, replicate in an independent cohort and associate with a five-fold reduced risk of developing ALS. IL18RAP 3'UTR variants modify NF-κB signaling, provide survival advantage for cultured ALS motor neurons and ALS patients, and reveal direct genetic evidence and therapeutic targets for neuro-inflammation.

Conclusions

We discovered an enrichment of rare variants in the IL18RAP 3'UTR, implicating the IL-18 pathway in ALS. This systematic analysis of the non-coding genome and specifically miRNA-networks will increase the power of genetic association studies and uncover mechanisms of neurodegeneration.

Non-invasive ventilation adaptation (NIV) is predicted by blood carbonate (HCO3-) and base-excess (SBE)

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Introduction

Non-invasive mechanical ventilation (NIV) is the treatment of choice for impending symptoms and signs of respiratory failure in Amyotrophic Lateral Sclerosis (ALS), improving patients' quality of life and survival [1]. Patients' compliance to NIV and adequate ventilator settings are crucial for improving long-term NIV efficacy [2].

Objective

To investigate the role of arterial blood gas analysis (ABG) parameters (blood carbon dioxide, pCO2; oxygen, pO2; carbonate, HCO3-; standard base excess, SBE) in monitoring respiratory function and ventilation compliance after NIV adaptation, predicting survival in ALS patients.

Methods

We selected the first ABG performed after NIV start in ALS patients followed from 2000 to 2015 in Turin ALS Centre. Correlations between ABG parameters and survival were calculated. Risk for death/tracheostomy was computed at modifying ABG parameters by using Cox regression models, adjusted for the main prognostic factors. Kaplan-Meier curves were then performed and compared.

Results

A total of 186 post-NIV ABGs were included. HCO3- and SBE showed a significant correlation with survival after NIV (respectively R= -0.183, p=0.018 and R= -0.200, p=0.010). Risk for death/tracheostomy after NIV was significantly higher at increasing HCO3- and SBE blood levels, especially when HCO3- was >29 mmol/L and SBE >4 mmol/L (respectively HR 1.466, 95% CI 1.068-2.011, p=0.018 and HR=1.411, 95% CI 1.030-1.32, p=0.032). Survival in NIV was higher in patients with HCO3- <29.0 mmol/L and SBE <4.0 mmol/L in comparison with those with both parameters increased (0.69 years, IQR 0.33-1.37 vs 0.37 years, IQR 0.12-0.77; p=0.013).

Conclusions

HCO3- and SBE blood levels are not only useful for NIV initiation [3], but they can be used as markers of ventilation compliance, tolerance and efficacy, being able to predict survival in NIV.

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Opportunistic assessment of motor unit firing behaviour during 'relaxed' muscle recordings

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INTRODUCTION: High-density surface electromyography (HDSEMG) permits non-invasive capture of motor unit activity at high temporal and spatial resolution. We recently designed SPiQE (Surface Potential Quantification Engine) to quantify fasciculations from relaxed muscle recordings. However, up to now, portions of data with inadvertent unrelaxed activity have been excluded from the analysis.

OBJECTIVES: In this study, we examined these excluded regions more closely, seeking potential correlations with disease parameters in ALS.

METHODS: In MATLAB, we analysed 30-minute HDSEMG recordings from the biceps and gastrocnemius of 20 ALS patients and 5 patients with benign fasciculation syndrome (BFS), each assessed at intervals of two months on a maximum of seven occasions. Periods of unrelaxed muscle activity were identified by SPiQE using a built-in, semi-automated process. Motor unit firing parameters were compared between the two muscles, across disease groups and according to riluzole status. Linear-mixed effect regression was employed in R to account for pseudoreplication.

RESULTS: The frequency of motor unit firing was highest in ALS gastrocnemius muscles (368 potentials per minute [ALS-GASTROC] vs 302 [ALS-BICEPS; p=0.004] vs 281 [BFS-GASTROC; p=0.043] vs 186 [BFS-BICEPS; p<0.0001]). ALS biceps muscles demonstrated higher motor unit firing frequencies in the weakest muscle group as compared with the strongest muscle group (377 vs 278 potentials per minute; p=0.012). Biceps motor unit median amplitude was greater in ALS patients than in BFS controls (55 vs 31μV; p=0.0257), although this did not alter according to disease stage in ALS patients. Riluzole status had no impact on the motor unit firing frequency or amplitude across both muscles.

CONCLUSIONS: This study demonstrated notable differences in motor unit firing behaviour according to muscle type and disease status. These changes could be attributed to differential composition of the two muscles tested, as well as compensatory mechanisms driven by progressive motor unit loss in ALS. This provides further validation of SPiQE and offers pathophysiological insight that could be translated into an accessible clinical biomarker, particularly in the context of future home-based assessments.

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Pallidal functional connectivity changes are associated with disgust recognition in pure motor amyotrophic lateral sclerosis

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Introduction. An altered ability to correctly recognize emotions, particularly disgust, has been reported in patients with amyotrophic lateral sclerosis (ALS). MRI studies in healthy and pathological conditions reported a crucial role of pallidum, insula/operculum and frontal regions in the facial recognition of disgust. In pure-motor ALS patients, we recently observed that smaller volume of the left pallidum was related with impaired recognition of disgust.

Objectives. To investigate the resting-state functional connectivity (RS-FC) of the pallidum in ALS compared to healthy controls, and the relationship between RS-FC changes and disgust recognition in patients.

Method. 19 ALS patients without cognitive/behavioral deficits and 52 age-, sex- and education-matched healthy controls underwent RS functional MRI and a neuropsychological assessment including the Comprehensive Affect Testing System (CATS), which investigates emotion recognition. In all subjects, a seed-based RS-FC analysis was run between the left and right pallidum and the rest of the brain, and was compared between groups. Finally, correlation analyses were run between the RS-FC significant changes and patients' performance in recognizing disgust.

Results. Compared to controls, patients were significantly less able to recognize disgust. In ALS compared to controls, the seed-based analysis showed reduced RS-FC between bilateral pallidum and right superior and middle frontal gyri, and increased RS-FC between bilateral pallidum and left superior temporal and postcentral gyri, and left Rolandic operculum. Furthermore, increased RS-FC was observed between left pallidum and left supramarginal gyrus and between right pallidum and contralateral insula and thalamus. In patients, lower performance in recognizing disgust was related with reduced RS-FC between bilateral pallidum and right middle and superior frontal gyri, and with increased RS-FC between bilateral pallidum and left postcentral gyrus and Rolandic operculum.

Conclusions. In a sample of cognitively unimpaired ALS patients, we demonstrated altered RS-FC between pallidum and the rest of the brain. Specifically, the reduced pallidum-frontal RS-FC and the increased pallidum-insular-thalamic RS-FC may suggest a fronto-striatal functional disconnection in ALS patients, which could have a role in the lower ability of patients in recognizing disgust. These findings offer new potential markers for monitoring extra-motor progression in ALS.

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Patient experiences with discussing personalized prognosis of survival in ALS

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Introduction

The ENCALS survival prediction model offers patients with ALS the opportunity to receive a personalized prognosis of survival at diagnosis.(1) We developed a communication guide to support physicians in tailoring prognostic discussion to the individual needs and preferences of patients with ALS.(2)

Objectives

To explore experiences of patients and their caregivers with discussing personalized prognosis in ALS. Method We are conducting a qualitative study using semi-structured interviews. Recently diagnosed patients with ALS, referred to one of three ALS care teams in the Netherlands and with whom the personalized prognosis was discussed, were invited to participate in this study. Interviews were conducted by a researcher not involved in the care of participants (RvE), and transcribed and analysed thematically (RvE, LK).

Results

Preliminary results of eight patients and four caregivers were available. The prognosis of included patients was shorter than average (n = 3), average (n = 1), or longer than average (n = 5). Three important themes with six subthemes emerged from the interviews. 1) Physician. Communication style: how the physician discussed the prognosis was crucial for the impact on the patient. 2) Patient. Previous disease experience (ALS and other) and personality and coping style affected how patients and their caregivers coped with the prognosis. 3) Effect on the patient. The emotional impact on patients ranged from happy and reassuring to regret, they found it helpful looking towards the future, and they reflected on the importance of quality over quantity of time left.

Conclusions

Personalized prognosis can safely be discussed with patients with ALS who want to know their life expectancy. Overall, patients reported positive and reassuring effects on their mental wellbeing and indicated that it helped them and their families to plan for the future. Patients underscored that the how the message was communicated was as important as or even more important than what was communicated (i.e. the prognosis). Additionally, many patients underscored that, in the time left to them, quality mattered more than quantity.

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Patient-centric measures to enhance participation in ALS research: Lessons from the multinational Phase 3 ORARIALS-01 study during the COVID-19 pandemic

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Introduction: The ORARIALS-01 study is an 18-month, randomized, double-blind, placebo-controlled trial of arimoclomol in patients with early ALS (NCT03491462). The study was prospectively designed in 2017/18 with multiple patient-centric elements to decrease the burden of participation, thus reducing non-adherence and drop-outs. A total of 245 participants were enrolled between July 2018 and July 2019 at 30 centers across Europe and North America. In early 2020, the global COVID-19 pandemic forced immediate measures to safeguard individuals at high risk of severe disease, thus impacting many aspects of trial conduct.
Objective: We report on the patient-centric aspects of the ORARIALS-01 study design that mitigated the impact of COVID-19 on participants, caregivers, and trial staff and, with minimal adaptation, allowed the study to overcome numerous practical challenges.
Methods: Prospective patient-centric elements in the study design included flexible in-person, telephone, or in-home visits; electronic expense reimbursement; portable spirometry; and fewer clinic visits over time. Following a risk assessment in response to COVID-19, the trial protocol was amended to further increase patient-centric elements, including expansion of home nursing and direct-to-patient services, remote clinical monitoring, and remote informed consent. Visit windows for central laboratory testing were increased and local laboratory use was instigated. The case report form was updated to capture COVID-19-related missed visits. Missed protocol assessments due to COVID-19 were permitted for exploratory endpoints while preserving assessment of safety and the primary endpoint (Combined Assessment of Function and Survival at Month 18).
Results: The risk of COVID-19 on study conduct was explicitly recognized in early February 2020. A COVID-19 clinical trial protocol addendum was issued on March 24, 2020, and implemented through national expedited regulatory routes where possible. To date, COVID-19 infection has been reported for two participants; one case required hospitalization, and both participants recovered and continued in the study. No participant discontinued from the study citing COVID-19 concerns. There have been no reports of confirmed COVID-19 infection in trial staff. The study is expected to complete in the first half of 2021, consistent with the original timeline.
Conclusions: The ORARIALS-01 study was prospectively designed to prioritize participants' trial experience and maximize retention. An additional benefit was that the study, with minimal adaptations to the design and operation, was able to preserve participant and trial staff welfare during the COVID-19 pandemic while maintaining scientific validity of safety and key efficacy endpoints. Many of these adaptations are applicable beyond the COVID19 era to further enhance ALS trial participation.

Perivascular fibroblasts activity precedes the onset of ALS neurodegeneration with high plasma SPP1 associated with short patient survival.

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Introduction:
For decades, ALS has been defined, diagnosed and evaluated based almost exclusively on symptoms of rapid, age-dependent degeneration of motor neurons. Apart from the well-defined neuron-centric factors, few reports consider that variability of sporadic ALS onset and progression can depend on the less-defined contributions from non-neuronal cell types including glia and blood vessels.

Objectives:
Unless we better understand how non-neuronal cells contribute to ALS aetiology, inaccurate survival prognosis will continue to confound clinical trial design and effective treatments will likely remain elusive.

Method:
Using single-cell-guided deconvolution of ALS transcriptomes, tissue histology and proteomic profiling of blood plasma, we aimed to investigate the role of non-neuronal cells in sporadic ALS patients and asymptomatic mouse models.

Results:
We found that perivascular fibroblast cell gene activity during presymptomatic disease stage remodels blood vessel matrix and provides distinct plasma protein biomarker that can independently predict short ALS patient survival at diagnosis. We inferred cell activity in ALS spinal cord transcriptomes using single-cell guided profiling. We determined that sporadic ALS patients present cellular changes consistent with the SOD1-G93A and TARDBP-Q331K mouse models in which gene expression patterns from vascular cells precede the microglial response and neuronal loss. Notably, perivascular fibroblast cells elicited the strongest pre-onset gene enrichments and their marker proteins SPP1 and COL6A1 accumulated in enlarged perivascular spaces in sporadic ALS patients. Moreover, in 574 ALS patients from four countries, increased plasma levels of SPP1 at disease diagnosis repeatedly predicted shorter survival with a stronger effect than established indications of bulbar onset or neurofilament levels in cerebrospinal fluid.

Conclusions:
We propose that the activity of the recently-discovered perivascular fibroblast cells can predict ALS patient survival and provide a novel conceptual framework to re-evaluate definitions of ALS aetiology. Since enlarged perivascular spaces consistently occur in both ageing and in numerous forms of neurodegeneration, perivascular fibroblast activity could represent a common underlying mechanism of cerebral injury response and become a novel therapeutic target.

Physical exercise is a risk factor for amyotrophic lateral sclerosis: Convergent evidence from mendelian randomisation, transcriptomics and risk genotypes.

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Introduction:
Amyotrophic lateral sclerosis (ALS) is determined by gene-environment interactions and improved understanding of these interactions may lead to effective personalised medicine. Physical exercise has been suggested as a potential modifiable risk factor for ALS but this remains controversial.
Objectives:
First, we sought to explore whether there is a causal relationship between exercise and ALS. Secondly, we aimed to identify genotypes that may place individuals at increased with of ALS within the context of exercise.
Method:
We dissected the exercise-ALS relationship through two-sample Mendelian randomisation (MR) experiments. Our primary MR analysis utilized genome wide association studies of strenuous sport and other exercise participation (124,842 cases and 225,650 controls) and the Project MinE ALS cohort (12,577 cases and 23,475 controls). Next, we tested for enrichment of ALS genetic risk within exercise-associated transcriptome changes. Finally, we applied a validated physical activity questionnaire in a small cohort of genetically selected ALS patients (17 C9ORF72-ALS patients, 34 non-C9ORF72-ALS patients and 34 neurologically normal controls) .
Results:
We present MR evidence supporting a causal relationship between genetic liability to frequent and strenuous leisure-time exercise and ALS (multiplicative random effects IVW, p=0.01). Transcriptomic analysis revealed that genes with altered expression in response to acute exercise are enriched with known ALS risk genes (permutation test, p=0.01) including C9ORF72, and with ALS-associated rare variants of uncertain significance. Questionnaire evidence revealed that age of onset is inversely proportional to historical physical activity for C9ORF72-ALS (Cox proportional hazards model, Wald test p=0.007, likelihood ratio test p=0.01, concordance=74%) but not for non-C9ORF72-ALS. Moreover, compared to non-C9ORF72-ALS patients and neurologically normal controls, C9ORF72-ALS cases reported the highest minimum average physical activity (20.9kJ/kg/day) consistent with an exercise threshold for penetrance.
Conclusions:
Our MR approach suggests a positive causal relationship between ALS and physical exercise. Exercise is likely to cause motor neuron injury only in patients with a risk-genotype. Consistent with this, we have shown that ALS risk genes are activated in response to exercise. In particular, we propose that G4C2-repeat expansion of C9ORF72 predisposes to exercise-induced ALS.

Positive Effect of Plasma Exchange with Albumin Replacement on Predicted versus Observed Survival and disease progression in ALS

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INTRODUCTION
Plasma exchange (PE) is an extracorporeal blood purification process that removes substances from the blood (along with potentially harmful metabolites) and, in this study, the fluid volume was replaced with albumin (PE-A). The objective of this post-hoc analysis was to assess the effect of PE-A on disease progression and observed survival versus predicted survival using the European Network for the Cure of ALS (ENCALS) model in patients with ALS.

METHODS
This was an open-label, non-controlled, single-arm, single-center, pilot study. Eligible ALS patients were 18-70 years old, with forced vital capacity (FVC)>70% of predicted value. Enrolled patients (n=13) received 6 months of PE-A (5% albumin) in two phases: a 3-week intensive phase with two PE-A per week followed by a 21-week maintenance phase with one weekly PE-A, and a 6-month follow-up. Observed survival was compared with survival predicted by the ENCALS model, which provides an estimated survival curve and categorizes patients based projected disease progression (very short, short, intermediate, long, very long survival). Three categories of disease progression based on the ALSFRS-R slope were defined: "Normal" from –0.8 to –1.33 points/month; "slow" if < –0.8 points/month; "fast" if > –1.33 points/month.

RESULTS
The age at onset for evaluable patients (n= 11) ranged 32.6-64.7 years. Progression rates range was 0.25-1.71 points/months. ENCALS predicted survival ranged from short (n=2) and intermediate (n=6) to long (n=1) and very long (n=2). For five of these patients (45% of the sample), observed survival was increased over predicted survival. For another five patients, observed survival was in the range predicted by the model. Only one patient had an observed survival less than predicted. ALSFRS-R scores declined throughout the study, although the median decline was less than expected in pre-treated patients. Seven patients had a slower decline than expected at the end of treatment and five patients had a slower decline at the end of study. Six patients remained in the same baseline slope progression category while four improved their slope category at the end of treatment.

CONCLUSION
These data may suggest that the PE-A treatment had a positive effect on survival in some patients from this pilot study. Most patients showed a slower than expected rate of decline at the end of treatment. Additional studies are needed to confirm this observation.

Preliminary evidence of neuroelectrical changes in asymptomatic C9ORF72 gene carriers using EEG

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Background:
Previous studies of asymptomatic carriers of C9ORF72 gene have reported the occurrence of cognitive and behavioural changes, as well as dysfunctional brain metabolism and structure degeneration [1]. However, the neurophysiological changes prior to the clinical manifestation of ALS are not adequately understood. Early detection and characterisation of disease manifestations in asymptomatic C9ORF72 carriers can underpin new therapeutic strategies that are based on early, targeted treatments.

Objectives:
To evaluate changes in the cognitive and motor control networks in C9ORF72 asymptomatic gene carriers (AGC) using electroencephalography (EEG).

Methods:
EEG (128 channels) was recorded during a randomised sustained attention to response task (SART) in 4 AGC and 8 controls. Participants were instructed to respond every time they saw a number (1-9, Go) except for the number 3 (NoGo). Source-localisation method was used to evaluate the differences between the two cohorts in the motor N2 (200-350ms post-stimulus) and the attentional P3 (350-500ms) event-related potential peaks, which are known to be impaired in ALS [2]. For both groups, a difference between NoGo and Go (NoGo-Go) activations during each peak were estimated. Mann-Whitney U test was used for statistical comparison.

Results:
Both cohorts showed increased P3 and N2 NoGo-Go activation in the frontal and frontocentral brain regions, respectively. The P3 activation was significantly lower in AGC compared to controls in the right superior and middle frontal gyri ($p < 0.01$). Similarly, N2 activation was lower and showed the most discriminatory power in the central regions bilaterally, albeit not statistically significant ($p = 0.1$).

Conclusions:
These preliminary results show the potential of EEG to capture functional changes associated with the disease in asymptomatic carriers of C9ORF72. The identification and characterisation of biomarkers that can be linked to the early development of ALS, can in turn help in the early diagnosis and enhance the understanding of causal physiological processes. This approach is important for early treatment strategies and to make ALS a potentially preventable disease.

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Pridopidine for the Treatment of Amyotrophic Lateral Sclerosis

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Introduction:
Pridopidine is a selective and potent Sigma-1 Receptor (S1R) agonist in clinical development for ALS and Huntington Disease (HD). The S1R is located at the interface of the endoplasmic reticulum (ER) and mitochondria, where it regulates key cellular processes relevant to neurodegenerative diseases that are impaired in ALS, including Ca2+ signaling, autophagy, mitochondrial function, and neurotrophic factor secretion.
The S1R is of particular interest in ALS. Complete loss of function (LOF) mutations cause juvenile ALS while partial LOF mutations cause late onset ALS. Thus, there is a gene dosage relationship between S1R activity and age of onset of ALS. S1R activation is neuroprotective, enhancing neuronal survival and function in preclinical models of multiple neurodegenerative diseases including ALS.
Pridopidine was chosen as one of the first four drug candidates to participate in the ALS Platform trial led by the Healey Center at the Massachusetts General Hospital. The first patient was randomized to the pridopidine regimen in January 2021.
Prior trials in HD patients show pridopidine maintains, or slows the decline in functional capacity at 52 weeks. Extensive long-term safety data show that pridopidine is safe and tolerable.
Objective:
To evaluate the neuroprotective effects of pridopidine in ALS models.
Method:
The effects of pridopidine on neuromuscular junction (NMJ) function, axonal transport and motor neuron (MN) viability were evaluated in primary MNs and muscle cultures from SOD1G93A mice. Pridopidine's effect on spinal cord aggregates of mutant SOD1 (mSOD1), muscle fiber wasting and NMJ structure was evaluated in SOD1 G93A mice treated daily, for 11 weeks with pridopidine (30mg/kg).
Results:
In vitro, SOD1G93A motor neurons show a significant reduction in BDNF axonal transport velocity ($p<0.001$), reduced innervation and myocyte contraction ($p<0.01$), and reduced MN survival ($p<0.01$). Pridopidine significantly enhances BDNF axonal transport (by 25% and 35%, $p<0.001$, at 0.1 and 1µM, respectively), restores NMJ dysfunction (by 50% at 0.1 µM, $p<0.001$ and 40% at 1 µM, $p<0.01$) and promotes neuronal survival via ERK pathway activation (2.9- fold at the 0.1 µM, $p<0.05$). In vivo, pridopidine reduces spinal cord aggregation of mSOD1 by ~50% ($p<0.05$), increases muscle fiber diameter by 4µm ($p<0.05$), and rescues NMJ loss ($p<0.05$). Pridopidine action is S1R-dependent, as its deletion inhibits the beneficial effects of pridopidine.
Conclusions:
Pridopidine demonstrates S1R-mediated neuroprotective effects in the SOD1G93A mouse model. It is a well-tolerated oral drug, which demonstrated a beneficial effect maintaining functional capacity in prior trials in HD patients. The therapeutic potential of pridopidine in ALS patients is currently evaluated in the Healey platform trial for ALS.

Proteins binding antisense RNA transcripts from C9orf72 gene mutation

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Introduction: The expanded repeat C9orf72 RNA transcripts are proposed to have toxic effect on cells through sequestration of RNA binding proteins, which disrupts their normal cellular functions. So far, extensive studies have been done on sense RNA transcripts and their interacting proteins, whereas antisense RNA interactions remain less investigated.

Objectives: Our aim was to determine the proteins interacting with long, disease relevant antisense RNA transcripts from the C9orf72 gene mutation and investigate their role in C9orf72 mutation-positive patient-derived cells.

Methods: We performed RNA pull-down assay on mouse brain lysates using 32xC4G2 RNA constructs and detected interacting proteins with mass spectrometry. We confirmed the interactions in RNA pull-down from human post-mortem brain tissue lysates. The co-localization of chosen proteins and antisense RNA in C9orf72 mutation-positive fibroblasts, lymphoblasts and iPSCs was detected with RNA fluorescence in situ hybridization (FISH) in combination with immunocytochemistry (ICC) and RNA-protein proximity ligation assay (PLA). We further investigated the function of the interacting protein Phe-tRNA synthetase in C9orf72 mutation-positive lymphoblasts with tRNA aminoacylation assay.

Results: We identified several strong protein interactors of C9orf72 antisense RNA and confirmed their interaction with RNA pull-down on human post-mortem brain tissue. The identified proteins play role in various cellular mechanisms such as protein synthesis, cytoskeletal stability and transport, RNA processing, myelinating cell functions, ribosome biogenesis, and protein quality control. Our research focused on cytosolic interacting protein - phenylalanine tRNA synthetase (FARS), which co-localized with antisense RNA in C9orf72 mutation-positive cells as shown by RNA-FISH/ICC. We also detected increased RNA-protein PLA signal in C9orf72 mutation-positive fibroblasts, lymphoblasts and iPSCs for FARSA-antisense RNA interaction. Moreover, we observed a significant increase in levels of uncharged Phe-tRNA in C9orf72 mutation-positive lymphoblasts relative to control with tRNA aminoacylation assay.

Conclusions: In this research, we determined novel proteins interacting with antisense RNA repeats. Furthermore, we showed co-localization of chosen proteins with antisense RNA by RNA-protein PLA, which enabled us to study cytoplasmic interactions for the first time. We revealed interaction of antisense RNA with cytoplasmic protein FARS, which is an important contribution to the field as aminoacyl tRNA synthetases are being increasingly mentioned in various impairments of the nervous system. Moreover, we found significant decrease in charging levels of Phe-tRNA in C9orf72 mutation-positive lymphoblasts, which indicates possible disruption in function of FARS protein in C9orf72 mutation.

Proteomic analysis of mutant FUS aggregates reveals factors and cellular pathways dysregulated in ALS-FUS

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Introduction. Formation of cytoplasmic RNA-protein structures called stress granules (SGs) is a highly conserved cellular response to stress. Abnormal metabolism of SGs may contribute to the pathogenesis of (neuro)degenerative diseases such as amyotrophic lateral sclerosis (ALS). Many SG proteins are affected by mutations causative of these conditions, including fused in sarcoma (FUS). Mutant FUS variants have high affinity to SGs and also spontaneously form de novo cytoplasmic RNA granules. Mutant FUS-containing assemblies (mFAs), often called "pathological SGs", are proposed to play a role in ALS-FUS pathogenesis. However, global structural differences between mFAs and physiological SGs remain largely unknown, therefore it is unclear whether and how mFAs may affect cellular stress responses.
Objectives. This study aimed to establish the differences and similarities between physiological SGs and pathological mutant FUS-containing assemblies.
Method. We used affinity purification to characterise the protein composition of normal SGs and mFAs purified from stressed cells, followed by validation studies in cultured cells, transgenic mouse tissue and transgenic flies.
Results. Comparison of the SG and mFA proteomes revealed that proteasome subunits and certain nucleocytoplasmic transport factors are depleted from mFAs, whereas translation elongation, mRNA surveillance and splicing factors as well as mitochondrial proteins are enriched in mFAs, as compared to SGs. Validation experiments for a hit from our analysis, a splicing factor hnRNPA3, confirmed its RNA-dependent sequestration into mFAs in cells and into pathological FUS inclusions in a FUS transgenic mouse model. Furthermore, silencing of the Drosophila hnRNPA3 ortholog dramatically enhanced FUS toxicity in transgenic flies.
Conclusions. Our study establishes molecular differences between physiological SGs and mFAs and identifies the spectrum of proteins and respective cellular pathways affected by mFAs in stressed cells. In particular, we demonstrate, in vitro and in vivo, that abnormal assemblies made of mutant FUS may cause loss of function for hnRNPA3 protein previously not implicated in ALS-FUS pathogenesis. In conclusion, we show that mFAs are compositionally distinct from SGs and that they cannot fully substitute for SG functions while gaining novel, potentially toxic functions in cellular stress response. Results of our study support a pathogenic role for stress-induced cytoplasmic FUS assemblies in ALS-FUS.

QRA-244 a Potent, Selective KCNQ2/3 Opener and a Potential Therapy for Motor System Hyperexcitability induced Disease Progression in ALS patients

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Recent studies have demonstrated that approximately half of ALS patients show hyperexcitability in the motor cortex and spinal motor axons, a phenotype that is linked to poor survival. ALS patients with motor system hyperexcitability can be targeted using neurophysiological biomarkers. In patient iPSC derived motor neurons this hyperexcitability leads to neurodegeneration and was traced to reduced Kv7.2/7.3 activity. This motor neuron degeneration was rescued by the Kv7.2/7.3 agonist Retigabine which also reversed hyperexcitability in a clinical trial of ALS patients. The trial demonstrated a statistically significant beneficial effect on several markers of excitability including short interval cortical inhibition (SICI) and strength duration time constant (SDTC), two biomarkers linked to patient survival. Despite these beneficial effects, retigabine was associated with significant adverse events consistent with its prior clinical use in epileptic patients which strongly limits its use as a therapeutic. We have been working to discover, characterize, and develop a novel KCNQ2/3 activator with an improved channel specificity, which is expected to translate into a better clinical safety profile with comparable or better efficacy. Here we show that QRA-244 activates KCNQ2/3 channels selectively in the KCNQ family, increases rheobase and decreases SDTC in rats. In side by side experiments with Retigabine we demonstrate a significantly improved safety profile in rat models of dizziness (rotarod) and fatigue (REM/NREM sleep). Unlike Retigabine, QRA-244 has no effect on human bladder strips at clinically relevant concentrations. Overall, QurAlis is developing QRA-244, a more potent and selective Kv7.2/7.3 activator aimed at normalizing excitability of the ALS motor system, with a significant reduction in off-target driven adverse events. We believe that this compound offers a promising therapeutic approach to counteract disease progression induced by hyperexcitability in ALS patients.

Randomized clinical trials in amyotrophic lateral sclerosis: the effect of disease duration and forced vital capacity cut-offs at trial entry.

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Introduction

The low success rate of clinical trials in Amyotrophic Lateral Sclerosis (ALS) could be related to the wide heterogeneity of both eligibility criteria used for recruitment and phenotypic characteristics of the disease. In addition, lack of stratification of patients into well-defined prognostic categories could hamper the identification of effective experimental drugs.

Objectives

To perform a randomized clinical trial (RCT) recruitment simulation to estimate patients' mortality rates and survival, based on different values of forced vital capacity (FVC) and disease duration at trial entry, in order to optimise the design of future RCTs.

Method

For each patient, a single spirometry was randomly selected among the ones performed during four time intervals from disease onset: 1) ≤12 months; 2) ≤18 months; 3) ≤24 months; 4) ≤36 months. Date of spirometry corresponded to date of recruitment, while onset-spirometry time interval to disease duration at enrolment. In this way we created four distinct groups of patients with different FVC values and disease duration at trial entry. Mortality rates from inclusion were computed at different time intervals, to simulate RCTs of different durations, depending on FVC values and disease duration at trial entry. Patients were then classified as slow (SPs), intermediate (IPs) and fast progressors (FPs), according to disease progression rates. Finally, survival from recruitment was calculated based on FVC, disease duration and disease progression rate at trial entry.

Results

We included 659 patients in group 1, 888 in group 2, 1019 in group 3 and 1102 in group 4. In each group, mortality rates increased when lowering the FVC% cut-off used for enrolment ($p<0.001$). Median survival decreased when lowering FVC% and disease duration cut-offs ($p<0.001$); a higher median disease progression rate of enrolled patients was related to a lower median survival from recruitment. Shortening disease duration and lowering FVC cut-off led to a greater proportion of recruited FPs.

Conclusions

We propose a straightforward model to establish trials' eligibility criteria, to tailor the population of recruited patients to RCTs primary endpoints and duration, reducing the risk of underestimating beneficial effects of drugs, limiting dropouts, and eventually allowing less restrictive inclusion criteria.

Regressive muscular changes associated with motoneuron deafferentation and gliosis occur in the spinal cord of C57BL/6J aged mice

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Aging is associated with sarcopenia, a decline in skeletal muscle mass, strength and function. The causative factors of aging sarcopenia are controversial and poorly understood, hampering the development of effective therapeutic interventions.

A detailed characterization of age-associated pathophysiological changes occurring simultaneously in distinct components of the neuromuscular system was performed. Motoneurons (MNs), glia, motor nerves, neuromuscular junctions (NMJs) and different types of skeletal muscles were analyzed in young, adult, middle-aged and old C57BL/6J mice.

Aging was not accompanied by a significant loss of spinal MNs, although a proportion of them in old mice exhibited an abnormally dark appearance. Morphological alterations in motor axons were already observed in adulthood but substantially increased with age. Old MNs were depleted of cholinergic and glutamatergic inputs. Prominent gliosis was found in old spinal cords, with increased density of pro-inflammatory microglial and astroglial phenotypes. Aging resulted in significant reductions in the nerve conduction velocity and the compound muscle action potential amplitude in old distal plantar muscles. Compared with adult muscles, old muscles exhibited significantly higher numbers of both denervated and polyinnervated NMJs, changes in fiber type composition, higher proportion of fibers showing central nuclei and lipofuscin aggregates, depletion of satellite cells, and augmented expression of different molecules related to development, plasticity, and maintenance of NMJs. Overall, these alterations occurred at varying degrees in all the muscles analyzed, with no correlation between the age-related changes observed and myofiber type composition or muscle topography.

Our data provide a global view of age-associated changes in the neuromuscular system of mice, some of them previously envisaged as controversial when different models and partial aspects of the aging process were assessed. Our results suggest that during aging, some MNs undergo early deleterious changes, which may not lead to MN death. Age-related MN dysfunction could be responsible for structural and molecular alterations in motor axons, NMJs, and skeletal muscles found in senescence.

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Remote monitoring of anxiety, depression, and caregiver strain in MND services: early learning from clinical implementation of the Telehealth in MND system

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INTRODUCTION:
People with motor neuron disease (pwMND) have a high level of medical need, with assessments and interventions focused on aiding mobility and communication, maintaining nutritional and respiratory needs, and reducing discomfort. Given the speed of change, it is difficult for healthcare professionals to keep up between appointments, and this is why the Telehealth in MND (TiM) remote monitoring was introduced.

It is also difficult for pwMND and their carers to cope with the relentless nature of MND and the losses they experience. However, psychological input into MND services is rare, and depression, anxiety, and carer strain can easily be missed in brief medical appointments. A recent survey by the MND Association found that only 38% of MND carers feel their needs are even considered by healthcare professionals.

To improve identification of distress and support needs, measures of depression, anxiety, and carer strain (for carers) were added to the TiM system.

OBJECTIVES:
TiM's primary use is as a clinical intervention to improve communication and care. However, learning from early implementation about the acceptability, feasibility, and utility of remotely monitoring psychological factors will inform ongoing clinical use and more widespread implementation.

METHOD:
At this stage, our results are based on clinical observations and informal feedback. However, we will be using quantitative methods to calculate completion rates, rates of depression, anxiety, and carer strain. Remote semi-structured interviews will be carried out to explore the experiences of users and to identify potential barriers; this will be analysed using thematic analysis.

RESULTS:
Further results will be presented at the meeting, but clinical observations and informal feedback suggest that pwMND and their carers are willing and able to complete psychological measures remotely, and this has enabled the team to identify needs and respond. Early results were often discussed with the Clinical Psychologist in the team, but the Specialist Nurses provided the follow-up care, and a telephone call was often enough to help the person feel better supported.

However, the specific measures used are not acceptable to everyone. For example, a question assessing suicidal ideation on the depression measure was removed after feedback from pwMND and carers. This question also generated concern within the clinical team, as it did not allow differentiation between active risk and more passive thoughts of death as an end to suffering.

CONCLUSIONS:
Remote monitoring of psychological distress is feasible and has enabled identification of psychological needs that would otherwise likely be missed. The Specialist Nurses have been able to respond to these

needs with support from the multidisciplinary team. Further work is required to evaluate and optimise the acceptability and utility of the measures used.

Reprogrammed astrocytes from symptomatic and pre-symptomatic individuals display differential C9orf72 pathology and motor neuron toxicity in co-culture

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Introduction 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 pre-symptomatic and symptomatic disease state, which emerges with age, are poorly understood. Recently published work from our group shows that induced neural progenitor cells (iNPCs) and their astrocyte derivatives (iNPC-As) retain the ageing features of the donor fibroblasts (Gatto et al., 2021). Reprogrammed human astrocytes contribute to motor neuron injury in in vitro co-culture systems (Meyer et al., 2014). Here we describe a family whose proband was diagnosed with ALS, discovered to carry a novel mutation in TARDBP and also a C9-HRE. One sibling also developed ALS and a further sibling was unaffected at time of biopsy collection, though both carried the TARDBP mutation and C9-HRE.
Objectives We aimed to generate iNPCs from the fibroblasts of all three individuals and characterise the derived iNPC-As in terms of the presence and quantity of RNA foci and the toxicity towards co-cultured motor neurons.
Methods Fibroblasts were directly converted as previously described (Meyer et al,. 2014). iNPCs were characterised by co-expression of PAX6 and Nestin and iNPC-As by expression of CD44 and Vimentin. C9orf72 RNA foci were assessed using fluorescent in situ hybridisation with RNA probes specific to the antisense and sense transcripts. Toxicity was determined by co-culture with murine HB9-GFP MN and counting of surviving motor neurons after 48 hours.
Results We first determined that all three siblings possess the C9-HRE and TARDBP A321V mutation. The iNPC-As from the two symptomatic iNPCs produced antisense foci in 1.2 and 2.5% of cells, and sense foci in 5.5 and 3.6% of cells, respectively. The iNPC-As from the pre-symptomatic individual produced significantly less than the symptomatic astrocytes (antisense 0.6% $P = 0.3654$ and $P = 0.0354$; sense 0.8% $P = <0.0001$ and $P = 0.0006$, respectively). The two symptomatic patient iNPC-As lines elicited toxicity when co-cultured with motor neurons, with significantly lower survival when compared to healthy controls ($P = 0.0058$ and $P = 0.0158$, respectively). The pre-symptomatic iNPC-As demonstrated survival of co-cultured motor neurons comparable to the levels seen in healthy controls (ns).
Conclusions Using direct conversion, we have generated in vitro astrocytes from a pre-symptomatic individual carrying two ALS genetic risk factors but showing markedly reduced pathology when compared to clinically affected siblings with the same pathogenic genetic changes. Further work is needed to accurately measure the C9 repeat size and additional pathological features including TDP-43 proteinopathy. This human co-culture model with retained features of ageing will help to provide new insights into the switch between pre-symptomatic and disease states in familial ALS.

Results of the Phase 3, Randomised, Placebo-Controlled Trial of oral Arimoclomol in Amyotrophic Lateral Sclerosis (ORARIALS-01)

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Introduction: Arimoclomol is an amplifier of the heat shock response under conditions of cellular stress. Heat shock response promotes clearance of intracellular protein aggregates, natural folding of nascent proteins, and refolding of misfolded proteins, reconstituting normal protein function - actions that are expected to have a disease modifying effect. A previous phase 2 trial in SOD1 ALS has shown encouraging results

Objective:

To determine the efficacy of chronic treatment with 1200 mg/day arimoclomol (400 mg t.i.d.) compared to placebo over 76 weeks in subjects with ALS as assessed by the Combined Assessment of Function and Survival (CAFS) and to evaluate PAV/tracheostomy free survival, disease progression as measured by change from baseline of the ALSFRS-R, progression of respiratory dysfunction as measured by change from baseline of SVC, and to assess the safety and tolerability of arimoclomol.

Methods:

ORARIALS-01 was an 18-month double-blinded, randomized 2:1, placebo-controlled phase 3 trial that enrolled 245 adult ALS patients. The trial was performed from July 2018 to December 2020. Eligible patients were aged ≥18 years who met the revised El Escorial criteria for clinically possible, clinically probable, clinically probable laboratory-supported or clinically definite ALS, or had familial ALS caused by a known pathogenic mutation. Patients were early in their disease course with less than 18 months since first appearance of weakness, had no more than mild to moderately impaired function as determined by a baseline ALSFRS-R of ≥35, and relatively preserved lung function with SVC ≥70% of predicted normal. The primary endpoint was the measurement of the CAFS in the arimoclomol treatment arm as compared to placebo after 18 months. Secondary endpoints included PAV/tracheostomy-free survival, change in ALSFRS-R and SVC.

Participants were evaluated in clinic every 8 weeks for endpoints, safety measures, quality of life and biomarkers for the first 52 weeks and then every 12 weeks. To reduce the drop-out rate, patients were assessed in their home if disease progression prevented their ability to attend the trial site. This provision also turned out to be valuable during the COVID-19 pandemic,

Results:

The results of the ORARIALS-01 phase 3 trial will be presented.

Revisiting the diagnosis of an ALS patient after gender reassignment– a case report.

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Introduction
Correctly diagnosing amyotrophic lateral sclerosis (ALS) is challenging due to the heterogeneous presentation of symptoms and to the presence of mimics such as multifocal motor neuropathy, primary lateral sclerosis and spinobulbar muscular atrophy. Up to 10% of patients are wrongly diagnosed with ALS; mimics may even fulfill the El Escorial criteria for probable ALS.

Case presentation
We discuss the case of a 60yo female patient who presented in 2007 with a slowly progressive paresis of the left arm and leg. Deep tendon reflexes were preserved bilaterally. Initial electrophysiology showed signs of acute and chronic denervation in 3 of 4 regions (bulbar, cervical and lumbar). Peripheral nerve conduction studies, somatosensory and motor evoked potentials, cranial and spinal MRI were unremarkable. The patient was diagnosed with a probable ALS according to the revised El Escorial criteria. ALSFRS-R score at first presentation was 42/48. There were no signs of respiratory involvement. Riluzole treatment was started.
In the following years, the patient presented a slow decline in motor function of the left arm and leg as well as a progressive trunk paresis, resulting in the need of a wheelchair. Bulbar involvement was never reported. However, symptoms of depression were present and treated with psychotherapy and trimipramine. The ALSFRS-R decreased about 4 points/year in the first 2 years and 1-2 points in the following years. Due to spasticity in the left arm and leg, repeated intramuscular botulinum toxin injections and oral cannabis were needed.
From 2011 on, the ALSFRS-R score remained stable (29-30). In 2013, a PEG tube was installed due to weight loss, but was never used and not replaced when dislocated in 2014.
In 2019, the patient decided to act upon a long-harbored gender dysphoria and started testosterone treatment. During the following months, a gradual improvement of both paralysis and spasticity to the full ambulation was documented. Botulinum toxin, cannabis, and Riluzole were discontinued. The ALS diagnosis was questioned.
In 2020, a new diagnostic work-up showed only a mild left trunk paresis and partially exaggerated deep tendon reflexes. Previously documented atrophies of the left arm and leg muscles were almost fully regressed. EMG was normal. In ongoing psychotherapy, the patient recently reported severe sexual abuse in early childhood.

Discussion
In retrospect, the diagnosis is still not clear. Normal neurographies at symptom onset discourage an inflammatory neuropathy, signs of myopathy were absent. Although the influence of testosterone treatment in motor neuron diseases remains ambiguous, a complete symptom reversion seems unlikely. The authors suspect a dissociative disorder based on early traumatization and gender dysphoria accompanied by depression. To our knowledge, such a severe psychosomatic mimic has not been reported before.

Social Cognition deficits in Amyotrophic Lateral Sclerosis: a cross-sectional population based study

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Objective. to assess Social Cognition (SC) abilities in a cohort of Amyotrophic Lateral Sclerosis (ALS) patients at diagnosis. Secondly, to assess possible differences in SC abilities depending on cognitive, behavioural and motor phenotype.
Background. Facial Emotion Recognition (FER) and Theory of Mind (ToM), has been intensively studied in the last decades in several neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS) and SC deficit has been included in ALS-FTD revised diagnostic criteria. There is, however, remarkable variability in SC impairment profile across different studies related to ALS cognitive and motor phenotypic heterogeneity and variability in Neuropsychological tools employed.
Methods. We included 72 patients attending the ALS Centre of Turin University Hospital between May 2019 and October 2020. All patients underwent neurological evaluation and cognitive evaluation, included SC assessment through Ekman 60 faces test (EK-60), Reading Mind in the Eyes task (RMET-36), Story-Based Empathy task-Emotion Attribution (SET-EA) and Intention Attribution (SET-IA).
Results. Out of 72 patients included, 3 (4%) were diagnosed with ALS-FTD, 7 (10%) ALScbi, 20 (28%) ALSci, 4 (5%) ALSbi and 38 (53%) were cognitively normal (ALS-CN), according to the revised ALS-FTD Consensus Criteria. Overall, 33 (46%) showed deficit in at least one SC test and, among these, 12 (36%) showed no further cognitive impairment or manifest behavioural changes. Multiple linear regression analysis showed weak not significant correlation between EK-60 corrected scores and other Neuropsychological tests scores. Conversely, RMET-36 corrected scores showed a positive significant correlation with Category Fluency test (p 0.03). SET-EA and SET-IA corrected score showed significant correlation with TMT B-A (p 0.04 and 0.003, respectively). Moreover, while EK-60 did not show any significant correlation with motor picture, RMET-36 showed significant correlation with onset site (p 0.004, with bulbar onset patients performing worse than spinal onset ones), and degree of severity of motor symptoms expressed through ALSFRS-S (p 0.009).
Discussion. Our findings reveals that ALS patients otherwise cognitive normal and without manifest behavioural changes can have a FER deficit at early disease stage. Deficits in affective and cognitive ToM are also present and they show significant correlation with executive functions. The presence of SC deficit in some cases elusive to the current cognitive classification system suggests the need of a SC systematic assessment in ALS patients, both to provide a more accurate definition of cognitive profile across ALS-FTD spectrum, and also to help clinicians to elaborate better communication and care strategies taking into account eventual deficits in managing social information in such patients.

Soluble wtSOD1 increase related to HSP70 activation in Peripheral Blood Mononuclear Cells of Sporadic Amyotrophic Lateral Sclerosis patients revealed through RNA-seq profiling

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Introduction. The involvement of wild-type Superoxide Dismutase 1 (wtSOD1) in sporadic cases of Amyotrophic Lateral Sclerosis (sALS) has been investigated and it was demonstrated that depending on SOD1 localization, sALS patients can be divided into two subgroups: those where the protein aggregates in cytoplasm and those where it relocates in nuclear fraction (Cereda et al., 2013). The protein re-localization as aggregates in insoluble fraction generates oxidative stress leading to DNA damage in contrast with the protective role that SOD1 acquires in the nucleus, preventing DNA damage (Bordoni et al., 2019). Moreover, issues in RNA processing have been associated to ALS (Levine et al., 2013) and changes of gene expression have been demonstrated in peripheral tissues (Mougeot et al., 2011; Gagliardi et al., 2018), confirming that RNA metabolism is relevant in ALS disease etiology (Kwiatkowski et al., 2009).
Objectives. We aimed to investigate the effect that higher concentration of soluble SOD1 in the nucleus exerts in Peripheral Blood Mononuclear Cells (PBMCs) of sALS patients by studying pathways activated when the protein localizes in this compartment.
Methods. We investigated pathways activated by nuclear SOD1 (nSOD1) in PBMCs of sALS patients by dividing them depending on the high or low concentration of nSOD1. PBMCs from sporadic ALS patients (n=18) and healthy controls (n=12) were collected to perform RNA-seq experiments and differential expression analysis. We validated the expression of selected genes and its interactors through qPCR and Western Blot analysis. Eventually, we performed a Comet assay to assess the impact of altered gene expression on DNA integrity.
Results. We obtained two different gene expression patterns for high and low nSOD patients. The most up-regulated genes in patients with high nSOD1 belong to HSP70 and HSP110 families and we also observed a greater phosphorylation of their activator HSF1, confirming their transcription is over-activated compared to both healthy controls and patients with low nSOD1. Investigating the possible effect of higher soluble SOD1 and HSP70, we demonstrated that in this group of patients less DNA damage is present even under oxidative stress condition.
Conclusions. HSP70s up-regulation has been associated to mutant SOD1 aggregation suppression and this mechanism may also be true for wtSOD1, leading to the possibility of fine-tuning the effect of soluble SOD1 on DNA integrity maintenance.

Some CSF kynurenine pathway intermediates associated with disease evolution in amyotrophic lateral sclerosis

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INTRODUCTION
Alteration of the kynurenine pathway (KP), highly linked to inflammation, has been reported in many neurological diseases and may play a key role in ALS.
OBJECTIVES : the aim of the study was to evaluate the KP and amino-acids profile in CSF of ALS patients at the diagnosis to early detect a putative disorder of these pathways and to assess their diagnostic or prognostic ability
METHODS
We explored amino acids (AA) and tryptophan metabolism intermediates, including KP using mass spectrometry, in the cerebrospinal fluid (CSF) of 40 ALS patients and 42 controls. Diagnostic and predictive values (based on body mass index, forced vital capacity, ALSFRS over 12 months, survival time) of these molecules were evaluated using univariate and multivariate analysis. Finally a cox analysis was performed from the most relevant metabolites commonly discriminant in each multivariate model evaluating each parameter of disease evolution.
RESULTS
Multivariate model to discriminate ALS and controls was not significant (accuracy 62%) but highlighted some KP metabolites (KYN, KYNA, 3-HK/KYNA) and AA (Lysine, asparagine). These findings revealed a probable KP impairment toward neurotoxicity in ALS patients and in bulbar forms (3-HK/KYN (FC: 1.44, p= 0.13), 3-HK/KYNA (FC: 1.44, p= 0.19) and QUIN/KYN ratios (FC: 1.35, p= 0.19) ratios). Regarding the prognostic effect of metabolites, we observed that the following metabolites were commonly discriminant for at least 3 or 4 disease evolution criteria : QUIN, 3-HK, 5OH-tryptophan, 5-HIAA, glycine, arginine, KYN, asparagine, Indole-3-acetic acid, serotonin, lysine and serine.
CONCLUSION
This early investigation in CSF was crucial as it did not show significant changes in concentrations of AA and KP intermediates in early ALS evolution. However some trends of KP modifications suggest further exploration in this way. The unclear kinetics of neuroinflammation linked to KP support the interest in exploring these pathways during disease evolution through a longitudinal strategy.

Spinal cord MRI for the description of the longitudinal evolution of presymptomatic pathology in c9orf72 mutation carriers: a three time-point study.

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Introduction: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal dementia (FTD) share genetic susceptibility and a large portion of familial cases are due to c9orf72 gene mutations. Brain and spinal cord (SC) imaging studies in asymptomatic c9orf72 carriers have demonstrated white (WM) and grey matter (GM) degeneration up to 20 years before the expected symptom onset1,2.
Objectives: The objective of this study was to longitudinally describe using quantitative MRI the evolution of SC degeneration over 36-months in a cohort of asymptomatic c9orf72 mutation carriers.
Methods: 72 asymptomatic individuals were enrolled in a longitudinal study of first-degree relatives of c9orf72-ALS and FTD patients. 40 (C9+) carried the pathogenic repeat expansion. Each subject underwent a 3T cervical SC MRI. Quantitative measures of GM and WM atrophy and DTI parameters were evaluated at baseline, after 18 and 36 months. Data were analysed on the total population and in two subgroups composed by subjects younger and older than 40 years of age.
Results: No significant difference in GM cross-sectional area was observed at baseline between C9+ and C9- subjects nor any evolution was identified over time. At baseline, significant WM atrophy was detected at each cervical vertebral level in C9+ subjects older than 40 years of age (p-value < 0.05), which was confirmed and remained stable after 18- and 36-months. A significant reduction of fractional anisotropy (FA) in the pyramidal tracts was observed in C9+ subjects older than 40 years. FA reduction was progressive over time with a significant difference between the baseline and the 36-months evaluation (p = 0.02).
Conclusion: Cervical SC imaging of c9orf72 hexanucleotide carriers detect a stable WM atrophy associated with progressive pyramidal tract FA reduction which seems to be continuous but not linear. Longer follow-up and combination with brain imaging will further shed light on the longitudinal degeneration profile of c9orf72 mutation carriers.

Structural hallmarks of Amyotrophic Lateral Sclerosis (ALS) disease aggressiveness in white matter

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Introduction

ALS is a progressive disease, characterized by high variability in phenotypes and disease courses. Numerous neuroimaging studies have reported links between structural changes and clinical data; however, heterogeneity of the disease has occluded robust associations. Biomarkers that reliably reflect disease progression are urgently needed for therapeutic management as well as future trials.

Objectives

The D50 disease progression model provides distinct parameters to quantify the individual disease courses of ALS patients. These were used here to probe associations with neuroimaging measures of structural integrity.

Methods

The value D50 indicates the individual overall disease aggressiveness of a patient (defined as time taken in months to lose 50% of functionality). The calculated functional loss-rate (cFL) describes the local slope of the curve and can be calculated for any given time-point. We conducted voxel based morphometry (VBM) using T1 data from 85 patients with ALS, for grey matter (GM) and white matter (WM) segments. Microstructural analyses were conducted via tract-based-spatial-statistics (TBSS) using diffusion tensor data from a cohort of 145 ALS patients. We thereby analyzed maps of fractional anisotropy (FA), mean (MD), radial (RD), and axial diffusivity (AD).

Results

Using VBM, ALS patients with higher disease aggressiveness (D50 < 30 months) showed widespread supratentorial white matter density decreases relative to patients with lower aggressiveness; no significant differences were observed for gray matter density. An inverse correlation between cFL and WM density in left-hemispheric association tracts was found, including parts of the superior longitudinal, inferior longitudinal and the fronto-occipital fasciculus. TBSS subgroup analyses revealed elevated DTI values in patients with high aggressiveness, with MD changes in the bifrontal, biparietal, and right-temporal lobes and both CSTs, while elevated AD values were mostly restricted to deep frontal/parietal WM. RD contrast clusters were only borderline significant within the callosal body and frontal WM and no significant differences were observed for FA values. Regression analyses confirmed negative correlations between D50 and MD/AD in the bilateral frontal and parietal pathways. Correlations with AD could also be revealed if using patients within Phase I or II separately, however, more specific correlations for Phase I patients could be shown using the cFL. Finally, in a sensitivity analysis, the D50 correlated well in AD/MD contrasts, whilst for the linearly approximated progression rate no significant correlations could be shown with any DTI-measure.

Conclusions

Application of the D50 model enabled to reveal strong correlations between structural WM changes and disease aggressiveness in ALS that propose potential as a readout for future therapeutic trials.

Structural MRI signatures of grey matter atrophy in genetic frontotemporal lobar degeneration

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Introduction. Genetic heterogeneity underlying different clinical presentations of the frontotemporal lobar degeneration (FTLD) spectrum, which includes both frontotemporal dementia (FTD) and motor neuron disease (MND) variants, hampers the identification of useful biomarkers that may be able to monitor disease progression.
Objectives. To assess cortical, subcortical and cerebellar grey matter (GM) atrophy using magnetic resonance imaging (MRI) in patients with disorders of the frontotemporal lobar degeneration (FTLD) spectrum with known genetic mutations.
Method. Sixty-six patients carrying FTLD-related mutations were enrolled, including 44 with pure MND and 22 with FTD. Sixty-one patients with sporadic FTLD (sFTLD) matched for age, sex and disease severity with genetic FTLD (gFTLD) were also included, as well as 52 healthy controls. A whole-brain voxel-based morphometry (VBM) analysis was performed. GM volumes of subcortical and cerebellar structures were obtained.
Results. Compared with controls, GM atrophy on VBM was greater and more diffuse in genetic FTD, followed by sporadic FTD and genetic MND cases, whereas sporadic MND (sMND) patients showed focal motor cortical atrophy. Patients carrying C9ORF72 and GRN mutations showed the most widespread cortical volume loss, in contrast with GM sparing in SOD1 and TARDBP. Globally, gFTLD patients showed greater atrophy of parietal cortices and thalami compared with sFTLD. In volumetric analysis, gFTLD patients showed volume loss compared with sFTLD in the caudate nuclei and thalami, in particular comparing C9-MND with sMND cases. In the cerebellum, gFTLD patients showed greater atrophy of the right lobule VIIb than sFTLD. Thalamic volumes of gFTLD patients with a C9ORF72 mutation showed an inverse correlation with Frontal Behavioral Inventory scores.
Conclusions. Measures of deep GM and cerebellar structural involvement may be useful markers of gFTLD, particularly C9ORF72-related disorders, regardless of the clinical presentation within the FTLD spectrum.
Study funding: Italian Ministry of Health (RF-2011-02351193; GR-2011-02351217; GR-2013-02357415) and the European Research Council (StG-2016_714388_NeuroTRACK).

Study of wt and C-terminally truncated FUS interactions

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Neuronal degeneration has been recognized as a predominant driver of disability and disease progression in central nervous system diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Successful treatments for these disorders have yet to be developed. The aggregation of RNA binding proteins (RBPs) has been recognized as a hallmark pathological feature in these disorders, defining them as proteinopathies. Fused in sarcoma (FUS), normally a nucleus residing RBP, is known to aggregate into physiological granules and pathological inclusions, which can impair cell homeostasis leading to neuronal cell death. Mutations in FUS that alter its C-terminal nuclear localization signal (NLS) are autosomal dominant in ALS and disrupt its nucleo-cytoplasmic shuttling leading to its cytoplasmic mislocalization. Since protein interactors of FUS and the exact signalling pathways involved in cytoplasmic toxicity of FUS remain unknown, we aimed to identify the interactomes of FUS and FUSdNLS (lacking NLS) proteins overexpressed in a model cell line. To reveal crucial and possibly distinct interactors of FUS and FUSdNLS we utilized the method of BioID2 proximity labelling. This technique harnesses the ability of the enzyme biotin ligase (birA) to biotinylate proximal endogenous proteins. We prepared constructs of the FUS and FUSdNLS conjugated to BioID2 enzyme by a flexible linker and transiently expressed them in HEK293T model cells. We cleared the biotinylated proteins from cell lysates and analysed them by mass spectrometry.
Our bioinformatic analyses of proteomic data identified interaction candidates involved in RNA processing and degradation, protein translation and various signal transduction pathways, pointing to differential involvement of wt and truncated FUS in cellular processes. We validated selected interactions by independent pull-down assay and performed cell co-localization analyses in vitro. The interactome differences detected between FUS and FUSdNLS, provide insight into FUS function most likely relevant to neurodegenerative diseases, that could be targeted in therapeutic interventions.

Systematic review of microRNA biomarkers for Motor Neurone Disease

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MicroRNAs (miRNAs) have been shown to have a crucial role in the development of Motor Neurone Disease (MND), however, their role in the diagnosis of patients remains at large, and as such is lacking a standardised approach to its research.

The aim of this systematic review is to analyse current clinical studies that identify miRNA's that have the potential to be diagnostic biomarkers for MND.

The review looked at studies with human extracellular miRNAs within blood plasma, cerebrospinal fluid (CSF), and peripheral blood serum samples, which were analysed using quantitative real-time PCR (qPCR) methodologies.

The review found a lack of reporting within the methodology and results of current literature, thus more research is needed in this area in accordance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE).

The review also identifies miRNAs that have the potential to be effective biomarkers for MND.

TDP-43 and metabolomic biomarkers in circulating cells for

ALS diagnosis and prognosis: a preliminary study.

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Introduction
ALS prognosis is variable between individuals, suggesting distinct pathological mechanisms in different groups of progression.
Objective
To study the potential use of platelet metabolomics for ALS prognosis, also with an examination of TDP-43 dependent phenomena.
Methods
We separated platelets from 15 ALS patients recently
diagnosed and 21 from healthy individuals. Western blot analysis was performed to quantify TDP-43 expression in platelets. We also analyzed the occurrence of TDP-43
controlled cryptic exon levels in selected mRNAs in corresponding peripheral blood mononuclear cells (PBMCs) by RT-qPCR. Mass-spectrometry coupled to liquid
chromatography was employed for metabolomic analyses in platelets.
Results
We demonstrate that cryptic exons, normally present in central nervous tissue of ALS patients, do not appear in platelets or PBMCs of this group. TDP-43 expression was
not altered in platelet fraction from ALS patients. Regarding metabolomic analyses, the concentration of several phospholipids differentiated platelets from ALS patients.
Greater differences were present when examining prognosis, with several metabolites differentiating ALS patients with variate prognosis, based on ALSFR-R changes.
Conclusions
All inall, these results support the potential existence of metabolomic prognostic biomarkers in platelets.

TDP-43 condensation properties specify its RNA-binding and regulatory repertoire

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Mutations causing amyotrophic lateral sclerosis (ALS) can affect the condensation properties of RNA-binding proteins (RBPs). However, it remains unclear how RBP condensation and RNA-binding properties are linked. We created mutants of the C-terminal domain of TDP-43 with a gradient from low to high condensation propensities, and found the same gradient also in nuclear mobility and foci formation, transcriptome-wide binding and regulation of RNA processing. Notably, binding to only a subset of RNA sites is sensitive to perturbed condensation, which depends on the length of the multivalent RNA sites, and the density and type of motifs within these sites. iCLIP experiments with RBP-chimera indicate that homomeric CTD-driven interactions promote TDP-43 assembly on these sites. Moreover, processing of a subset of bound transcripts is sensitive to perturbed condensation, including autoregulation of TDP-43 mRNA. These findings link RBP condensation to selective remodelling of RNA regulatory networks, with relevance for signaling, disease and evolution.

TDP-43 cytoplasmic inclusions in the skin of ALS patients

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Background. To identify and quantitatively analyze cytoplasmic TDP-43 inclusions in epidermis and dermis of the skin of ALS patients.
Methods. Skin biopsies from 64 subjects were analyzed: 44 ALS patients, 10 healthy controls (HC) and 10 neurological controls (NC) (5 patients with Parkinson's disease and 5 with multiple sclerosis). TDP-43 immunoreactivity in the epidermis and dermis was analyzed, as well as the percentage of cells with TDP-43 cytoplasmic inclusions in predefined areas of epidermis and dermis (papillary and reticular). ROC analyses of these data were also performed. A subset of ALS patients was again biopsied 12 months later for comparison over time.
Results. We detected higher expression of TDP-43 in the epidermis ($p<0.001$) and in both layers of the dermis ($p<0.001$), as well as higher percentage of TDP-43 cytoplasmic positive cells ($p<0.001$) in the ALS group compared to HC and NC groups. Dermal cells containing TDP-43 were fibroblasts as identified by co-labeling against vimentin. ROC analyses (AUC 0.867, $p<0.001$; CI 95% 0.800-0.935) showed that detection of 24.1% of cells with cytoplasmic TDP-43 positivity in the dermis had 85% sensitivity and 80% specificity for detecting ALS. We did not find significant correlation with clinical features, including disease onset and ALSFR-R slope.
Conclusions. In this study we have identified significantly increased TDP-43 expression in the epidermis and cytoplasmic aggregations of TDP-43 in the dermis of ALS patients. Our findings provide insight into the TDP-43 expression in non-neural tissue in ALS and support its use as a potential biomarker.

TDP-43 is located in endoplasmic reticulum and mitochondria-associated membranes in central nervous system from mammals

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INTRODUCTION
TDP-43 is frequently mislocalized in nervous tissue from ALS patients. One of the extranuclear locations where this protein could contribute to pathophysiology is the mitochondrial one. Mitochondria show intimate contacts with endoplasmic reticulum (ER) domains termed mitochondrial-associated membranes (MAMs), which are detergent-resistant (i.e. intracellular lipid rafts) considered key hubs in lípid metabolism,autophagy and calcium homeòstasis
OBJECTIVES
To evaluate the potential presence of TDP-43 in MAMs in vivo, and infer its potential dysfunction in ALS
METHOD
We have evaluated -by subcellular fractionation and sucrose gradients in detergent resistant membranes- the presence of TDP-43 in several subcellular locations, including ER, MAMs, nuclei and mitochondria. We have explored this characteristic in brain cortex samples from ALS, ALS-FTD complex patients, as well as in the transgenic B6N.Cg-Tg(Prnp-TARDBP*Q331K)103Dwc/J (TDP-43 Q331K) mice. Also, we evaluated the distribution of TDP-43 in situations leading to MAM disturbances.
RESULTS
Subcellular fractionation results show that highly pure MAM and ER fractions, exhibit anti-TDP-43 immunoreactivity in all the models evaluated (both endogenous TDP-43 in human samples, endogenous tdp-43 and human TDP-43 in transgenic mice). Thought a minor lysosomal and stress-granule contribution can not be ruled out in MAM-enriched preparations, ER shows a prominent enrichment of TDP-43 in all samples evaluated, and in brain samples from the transgenic mice shows an enhanced concentration in this subcellular fraction. Of note, we did not observe an enhanced concentration of TDP-43 in the MAMs isolated from brain cortex of patients with ALS or ALS/FTD. Strikingly, perturbation of ER homeostasis by ER stressors, induce an enhanced phosphorylation of TDP-43, but no major changes in TDP-43 in MAMs.
CONCLUSIONS
TDP-43 is present in subcellular locations, associated with detergent resistant membranes (MAMs) and in ER , both in transgenic models of overexpression of human TDP-43 and in samples from several nervous tissue locations in humans.

Teaching an Old Dog New Tricks: Serum Troponin T as a Biomarker in Amyotrophic Lateral Sclerosis

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Introduction
Amyotrophic lateral sclerosis (ALS) is characterized by progressive loss of upper and lower motor neurons. Diagnosis, management and therapeutic trials are hampered by a lack of informative biomarkers. Troponins (Tn) are components of skeletal and cardiac muscles. Acute elevation of cardiac isoforms of troponin I (cTnI) and T (cTnT) in serum indicates myocardial injury. Case reports suggested that serum levels of cTnT, but not cTnI are chronically elevated in ALS and other neuromuscular disorders.

Objectives
We sought out to evaluate the informative potential of tracking TnT and TnI levels in ALS patients for determining their diagnosis and prognosis.

Method
Using standard clinical laboratory tests we measured serum troponin levels in a multicentric cross-sectional cohort of 75 ALS patients and sixty controls (DESCRIBE-ALS cohort) and in a real-world cohort of 179 consecutive patients from our ALS clinic at the University Hospital Bonn.

Results
We found that serum cTnT, is elevated in >60% of ALS patients while cTnI is always normal. Serum cTnT levels increase over time and correlate with disease severity as measured with the revised ALS FRS score. There was no correlation with the phosphorylated neurofilament heavy chain (pNfH) levels in the cerebrospinal fluid.

Conclusions
We propose that cTnT elevations in ALS are of non-cardiac origin and may serve as a proxy of lower motor neuron or skeletal muscle involvement. They potentially help to stratify patients according to lower motoneuron involvement. Further research will determine the biological origin of the cTnT elevation and its validity as a diagnostic and/or prognostic marker. Our finding also serves as a reminder to interpret cTnT with caution elevations in patients with neuromuscular diseases.

Telehealth in Motor Neuron Disease (TiM): Barriers, facilitators, and early engagement.

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Introduction
Even before Covid and the restriction of face-to-face appointments, clinical care for people with Motor Neuron Disease (pwMND) was problematic and often unresponsive to individual needs.

To enable greater communication between pwMND, their carers, and healthcare professionals (HCPs), through a user-centred design approach, we had developed a remote monitoring system, Telehealth in MND (TiM). As a response to Covid, we have now rapidly implemented TiM as a clinical service. TiM regularly asks pwMND and carers about their symptoms and wellbeing through questionnaires. Answers are reviewed weekly by HCPs who take action as appropriate.

Objectives
Explore key barriers and facilitators of engaging with a rapidly implemented TiM system experienced by HCPs.
Investigate the level of engagement of users.

Method
Remote observations and semi-structured interviews were conducted with HCPs who were using TiM, analysed using summative content analysis and reflexive thematic analysis, respectively. Anonymised, quantitative data on how pwMND and carers used the system was collected and averages calculated to provide a general profile of TiM users.

Results
More results will be available at the meeting.

The observations and interviews identified barriers and facilitators to implementing a telehealth service. HCPs were anxious regarding the amount of time involved in reviewing answers on TiM and suggested solutions to reduce the demand on time. HCPs highlighted how TiM facilitated the early identification of patient clinical decline. Additionally, the system has been instrumental in identifying the current strain of carers, which has been increased by Covid.

We found that the system has several different types of user, for example some people use TiM at regular intervals (i.e. weekly, fortnightly, or monthly), whilst others only enter in information when they want to highlight a change in a symptom. In all, the service receives good engagement, with 126 current users and over 300 completed questionnaires per week.

Conclusions
This project highlighted barriers and facilitators to engaging with novel telehealth services that are beneficial to the TiM project and the wider clinical and research community.

Findings demonstrate several types of TiM user, highlighting the need to provide adaptable services for pwMND and carers. This learning is being used and built upon as TiM is implemented in other MND centres in the UK and Europe.

Test-Retest Reliability of EEG Markers of Cognition

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Introduction: Research into longitudinal changes to cognition in ALS depends on the identification of biomarkers that are stable over repeat testing in the absence of cognitive change. EEG is safe, well-tolerated, and cost-effective, making it well-suited to longitudinal research.

Objectives: This study aims to evaluate the reliability of EEG-based markers of cognition in ALS. Earlier work suggests that the P300 and MMN should be stable over time[1].

Methods: The recruitment of a total of 10 healthy young adults is currently in progress. The participants complete a battery of EEG tests on two consecutive days. Time of day and experimenters are kept constant between days. Participants are asked to complete a frequency oddball passive listening task, the Sustained Attention to Response Task (SART), and blocks of resting EEG with eyes opened and closed. The mismatch negativity (MMN) and P300 wave are identified from the auditory oddball and SART, respectively. The two-way random effects absolute agreement intraclass correlation coefficient (ICC) will be calculated for the peak and mean amplitude, peak and median latency, and area under the waveform of the MMN and P300 obtained on day 1 and day 2.

Results: Qualitative visual results indicate the consistency of the MMN and SART on two consecutive days. Preliminary pairwise comparisons (hotelling T2 test, Mann Whitney) failed to find differences between any features of the MMN or SART on days 1 and 2 ($p>0.05$). Quantitative ICC results will be obtained for the test-retest reliability of the MMN and P300 to verify these results.

Discussion: Longitudinal EEG has the potential to be a reliable method of tracking changes to cognition in ALS, aiding the development of cognitive treatments. Our data demonstrate stability of these measures over time in healthy individuals. Good test-retest reliability justifies the use of EEG markers of cognition in longitudinal analyses.

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The Cutaneous Silent Period in Motor Neuron Disease

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Introduction
Voluntary contraction of limb muscles can be briefly interrupted after peripheral nerve stimulation. The stimulus most frequently used is an electrical stimulus delivered to cutaneous nerves of the hand fingers, while recording EMG activity from the small muscles of the hand.
This reflex, termed the cutaneous silent period (CSP), represents a spinal inhibitory circuit, mediated primarily by small myelinated A-δ fibers and spinal inhibitory interneurons.
Despite the changes in central inhibitory circuits inherent to ALS pathology, the CSP has scarcely been studied in this disease.

Objective
To investigate the cutaneous silent period by measuring its onset latency, duration and amount of signal suppression in patients with motor neuron disease (MND) grouped according to the intensity of upper motor neuron involvement (UMN), and to test the effect of a contraction of the contralateral hand.

Methods
Painful stimulation was applied at the V finger, and contraction recorded from the abductor digiti minimi (ADM) muscle with surface EMG(baseline condition).
Afterwards, CSP was studied during strong contralateral ADM contraction (test condition).
Ten to fifteen consecutive traces were recorded for each condition, signals were rectified, averaged, and analyzed offline.

Results
46 patients were included, 15 with progressive muscular atrophy (PMA), 16 with typical ALS, 15 with primary lateral sclerosis/predominant UMN-ALS (PLS-UMN-ALS), and 28 controls.
In the baseline condition, all MND groups showed delayed onset latencies ($p=0.001$). There was no significant difference in the CSP duration. Suppression was lower in the PLS+UMN-ALS group ($p=0.004$). In the control group, contralateral contraction did not change CSP, but onset latency shortened significantly in the PMA group.

Conclusions
CSP onset latency is delayed in all investigated groups of MND, including in PMA, indicating subclinical UMN involvement in PMA.
Transcallosal ipsilateral corticospinal tract activation by contralateral hand contraction can partially compensate UMN lesion.

The effect of C9orf72 repeat expansion and expression on brain morphology in the asymptomatic: a longitudinal MRI study

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The C9orf72 repeat expansion (RE) is a highly penetrant mutation in amyotrophic lateral sclerosis (ALS). Previous imaging studies have shown that ALS patients carrying the mutation have widespread cortical thinning and subcortical volume loss when compared with non-carriers. This has also been found in a large family of asymptomatic C9orf72 RE carriers. However, since then, no new studies covering more families have been conducted, and no longitudinal studies covering the subject have emerged. The pathogenetic mechanism of how C9orf72 RE relates to alterations in brain morphology still remains largely unknown. A possible approach to elucidate this involves the examination of brain phenotype in context of transcriptional profiles.

To increase our understanding of the effects of the C9orf72 RE before disease onset, we assessed brain morphology in asymptomatic carriers longitudinally and performed a cross-correlation study on gene expression.

We included 62 subjects with C9orf72 RE, and 182 without. All were asymptomatic family members (AFM) of ALS patients, where asymptomatic was defined as a normal neurological exam and ECAS performance. Participants underwent multiple 3T MRI scans and brain measurements were assessed using T1 and DWI data. Cortical thickness, subcortical volumes and white matter connectivity were derived for each group and compared using mixed-effects models incorporating kinship matrices. Outcomes were found statistically significant after FWER corrected p value <0.05. Transcriptional profiles for C9orf72 expression were obtained from the Allen Human Brain Atlas. Correlation between gene expression and regional cortical thinning was evaluated with meta-regression.

AFM with C9orf72 RE had quite symmetrical significantly thinner cortex, mostly in the posterior regions of the brain (parietal, occipital, inferior temporal). Multiple subcortical grey matter structures showed decreased volume. A significant connected component of temporal connections reflected loss of white matter in this area. Apart from ageing, no significant cerebral changes occurred over time that could be attributed to C9orf72 RE carriership.
There was a negative linear correlation between C9orf72 gene expression and cortical thickness between carriers and non-carriers ($\beta = -0.68$, $R^2 = 0.62$, $p = 0.008$).

Our results underline the suggestion that effects of C9orf72 RE are already present before clinical disease onset, mainly in the posterior parts of the brain. Notably, the motor cortex is spared. The mutation does not seem to influence the longitudinal morphological changes. The negative correlation between transcriptional profile and cortical thinning suggests that not only the presence of the mutation itself, but also the level of C9orf72 expression could be of importance in the pathogenic mechanism underlying the development of ALS.

The endoplasmic reticulum-mitochondria axis in C9orf72-related ALS/FTD

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INTRODUCTION
The ER and mitochondria form close physical contacts that regulate a number of key cellular functions. Many of these functions are damaged in neurodegenerative diseases, including amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD). Importantly, ER-mitochondria signalling is known to be perturbed by several ALS/FTD–linked insults. A pathogenic repeat expansion in the C9orf72 gene is the most frequent cause of familial ALS/FTD. The expansion produces dipeptide repeat proteins (DPRs) that are a pathology of ALS/FTD.

OBJECTIVES
To investigate the ER–mitochondria association in C9orf72 ALS/FTD models, including human disease-relevant cells and C9orf72 transgenic mice.
To explore the effect of DPRs expression on ER-mitochondria interaction and signalling, studying the calcium exchange between the two organelles.

METHODS
ER-mitochondria contacts were examined in C9orf72 iPSC-derived neurons and transgenic mice harbouring pathogenic C9orf72 expansions. Primary-cultured rat neurons and SH-SY5Y cells transfected with control EGFP vector or EGFP-DPRs involving 125 repeat poly-GP, -PA, -GA, -PR or –GR from a non-G4C2 sequence were also examined. Proximity ligations assays and super resolution structure illumination microscopy were utilized to quantify ER-mitochondria contacts. As a physiological readout of ER-mitochondria signaling, delivery of calcium from ER stores to mitochondria was monitored.

RESULTS
ER-mitochondria contacts were reduced in C9orf72 cell and transgenic mice. Expression of known pathogenic DPRs also damaged ER-mitochondria signalling in SH-SY5Y and rat cortical neurons.

CONCLUSIONS
These results show that ER-mitochondria contacts and linked signalling are disrupted by mutant C9orf72. Since other ALS/FTD genetic insults also disrupt ER-mitochondria signaling, correcting this damage may be beneficial in ALS/FTD.

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The inhibitor of fatty acid amide hydrolase (FAAH) URB597 is neuroprotective in a TDP43-based transgenic mouse model recapitulating frontotemporal dementia

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Introduction: Frontotemporal dementia (FTD) is a group of diseases characterized by degeneration of the frontal and temporal lobes of the brain leading progressive decline in cognition and language with alterations in behaviour and personality. Due to overlap in clinical presentation, pathology and genetics, FTD is closely related to amyotrophic lateral sclerosis (ALS) with both diseases being considered a continuous spectrum disorder. In last years, our group has provided experimental support for the therapeutic potential of the endocannabinoid system (ECS) in ALS. The ECS is an intercellular system involved in several functions as the maintenance of neuronal homeostasis and integrity. It is well-known that, during neurodegenerative events, several elements of ECS alter their expression, which may be interpreted as an endogenous neuroprotectant response against excitotoxicity, oxidative stress and/or neuroinflammation occurring in these diseases. In this sense, preliminary data in a TDP-43-based FTD mouse model showed changes in fatty acid amide hydrolase (FAAH), the enzyme that inactivate the endocannabinoid anandamide (AEA), that may be interpreted as a protective response aimed at elevating AEA levels in the affected brain structures. If that is true, the pharmacological management of these changes by inhibiting FAAH enzyme may have therapeutic value in this disease.

Objectives: To explore the therapeutic potential of enhancing the endocannabinoid tone in a FTD-TDP43 transgenic mouse model, through the selective inhibition of the degradation enzyme FAAH.

Method: We used FTD-TDP-43 transgenic mice having overexpression of TDP-43 protein exclusively in the forebrain, which recapitulate major signs of FTD (Tsai et al., J Exp Med 2010). Mice were treated intraperitoneally with the selective FAAH inhibitor URB597 (0.2 mg/kg) or vehicle on alternate days from 45 (pre-symptomatic stage) to 90 days of age (symptomatic stage). The cognitive status of animals was recorded at two time points (60 and 90 days of age) using the Novel Recognition test (NOR). At the end of treatment, mice were euthanized and their prefrontal cortex and hippocampus were used for histopathological analysis using specific cellular markers of neurons (NeuN, Ctip2), astrocytes (GFAP, S100β) and microglia (Iba1).

Results: We found that URB597 treatment improves early cognitive deficits in FTD mice compared with wildtype animals. Such behavioural improvement was likely associated with an increase in the survival of pyramidal neurons and a reduction of astroglial and microglial reactivity in the prefrontal cortex and the hippocampus.

Conclusions: We conclude that FAAH enzyme may be a promising target for the treatment of TDP-43-induced neuropathology in FTD since their inhibition may serve to improve cognitive deficits and to reduce neuronal death and inflammatory processes.

The involvement of RNA G-quadruplex structures in stress granule dynamics

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Introduction: Stress granules (SGs), ALS-associated assemblies, are cytoplasmic biomolecular condensates that regulate the cellular RNA metabolism in health and disease. These membrane-less organelles are formed under stress by complex interactions of RNA-binding proteins (RBPs) and mRNAs that are mostly halted from translation. The SG-transcriptome may organized in different structures, such as RNA G-Quadruplexes (rG4s). rG4s are guanine-rich RNA non-canonical secondary structures that have regulatory roles in central cellular processes.
Objectives: Although formation of G4s within 5'UTR of mRNAs is suggested to block ribosome scanning, which lead these G4-enriched transcripts to SGs, little is known about direct roles of rG4s in SG biology. Here, we hypothesize that rG4s may play a direct role in SG dynamics.
Methods: Using small-molecules that specifically bind to rG4s, and imaging of fixed and live G3BP1-GFP-expressing U2OS cells under different stresses and conditions, we characterize a potential direct function of rG4s in the assembly of SGs.
Results: Our preliminary results show in fixed cells an enrichment of rG4s in G3BP1-GFP-enriched SGs by fluorescently-labeled preferential rG4 binders, bio-TASQ and QUMA-1. The colocalization between rG4s and SGs is found under different stresses. Moreover, we demonstrate that bio-TASQ, QUMA-1 and the rG4 stabilizer, cPDS, reduce the assembly dynamics of SGs under Sodium arsenate stress in live cells.
Conclusions: Thus, these results suggest that RBPs-rG4 interactions contribute to the SG formation and rG4s may regulate SG biology through this aspect in addition to the already known translation-associated aspects. This study sheds a new light on the regulatory roles of rG4-RBP interactions in general. Particularly, this work contributes to a better understanding of the molecular mechanisms underlying SG biology, under stress and potentially with association to disease.

The Italian version of self-administered ALSFRSr scale: a validation study

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Introduction: The most widely used functional rating scale (FRS) for global assessment of disease in Amyotrophic Lateral Sclerosis (ALS) is the ALSFRSr score. It is a 12 items scale, evaluating bulbar, upper and lower limb, and respiratory functions. A self-administered version has been already validated in some languages.

Objectives: In this work we wanted to develop and assess the reliability of the Italian version of the self-administered ALSFRSr scale, considering patients' clinical and cognitive features and caregiver's help.

Methods: During the COVID-19 pandemic, from 27th March 2020 to 5th May 2020, we visited in telemedicine most of patients regularly followed-up in our Center. We created the Italian version of self-administered ALSFRSr following ENCALS recommendation. The self-administered version of ALSFRSr scale and the standard telephone-administered ALSFRSr scale were evaluated in 70 ALS patients. Overall scores, single item scores, ALSFRSr domain scores, King's and MiToS stage inter-rater agreement, and reliability were assessed. Data were analysed by using spearman's rho, Bland–Altman difference plots, Cronbach's alpha coefficient, and Intraclass correlation coefficient (ICC).

Results: Correlation between the two scales was 0.94 and no systematic directional bias was found. 47% of patients answered the self-administered ALSFRSr version questionnaire with the caregiver's help. The ICCs for ALSFRSr scale, for single items and for domain score were very high (>0.90) and did not differ according to gender or cognition, although it was higher when patients were helped by their caregiver (0.95). Also, the reliability for both King's staging system and MiToS was very high (ICC of 0.96 for King's and 0.94 for MiToS total scores).

Conclusions: We validated the online Italian version of the self-administered ALSFRSr scale and we demonstrated that it is a valid tool to stratify ALS patients into clinical stages. This reliability is increased with the help of caregiver in filling the questionnaire. Finally, it is a way to implement the monitoring of patients in telemedicine.

The pleiotropy of neurodegenerative repeat expansions in ALS

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Introduction: The progressive atrophy and/or loss of functions or neurons is defined as neurodegeneration, and more than 40 diseases that affect the nervous system is caused by repeat expansions (REs). The most common neurodegenerative repeat expansions (NDREs) diseases are Huntington's Disease (HD), Spinocerebellar Ataxias (SCA), Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disorder which causes the death of neurons controlling voluntary muscles and ultimately the death of the patient. ALS has no cure, and its underlying cause is mostly unknown, although a strong genetic component is known to play a role. Several REs are pleiotropic; for example, GGGCC RE in C9orf72 is associated with FTD/ALS and CAG RE in ATXN2 causes SCA2/ALS. The gene ATXN2 normally has a repeat structure of around 22-23 triplets encoding for glutamine (CAG) within the reading frame of the gene encoding the ataxin two protein. Studies have shown that harbouring more than 40 repeats causes spinocerebellar ataxia type 2 (SCA2). Recently, it was discovered that intermediate-length repeat expansions (27-33 repeats) in ATXN2 are significantly associated with the risk of developing ALS. Therefore, pleiotropy might be common in ALS.
Objectives: This study aims to genotype 34 neurodegenerative genes that harbour REs, in a cohort of 1000 controls and 1000 patients from the Irish ALS bank to assess the association between expanded genotypes and ALS.
Materials and Methods: The measurement of the repeat of each NDRE gene and its possible repeat expansions was determined by optimizing protocols of PCRs, Repeat Primed-PCR (RP-PCR), agarose gel electrophoresis and fragment length capillary (FLA) electrophoresis.
Results: In an Irish population, ALS might be driven by multiple intermediate-length repeat expansion in likely 10 NDREs genes: ATXN2, ATN1, DIP2B, FRA10AC1, FRA11A, NUTM2B-AS1, PABN1, TCF4, TK2-BEAN and ZNF713.
Conclusions: ALS is a very complex disease that might be caused by pleiotropy of multiple REs and factors.

The psychosocial impact of rehabilitative and recreational physical exercise in amyotrophic lateral sclerosis.

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Introduction: Physical exercise in rehabilitaion is among the most frequently prescribed symptomatic measures in ALS to minimize disease-related symptoms and to eventually secure independence as long as possible. How it promotes wellbeing, including quality of life and depressiveness is poorly understood.

Objectives: This is an observational, cross-sectional study on the relationships between physical exercise and psychosocial adaptation in patients with amyotrophic lateral sclerosis (ALS) in a German sample within the EU Joint Programme–Neurodegenerative Disease Research study NEEDSinALS (NEEDSinALS.com).

Methods: Patients with ALS (n = 85) with median ALS-FRS= 34.00, indicating moderate physical impairments, were interviewed on well-being (quality of life, depression) and physical exercise. Additionally, clinical (disease parameters, ALS Functional Rating Scale - revised version) and sociodemographic data were recorded. Standardized questionnaires on subjective QoL (Anamnestic Comparative Self-Assessment, ACSA; Schedule for the Evaluation of Individual Quality of Life-Direct Weighting, SEIQoLDW) and affective state (ALS Depression Inventory–12 items, ADI-12) were determined as measures of patient's wellbeing. For physical exercise, the Patient's Physical Activity Questionnaire, EPIC-PAQ) was used. Statistical analysis included Kruskal-Wallis and Mann-Whitney tests.

Results: The median intensity of daily physical activity was 2.00, corresponding to a sedentary activity level. None of the respondents was capable of high-intensity daily physical activity. In total, 81% of the patients had access, at a minimum, to one form of rehabilitation exercise. 49% of participants reported regular physical activity not related to activity of daily life and therapy. In this group, the activity hours were significantly positively correlated with gQoL (p = 0.039). There was also a significant correlation between the rehabilitation exercise and subjective well-being (r= p=). For 21% of the patients, physical activity was one of the five most important areas of their lives. The median satisfaction with physiotherapy was 90%.

Conclusion: Our study demonstrates that recreational and rehabilitative physical activity was associated with better quality of life in patients with amyotrophic lateral sclerosis.

the role of intestinal permeability in the development and chronicity of SLA

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The role of intestinal permeability in the development and chronicity of SLA
PLL-THERAPEUTICS
Jean-Pascal Zambaux

Introduction
More and more the Gut is highlight in many diseases as playing a key role in Parkinson or SLA by being the open gate for many components as bacteria, LPS, toxins and chemicals to move in the blood and in the brain which can have a role in the development of the disease and in its chronicity.

Objectives
The idea was to develop our Drug product around the Neuro metabolism link to ALS as tryptophan, NO… and to develop our formulation with the target to solve the gut leaking and avoid, due to some reasons in the change of microbiome for example, the increase of the intestinal permeability.

Method and Results
Our first target was to validate the marker as Circulating Antibodies in our catalogue which are the reflect of gut permeability and ALS disease and to formulate our DP with component able to increase gut permeability and decrease our ALS marker which will prove the stabilisation of the disease.
We did launch SOD1 mice trial and collect their serum to evaluate how our marker evolve and it's still under evaluation at he time I write this note.
The same trial was done on inflamed Rats with DSS to evaluate the effect of our DP and 7 days of inflammation.
After 3 days of treatment the gut permeability of the rat came back to normal (as on witness) and the behaviour became normal and the inflammation disappear.
The collect of serum is done and the evaluation of the gut permeability marker will be presented.

Conclusions
This development is unique, simple and without side effect.
The regulatory tox is in progress on our DP.
This concept will allow us to launch a clinical trial on ALS by the end of 2020.
Our goal is to focus on the origin of the disease and the gut leaky is the key.

The split-hand syndrome as a prognostic biomarker in amyotrophic lateral sclerosis.

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Objective: The split-hand syndrome (SHS) is a characteristic feature in amyotrophic lateral sclerosis (ALS). The objective of this study is to assess the role of some neurophysiological measures as early prognostic biomarkers.

Methods: ALS patients evaluated in our tertiary hospital from 2010 to 2019, meeting Awaji criteria and with an available compound muscle action potential (CMAP) of the abductor pollicis brevis (APB) and the abductor digiti minimi (ADM) within six months from diagnosis, were included in this study. The SHS was defined as an APB/ADM ratio <0.6. Demographic and clinical data at diagnosis, as well as the forced vital capacity (FVC) were recorded. Patients were followed-up for death or tracheostomy until March 15th 2021. Linear regression models were used to assess the association of APB, ADM and the APB/ADM ratio and clinical variables. Kaplan-Meyer curves and Cox regression analysis were performed to assess the effect of neurophysiological biomarkers in survival.

Results: From 464 medical records reviewed, 197 patients were finally included and 35% of them had SHS. The CMAP of APB and ADM and the APB/ADM index were lower in upper limb onset patients. A decreased CMAP of the APB and the ADM and the SHS were independently associated with the disease progression rate and the SHS was associated with worse survival (30.7 versus 42.4 months, p=0.011). However, in the multivariable analysis, the CMAP of the ABP was the only independent biomarker of survival.

Conclusion: The presence of SHS is associated with faster disease progression and worse survival. However, the CMAP of APB is the only independent biomarker of survival. Our study supports the incorporation of easy-to-obtain neurophysiologic biomarkers for stratification purposes in clinical trials.

Transcriptomic analysis of small non-coding RNA and mRNA profiles in an in vitro model of C9orf72 dipeptide repeat protein toxicity

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Introduction: Small non-coding RNAs (sncRNAs) have emerged as important regulators of gene expression in the nervous system and their dysregulation has been implicated in various neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). A growing body of evidence suggests that sncRNAs, including microRNAs (miRNAs) and the newly discovered class of transfer RNA-derived small RNAs (tsRNAs), may be involved in various aspects of ALS pathogenesis. However, the full spectrum of sncRNAs and their regulatory roles in ALS remain largely unknown.

Objectives: This study aims to identify the transcriptomic profile of sncRNAs and mRNAs in a cellular model of C9orf72 dipeptide repeat protein (DPR) toxicity, and to explore biological pathways and functions that may be dysregulated by sncRNAs in C9orf72-mediated ALS and frontotemporal dementia (FTD).

Method: To model C9orf72 toxicity in vitro, cultures of mouse primary cortical neurons were exogenously treated with a synthetic proline-arginine DPR of ten repeats (PR_{10}). RNA sequencing (RNA-seq) and small RNA-seq were performed on the PR_{10}-treated neurons (n=3) and vehicle (PBS)-treated control neurons (n=3) followed by differential expression analysis, sncRNA target prediction and gene-set enrichment analysis (GSEA).

Results: Small RNA-seq analysis of the PR_{10}-treated neurons compared to control identified a set of deregulated sncRNAs. GSEA revealed that the putative target genes of the top deregulated tsRNAs and miRNAs were significantly enriched in gene annotation terms and pathways related to nervous system development. RNA-seq analysis of the PR_{10}-treated neurons identified differential expression of genes functioning in endoplasmic reticulum stress, unfolded protein response and amino acid transport. Furthermore, seven of the significantly downregulated genes in response to PR_{10} were the predicted targets of miR-298-5p, the top upregulated miRNA from the sncRNA profile.

Conclusions: These results provide further insights into the functional roles of sncRNAs in C9orf72-mediated ALS/FTD and highlights miR-298-5p as a potential regulator of gene expression in response to C9orf72 DPR neurotoxicity.

Trehalose treatment finely tunes the mitophagy pathway in PBMCs of sALS patients

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Background. Mitophagy is the pathway responsible of the elimination of damaged mitochondria and its impairment could lead to the accumulation of inefficient mitochondria. Mechanistic and genetic evidences strongly support a critical contribution of mitochondria to ALS pathogenesis, but many questions are still unresolved. Mitochondria alterations can be found in both in ALS animal models and proximal axons of ALS patients, while no data are reported in other patients cells types, like peripheral blood mononuclear cells (PBMCs).

Objectives. Aim of this work is to investigate mitophagy in PBMCs of sALS patients and how the pathway can be pharmacologically tuned.

Method. Mitochondria morphology and distribution was analyzed in PBMCs of sALS patients and healthy controls by Transmission Electron Microscopy (TEM) and MitoTracker® Red CMXRos. Moreover, we investigated the percentage of healthy mitochondria by cytofluorimetric analysis using Mitotracker and TMRE. Then, mitophagy was also studied by both WB and immunofluorescence analysis. Finally, we tuned mitophagy pathway by treating cells with NH4Cl, rapamycin, and trehalose followed by immunofluorescence and WB analysis.

Results. In patients PBMCs, TEM analysis evidenced the presence of morphologically atypical mitochondria, with the presence of an increased number of vacuoles and damaged cristae. We confirmed abnormalities using also MitoTracker. Moreover, we found a decreased number of healthy mitochondria in sALS PBMCs. By WB and immunofluorescence, we found an impairment of mitophagy caused by possible lysosomes dysfunction. After NH4Cl treatment we found a lower increase of LC3 marker in sALS PBMCs, while after rapamycin treatment we found a higher increase of LC3 marker in sALS PBMCs. Finally, after trehalose treatment, we found no modulation of lysosomes level, on the contrary we found a significant decrease in lysosomes levels in sALS PBMCs.

Conclusions. In conclusion, our data suggest that the presence of morphologically altered mitochondria and an inefficient turnover of damaged mitochondria in PBMCs of sALS patients relies on an impairment of the mitophagy pathway. In particular, the induction of mTOR-independent autophagy pathway, caused by trehalose treatment, leads to a decrease of lysosomes levels, suggesting a more sensitivity of sALS PBMCs to the effect of trehalose. Such evidences suggest that trehalose can exert protective effects in sALS, representing a potential therapeutic strategy.

Ultrasonographic and manometric study of the tongue as biomarkers of bulbar dysfunction in patients with amyotrophic lateral sclerosis

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Introduction: Amyotrophic lateral sclerosis (ALS) is a disease with significant phenotypic variation characterized by progressive weakness, muscle atrophy, dyspnea, and bulbar dysfunction (dysarthria, dysphagia). One-third of patients, at baseline, and 85% throughout the disease, have bulbar dysfunction. Dysphagia has a significant impact on mortality (malnutrition, dehydration, pneumonia) and significantly affects the patient and caregivers' quality of life and social relationships.

The possibility of having fast, which can be carried out in the outpatient clinic, and minimally invasive methods to assess bulbar function quickly, reliably, and prospectively can facilitate follow-up and perform therapeutic interventions at faster and earlier stages.
Oropharyngeal dysphagia in ALS is caused by a combination of facial, lingual, pharyngeal, and laryngeal muscle stiffness/weakness, respiratory muscle weakness, and laryngeal sensory disturbances. Although all aspects of swallowing are altered, the oral phase is the first to be affected in ALS patients.
Thus, since the tongue plays an essential role in the oral phase of swallowing, its study can be of great relevance for patients' follow-up.

Objective: The main aim is to study the association between tongue strength and thickness with VDF findings in ALS patients. The secondary objective is to investigate whether tongue strength is a marker of preclinical bulbar dysfunction.

Methods: Patients with a definitive or probable ALS diagnosis have been studied, according to the El Escorial criteria. Ultrasound tongue thickness and tongue strength measurements, using the Iowa Oral Performance Instrument (IOPI®) system, have been performed.

Results: Of the 22 patients studied, 10 were women (45.5%), 9 presented bulbar forms (40.9%) and 13 spinal forms (59.1%).
There is a correlation between patients with qualitative alterations in oral transit and the anterior lingual strength values (60% 24-83 vs 99%, IQR 97-100 vs; p = 0.05). There was a similar trend with the posterior lingual strength although it did not reach statistical significance (74%, IQR 10-88 vs 106%, IQR 100-113; p = 0.07).
No correlation was found between tongue thickness and qualitative assessment of VDF.
There is no correlation between anterior/posterior lingual strength and quantitative parameters of VDF.

Regarding the secondary objective, a significant relationship has been found between the ALSFR-R scale and the anterior (R 0.581 p = 0.007) and posterior (R 0.583 p = 0.009) lingual strength.

Conclusions: The functional study of the tongue using the IOPI® system is an easy to perform, reproducible and non-invasive way that allows selecting those patients who need an adaptation of the diet and gastrostomy placement.
In our cohort, there was no correlation between the morphological study of the tongue, with ultrasound, and the VDF.

Ultrasound of peripheral nerves as preclinical marker for disease progression in mutant SOD1G93A mice?

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Introduction & Objectives:
In the last decade few studies have been published on sonographic evaluation of peripheral nerves in patients suffering from ALS. In the majority, the cross-sectional area (CSA) of a variety of different nerves was analysed in ALS patients compared to controls or throughout disease progression. Most studies showed CSA reduction in ALS patients. Schreiber et al., on the other hand, described an inflammatory subtype of ALS patients with increase in CSA, that also was detected in a large proportion of patients carrying SOD1 mutations. In relation to these findings we are currently investigating the sciatic nerve of ALS transgenic mice by ultrasound.
Methods:
Ultrasound data are set in the context of standard parameters in this animal model at different disease stages: Motor function tests, phenotypic data, nerve conduction studies and histological data from the sciatic nerve, spinal cord and gastrocnemius muscle.
Diameter and CSA of sciatic nerve are evaluated and grey-scale analysis of CSA is performed.
Results:
Our preliminary results indicate that ultrasound measurements can be used as sensitive and non-invasive (pre-) clinical marker of disease progression.
Conclusion:
A possible effect of an experimental treatment could therefore be detected earlier and more sensitively. Moreover, our multi-modal analyses will provide further insight into the correlation of neurophysiological and morphological/histopathological data in the mutant SOD1 mouse model of ALS.

Understanding the role of ALS/FTD gene TBK1 in neuronal response to stress.

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Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) have differing clinical presentations with distinct neuronal populations undergoing cell death in each disorder. In contrast to mutations in TDP43 and FUS, where both gain- and loss-of-function contribute to pathogenesis, genetics suggest TBK1 haploinsufficiency causes disease, conceivably through the loss of protective functions in ageing neurons. Despite well-characterised roles in innate immunity and autophagy, and interactions with ALS/FTD genes OPTN and SQSTM1, non-neuronal studies suggest TBK1 interacts with a wide range of proteins and phosphorylates an extensive range of substrates.
This research project aims to understand how TBK1 contributes to cellular response to age-related disorder and in doing so identify protein interactions associated with its stress-induced activation.
Thus far we have demonstrated that inhibition of TBK1 kinase function in differentiated SH-SY5Y cells leads to altered response to compound stresses in a range of high-content assays.
Cytotoxicity screening of an autophagy-modifying compound library identified susceptibility to late-stage autophagy inhibition, unfolded protein response and ER stress-induced cell death. Pilot studies utilising the Cell Painting assay present dramatically altered morphological profiles when exposed to sub-lethal doses of select compounds. Finally, bioenergetic analysis reveals reduced basal respiration and ATP production, suggesting a previously unidentified role in neuronal respiration.
Taken together our data suggest novel and diverse roles for TBK1 in neuronal response to stress, with haploinsufficiency presumably sensitising neurons to ageing-induced dysfunction.
We now aim to generate a novel TBK1-interactor dataset to understand how these interacting proteins contribute to identified stress phenotypes and resulting neurodegeneration. As TBK1 interacts with other disease genes, it is feasible that we may identify common targets for therapeutic intervention in ALS/FTD. In addition to translational biological relevance, our optimised protocol for Cell Painting high-content image analysis of the widely used SH-SY5Y cell line has transferable application in pre-clinical neurodegenerative disease research.

Unique RNA dysregulation in resistant and vulnerable motor neurons in mutant SOD1 ALS

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We have investigated the longitudinal transcriptional response of resistant and vulnerable motor neuron populations to the SOD1G93A ALS-causative mutation. Specifically, we have conducted RNA sequencing of motor neurons isolated from the SOD1G93A mouse at presymptomatic (p56) and symptomatic (p112) stages using laser capture microdissection. Oculomotor and trochlear (CN3/4) motor neurons, which regulate eye movement, were used as resilient neuron groups as were the visceral motor neurons from the vagus nerve (CN10). Motor neurons from the lumbar spinal cord (SC) and hypoglossal (CN12) nucleus were utilized as two vulnerable populations. Differential gene expression analysis showed that spinal motor neurons display the most gene dysregulation among the isolated neuron populations. Each disease stage showed distinct gene regulation with only a minority of genes being regulated across stages. Multiple identified dysregulated pathways were consistent with other published data set across SOD1 mutations that could explain their sensitivity in ALS. Resilient CN3/4 motor neurons showed very minor gene dysregulation and appeared unphased by mutant SOD1 overexpression at the investigated time points. Visceral CN10 motor neurons on the other hand showed dysregulation of a number of targets. We are currently in the process of investigating their involvement in presumably protective responses in these unaffected neurons. In conclusion our preliminary transcriptomic analysis in mutant SOD1 ALS expands our knowledge on the molecular pathophysiology during ALS disease progression and gives clues to motor neuron resilience and vulnerability.

Use of ALS-related biomarkers in SMA progression in adult patients treated with nursinersen

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The antisense-oligonucleotide drug administered intrathecally was a possible treatment for motorneuron disorders and was approved for the treatment of SMA Spinal muscular atrophy. SMA is a severe autosomal recessive inherited disorder characterized by degeneration of alpha-motor neurons that results in proximal spinal and bulbar muscle weakness and atrophy. Clinical studies show improvement in motor function, in patients with treatment, particularly in SMA type 1. However, the effect of nusinersen in adult patients with SMA types 2, 3 and 4 is practically unknown. The present work evaluates the modulation of ALS-related biomarkers, another neurodegenerative motorneuron disease, in SMA adult patients and how they response to nusinersen therapy. Thus, the evaluation of specific biomarkers linked to target pathways of other neurodegenerative disorder, in SMA patients, may be a useful tool to assess target engagement in therapeutic trials and the particular treatment response in each individual as well.

Conclusions:

a. Protein levels of studied putative biomarkers for neurodegenerative disorders revealed that pNFH and sAPPβ levels are modulated by nusinersen treatment, being reduced with its administration.

b. There is no correlation between clinical and molecular data, however, our results indicate that there is a relation between pNFH levels in CSF and disease time and age of onset; but not between pNFH levels and age.

c. Stablished biomarkers for ALS and other neurodegenerative disorders are partially useful as SMA-2 and -3 biomarkers due to they do not recapitulate the observed alterations. Further studies are required with young age-matched controls to consolidate results.

Using Telehealth for Delivery of an Intervention-Based RCT

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Introduction

Telehealth enables continuing clinical care and has been widely adopted in ALS centres as a direct result of the COVID-19 pandemic. Prior to the pandemic, uptake of telehealth was slower than anticipated, despite advances in the relevant technology. Factors such as ambivalence on the part of health professionals and inertia in healthcare systems are some of the suggested reasons for this. Research shows telehealth approaches are generally well accepted by ALS patients and caregivers.

Objectives

The aim of this study is to outline the learning emerging from an RCT of two psychosocial interventions with ALS caregivers in Ireland, that moved from in-person to online during the COVID-19 pandemic. The learnings are placed within the context of recent evidence on the use of telehealth approaches in ALS research and care.

Method

The iterative adaptation of an RCT intervention from in-person to an online format is described, with a review of in-person versus online metrics and participant feedback. A synopsis of recent literature in the field of ALS and telehealth contextualises the findings from the adaptation of this study.

Results

The adaptation from in-person to online included moving from clinic-based recruitment to liaising with the local MND charity and contacting potential participants via social media. Ethical amendment was required to accommodate electronic consent (eConsent) and online delivery. Standardised psychometric measures were adapted for online administration. Protocols were developed for risk management and establishing online boundaries.

Conclusions

This study, and other relevant evidence from the literature explores the benefits, challenges, and opportunities of telehealth approaches in ALS that enable continued research and clinical practice amid the global pandemic. The migration of the RCT to online shows the significant potential of telehealth approaches in ALS as well as highlighting the possible losses and challenges involved in moving interventions online.

Validation of the Italian version of the Rasch-Built Overall Amyotrophic Lateral Sclerosis Disability Scale (ROADS).

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Introduction.
The ALS functional rating scale - revised (ALSFRS-R) is the most widely used tool for the clinical monitoring in ALS patients. Despite its usefulness as a multidimensional scale, the combined score derived from different domains is not linearly related to symptom severity. The Rasch-Built Overall ALS Disability Scale (ROADS), which is linearly weighted and unidimensional, has recently been developed to overcome some of these limitations [1].

Objectives.
To validate the Italian version of the ROADS scale and assess the reliability of its administration to patients versus their respective caregivers and the correlation to the corresponding ALSFRS-R.

Methods.
The ROADS Scale questionnaire was administered together with ALSFRS-R to 55 ALS patients during regular follow-up assessments in the Turin ALS Centre. The same questionnaire was administered also to the caregivers, instructing them to compile it according to their own assessment of the patients' functional status. Correlation analysis was performed using Spearman's rho, Bland-Altman difference plots, Cronbach's alpha coefficient and Intraclass correlation coefficient (ICC), one-way random effects were used for proper comparison as appropriate. A value of $p < 0.05$ (two-tailed) was considered significant.

Results.
The median normalized ROADS values did not differ significantly between patients and caregiver (73.0, IQR 60.0-86.0 vs 70.0, IQR 57.0-82.0, p=0.524). Their correlation coefficient was found to be very high (ICC 0.95, p<0.001; Cronbach's alpha coefficient 0.94) and the total score agreement based on Bland-Altman showed no systematic directional bias (mean difference: 0.82, 95% limits of agreement: -15.9-17.6). Stratificating for sex, age classes and caregiver type, we found high ICC values, that did not change significantly among the considered categories. We also found a high correlation between ROADS and ALSFRS-R total score (patients' correlation coefficient: 0.88; caregivers' correlation coefficient: 0.87).

Conclusions.
The ROADS scale is a valid and reliable tool to monitor disease burden, showing a high level of agreement between the responses given by patients and caregivers.

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Validity and feasibility of unsupervised home-based vital capacity testing in patients with MND

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Introduction. Remote monitoring of respiratory function is important in patients with motor neuron disease (MND), especially in individuals who are not able to visit a multidisciplinary clinic. However, its use in MND care is lacking, due to the limited data on validity and feasibility of home-based respiratory function testing.
Objective. We aim to assess the validity and feasibility of unsupervised home-based vital capacity (VC) testing in patients with MND.
Methods. We included 33 patients with MND from two medical centers. During the initial home visit, patients performed a supervised VC test, and filled out the ALSFRS-R and MND dyspnea scale. Patients received instructions from the investigator on proper use of the portable spirometer and practiced performing the VC test. The following day patients performed the unsupervised VC test. The follow-up period was 12 weeks, consisting of 4-weekly unsupervised VC testing, and self-administration of the two questionnaires. At each follow up, patients received a notification by text message or email. All VC tests were performed using a full-face mask, to enable testing in patients with bulbar involvement. At final follow-up the investigator visited patients' homes to perform a supervised VC test and administer a survey on user-experiences. Validity was assessed by comparing the supervised and unsupervised VC at baseline and final follow-up using a paired t-test and Bland-Altman plot. Feasibility was evaluated through user-experiences and adherence.
Results. Preliminary data from 24 patients (mean age=62 years, male=78%, mean ALSFRS-R=35.1, spinal onset=70%) showed that the supervised and unsupervised VC were not significantly different (mean difference=0.064, 95% CI [-0.012 - 0.140], p=0.097), and showed good agreement (SE=0.177). Five patients were assisted with VC testing by a caregiver. Adherence to the 4-weekly VC testing was 100%, and most patients did not perceive it as burdensome (77%). The majority of patients were confident in their ability to perform a VC test (74%) and would like home monitoring of VC in MND care (86%).
Conclusion. Home-based VC testing, with prior training and notifications during follow-up, is a valid method for remote monitoring of respiratory function, and well-received by patients with MND in our cohort.

Variable number tandem repeats in motor neuron disease (MND)

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Introduction:

Tandem repeat sequences are genomic loci composed of a repeating nucleotide motif of variable length. These repeat sequences may be classed by the length of their repeating motif as short tandem repeats, with motifs of up to 6 nucleotides, or variable number tandem repeats (VNTRs) with motifs of >6 nucleotides. To date, more than 40 short tandem repeats are known to cause neurological disease due to repeat expansions, of which 2 have been studied in relation to MND: a hexanucleotide intronic repeat in C9orf72 which represents the largest genetic cause of the disease, and a trinucleotide repeat in ATXN2 which increases susceptibility to ALS. There exist more than 80,000 VNTRs across the genome, however, they have previously proven difficult to study due to their longer repeat length and variable internal structure. Thanks to new sequencing techniques the first VNTR associated with MND was identified in 2019, a 69 nucleotide repeat in the last intron of the WDR7(1). We hypothesise there are more pathogenic VNTRs awaiting discovery.

Objectives:

We aimed to identify novel pathogenic VNTRs associated with MND.

Methods:

We performed the first genome wide screen of VNTRs in any disease, including 30,000 VNTRs, to identify association with MND using whole genome sequencing from the New York Genome Consortium and the Project MINE cohorts. We further refined our screen using epigenetic profiling of motor neurons to focus on areas of the genome most likely to harbour pathogenic variants.

Results and conclusions:
We have continually developed our methods to account for confounding factors. We have found measurements of VNTRs are significantly affected by the tissue source used for sequencing, sequencing platform, and population background and are now able to take these into account in our analyses. We have identified candidate pathogenic VNTRs for further investigation.

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What is Amyotrophic Lateral Sclerosis prevalence?

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Introduction. ALS prevalence is usually calculated considering all patients alive at the last day of the study period among those incidents through out the same interval. Prevalent cases whose diagnosis is performed before the beginning of the study period are excluded. Because of both the short survival and the low frequency of the disease, the exclusion of these cases should lead to a negligible bias in ALS prevalence estimates. However, survival has been proved to largely vary among ALS patients.
Objectives. To assess ALS prevalence and to analyze how this estimate vary according to the historical depth of data collection.
Methods. Data from the PARALS register have been used. Crude prevalence ratio was estimated on December 31, 2015, separately for each of the following time intervals: 2013-2015, 2010-2015, 2007-2015, 2004-2015, 2001-2015, 1998-2015, and 1995-2015. For each time interval, prevalence ratio was calculated globally and stratified by sex, age at diagnosis, and phenotype. Prevalence was also calculated considering patients who underwent tracheotomy during the same study period.
Results. Prevalence ratios increased proportionally to the length of the time period considered, ranging from 6 (95% CI 5.3 - 6.7) when considering the 2013-2015 period to 12.1 (95% CI 11.1 - 13.1) when considering the 1995-2015 period. Prevalence ratio increase was inversely proportional to age at diagnosis, being null in the >85 years class and maximal in the]25-35] age class (+1700%). Among phenotypes, predominant UMN showed the highest increase (from 0.5, 95% CI 0.3 – 0.8, to 2.1, 95% CI 1.7 – 2.6, +320%).
Discussion. Because of the variability of ALS survival, prevalence ratio strongly depends on the length of the follow-up period. A 12-year period resulted to be sufficient to get a reliable estimate of ALS prevalence including long-survival patients.
We believe that our results could be useful for a better definition of ALS burden. Furthermore, since prevalence could be used as a pre-test probability, a more reliable estimate will be crucial to better assess the predictive values of potential diagnostic markers along with other significant measures such as the assessment of heritability.

Zebrafish for the functional analysis of variant pathogenicity in ALS associated genes

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Introduction
Considering the inheritance of Amyotrophic Lateral Sclerosis (ALS), 90% of cases are sporadic and 10% of cases are familial. More than 30 genes have been associated with familial ALS, the major ones being C9ORF72, SOD1, FUS and TARDBP. Mutations within the SOD1 gene account for up to 20% of familial ALS cases worldwide. Genome-wide association and next generation sequencing led respectively to an increasing number of ALS-associated genes and the discovery of multiple new variants.

Molecular diagnosis of ALS is based on the analysis of patients' genetic sequences by seeking a disease-causing variant. The interpretation of variant pathogenicity is referring to an international classification system. According to this system, functional analysis is considered an essential element for variant classification. One of the main issues in molecular diagnosis is the lack of experimental evidence for the effect of rare variants, which complicates the interpretation of their pathogenicity.

Objectives
Our objective is to help molecular diagnosis of ALS by developing Zebrafish as a tool for functional analysis variant pathogenicity.

Method
Candidate variants in SOD1 and OPTN genes are identified by routine genetic testing at Nîmes University Hospital. Variants are overexpressed by microinjection of mRNA in 1-cell stage Zebrafish embryos. Locomotion is assessed in larvae at 2 days post fertilisation using Touch Evoked Escape Response Assay (TEER), followed by analysis of motor neuron morphology by immunostaining.

Results
We validated our model using well known ALS-causing mutations in SOD1 gene: A5V and G94A. Here, we present the study of 4 patient-derived variants of SOD1 gene: D126N; E134del; K137*; I150M. This approach is further applied to OPTN gene using the pathogenic E478G as a control to test the patient-derived variant L492R. Transient overexpression of all tested variants in Zebrafish embryos led to significant reduction of swimming speed and distance in 2 days old larvae. Locomotor impairments were consistent with shorter axonal projections and increased axonal branching in all larvae expressing candidate variants, showing a functional in vivo effect in Zebrafish.

Conclusions
Our approach allows to overcome the challenge of variant pathogenicity interpretation in a simple and efficient way and can be applied to multiple genes. We therefore consider Zebrafish as an efficient tool for

molecular diagnosis of ALS. Moreover, our system has the potential to take patients a step further to treatment in view of the emerging antisense therapies.