UNIVERSITÀ DI MILANO
"CENTRO DINO FERRARI"
PER LA DIAGNOSI E LA TERAPIA DELLE MALATTIE
NEUROMUSCOLARI, NEURODEGENERATIVE E CEREBROVASCOLARI

FONDAZIONE I.R.C.C.S. CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
ISTITUTO DI RICOVERO E CURA A CARATTERE
SCIENTIFICO DI NATURA PUBBLICA

COLLABORAZIONI INTERNAZIONALI

E

FRONTESPIZI

LAVORI SCIENTIFICI

2020

"CENTRO DINO FERRARI"

Sezione di Neuroscienze
Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti
Università degli Studi di Milano
Fondazione I.R.C.C.S. Ca' Granda - Ospedale Maggiore Policlinico

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BRAIN
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New genotype-phenotype correlations in a large European cohort of patients with sarcoglycanopathy

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Sarcoglycanopathies comprise four subtypes of autosomal recessive limb-girdle muscular dystrophies (LGMDR3, LGMDR4, LGMDR5 and LGMDR6) that are caused, respectively, by mutations in the *SGCA*, *SGCB*, *SGCG* and *SGCD* genes. In 2016, several clinicians involved in the diagnosis, management and care of patients with LGMDR3–6 created a European Sarcoglycanopathy Consortium. The aim of the present study was to determine the clinical and genetic spectrum of a large cohort of patients with sarcoglycanopathy in Europe. This was an observational retrospective study. A total of 33 neuromuscular centres from 13 different European countries collected data of the genetically confirmed patients with sarcoglycanopathy followed-up at their centres. Demographic, genetic and clinical data were collected for this study. Data from 439 patients from 13 different countries were collected. Forty-three patients were not included in the analysis because of insufficient clinical information available. A total of 159 patients had a confirmed diagnosis of LGMDR3, 73 of LGMDR4, 157 of LGMDR5 and seven of LGMDR6. Patients with LGMDR3 had a later onset and slower progression of the disease. Cardiac involvement was most frequent in LGMDR4. Sixty per cent of LGMDR3 patients carried one of the following mutations, either in a homozygous or heterozygous state: c.229C>T, c.739G>A or c.850C>T. Similarly, the most common mutations in LMGDR5 patients were c.525delT or c.848G>A. In LGMDR4 patients the most frequent mutation was c.341C>T. We identified onset of symptoms before 10 years of age and residual protein expression lower than 30% as independent risk factors for losing ambulation before 18 years of age, in LGMDR3, LGMDR4 and LGMDR5 patients. This study reports clinical, genetic and protein data of a large European cohort of patients with sarcoglycanopathy. Improving our knowledge about these extremely rare autosomal recessive forms of LGMD was helped by a collaborative effort of neuromuscular centres across Europe. Our study provides important data on the genotype-phenotype correlation that is relevant for the design of natural history studies and upcoming interventional trials in sarcoglycanopathies.

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Keywords: limb girdle muscular dystrophies; sarcoglycan; registries; treatment; cohort

Abbreviation: LGMD = limb girdle muscular dystrophy

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Muscle MRI in two SMA patients on nusinersen treatment: A two years follow-up

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ABSTRACT

Introduction: The effects of nusinersen in adults with SMA rely on neuromotor function scales and qualitative assessments. There are limited clinical or imaging data on muscle changes over time.
Methods: Two adult SMA patients underwent clinical assessments including measures of upper and lower limb function with Revised Upper Limb Module (RULM) and Hammersmith Function Motor Scale Expanded (HFMSE); both patients were also studied with whole-body muscle MRI (T1-weighted and Diffusion Tensor Imaging/DTI sequences), at baseline and after 10 and 24 months from the beginning of treatment with nusinersen.
Results: After two years of treatment, HFMSE and RULM scores were stable in both patients. DTI sequences revealed an increased number, length and organization of muscle fiber tracks, and Fractional Anisotropy (FA) values showed a significant reduction after 10 and 24 months from baseline, in their corresponding maps.
Discussion: Muscle DTI imaging seems to play an interesting role to monitor treatment effects over time in adult SMA patients.

1. Introduction

Spinal Muscular Atrophy (SMA) is an autosomal-recessive disorder caused by mutations in *SMN1* gene, leading to degeneration of alpha motor neurons in the spinal cord resulting in progressive muscle weakness and disability [1–3]. The results from the pivotal [4] and more recent trials [5] with intrathecal nusinersen treatment, as well as the results from real world data [6], have created great expectations in older patients having long-lasting muscular atrophy and weakness; in fact there is growing evidence that treatment is at the very least stabilizing or providing some degree of improvement in motor function [7]. However, there are still some critical issues in understanding results in adults, including the lack of natural history data and outcome measures to monitor disease progression [8]. Furthermore limited data are available on muscle degeneration and neurophysiology over time, and this is often a limiting factor in interpreting the impact of any treatment in these patients. In several neuromuscular disorders, muscle MRI is increasingly used to identify a specific pattern of muscular involvement [9,10], to monitor progression [11], to quantify the effects of treatment on muscle structure [12], and in some pharmacological trials as a potential biomarker [13]. Only a few MRI studies have described the pattern of muscular involvement in the different subtypes of SMA [14–16]. More recently, Brogna and colleagues [17] described the pattern of muscular involvement on MRI and its large variability in both type 2 and type 3 SMA patients.

MRI performed with conventional pulse sequences (e.g., T1 and T2-weighted sequences) provides only gross information on muscle structure; more specific technique such as Dixon MRI sequence allows to quantify the amount of fat present in an area of skeletal muscle of interest [18], however, since early pathological changes often start at cellular or fascicular level, it is not able to detect early or subtle changes [19]. Diffusion tensor imaging (DTI) is a relatively new, quantitative,

Abbreviations: DTI, Diffusion Tensor Imaging; FA, Fractional Anisotropy; HFMSE, Hammersmith Function Motor Scale Expanded; MRC, Medical Research Council; MRI, Magnetic Resonance Imaging; RULM, Revised Upper Limb Module; SMA, Spinal Muscular Atrophy; *SMN1*, Survival of Motor Neuron 1 gene

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RESEARCH ARTICLE

Genetic modifiers of respiratory function in Duchenne muscular dystrophy

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[Corrections added on 14 May 2020 after first publication: The 5th affiliation has been corrected.]

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Abstract

Objective: Respiratory insufficiency is a major complication of Duchenne muscular dystrophy (DMD). Its progression shows considerable interindividual variability, which has been less thoroughly characterized and understood than in skeletal muscle. We collected pulmonary function testing (PFT) data from a large retrospective cohort followed at Centers collaborating in the Italian DMD Network. Furthermore, we analyzed PFT associations with different *DMD* mutation types, and with genetic variants in *SPP1*, *LTBP4*, *CD40*, and *ACTN3*, known to modify skeletal muscle weakness in DMD. Genetic association findings were independently validated in the Cooperative International Neuromuscular Research Group Duchenne Natural History Study (CINRG-DNHS). **Methods and Results**: Generalized estimating equation analysis of 1852 PFTs from 327 Italian DMD patients, over an average follow-up time of 4.5 years,

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ORIGINAL COMMUNICATION

MRI patterns of muscle involvement in type 2 and 3 spinal muscular atrophy patients

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Abstract

Only few studies have reported muscle involvement in spinal muscular atrophy using muscle MRI but this has not been systematically investigated in a large cohort of both pediatric and adult patients with type 2 and type 3 spinal muscular atrophy. The aim of the present study was to define possible patterns of muscle involvement on MRI, assessing both fatty replacement and muscle atrophy, in a cohort of type 2 and type 3 spinal muscular atrophy children and adults (age range 2–45 years), including both ambulant and non-ambulant patients. Muscle MRI protocol consisted in T1-weighted sequences acquired on axial plane covering the pelvis, the thigh, and the leg with contiguous slices. Each muscle was examined through its whole extension using a grading system that allows a semiquantitative evaluation of fatty infiltration. Thigh muscles were also grouped in anterior, posterior, and medial compartment for classification of global atrophy. The results showed a large variability in both type 2 and type 3 spinal muscular atrophy, with a various degree of proximal to distal gradient. Some muscles, such us the adductor longus and gracilis were always selectively spared. In all patients, the involvement was a combination of muscle atrophy and muscle infiltration. The variability observed may help to better understand both natural history and response to new treatments.

Keywords Spinal muscular atrophy · Magnetic Resonance Imaging · Fatty infiltration · Muscle atrophy

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder caused by mutations in the survival motor neuron 1 (SMN1) gene, which result in insufficient expression of the survival motor neuron (SMN) protein leading to spinal motor neuron degeneration and subsequent muscle atrophy and weakness [1]. SMA is classified by the maximal achieved motor milestone in three different forms: Type 1 (patients never achieve the ability to sit independently), Type 2 (sit but never walk independently), Type 3 (stand and walk independently) [2].

Over the last few years, new therapeutic approaches have become available [3–7]. The results of the clinical trials and of the first real world studies clearly indicate efficacy of the new treatments, even though there is some variability in the responses observed after treatment [3–7]. It has been hypothesized that the different responses may at least be partially due to a different involvement of the skeletal muscles. Patients showing diffuse atrophy and intramuscular fatty replacement are potentially more at risk of showing less functional improvements than those with relatively preserved muscles. It has therefore become important to define the spectrum and the overall pattern of muscle involvement using muscle imaging tools such as muscle Magnetic Resonance Imaging (MRI). Only a few studies have reported MRI findings in in the three types of SMA: a seminal paper in the early 1990s [8] reports that in type 1 there is a severe and

Claudia Brogna, Lara Cristiano, and Tommaso Verdolotti first authors.

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Meta-Analysis

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Journal of Comparative Effectiveness Research

Meta-analyses of ataluren randomized controlled trials in nonsense mutation Duchenne muscular dystrophy

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Aim: Assess the totality of efficacy evidence for ataluren in patients with nonsense mutation Duchenne muscular dystrophy (nmDMD). **Materials & methods:** Data from the two completed randomized controlled trials (ClinicalTrials.gov: NCT00592553; NCT01826487) of ataluren in nmDMD were combined to examine

Future Medicine

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Primary mitochondrial myopathy

Clinical features and outcome measures in 118 cases from Italy

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Abstract

Objective

To determine whether a set of functional tests, clinical scales, patient-reported questionnaires, and specific biomarkers can be considered reliable outcome measures in patients with primary mitochondrial myopathy (PMM), we analyzed a cohort of Italian patients.

Methods

Baseline data were collected from 118 patients with PMM, followed by centers of the Italian network for mitochondrial diseases. We used the 6-Minute Walk Test (6MWT), Timed Up-and-Go Test (x3) (3TUG), Five-Times Sit-To-Stand Test (5XSST), Timed Water Swallow Test (TWST), and Test of Masticating and Swallowing Solids (TOMASS) as functional outcome measures; the Fatigue Severity Scale and West Haven-Yale Multidimensional Pain Inventory as patient-reported outcome measures; and FGF21, GDF15, lactate, and creatine kinase (CK) as biomarkers.

Results

A total of 118 PMM cases were included. Functional outcome measures (6MWT, 3TUG, 5XSST, TWST, and TOMASS) and biomarkers significantly differed from healthy reference values and controls. Moreover, functional measures correlated with patients' perceived fatigue and pain severity. Patients with either mitochondrial or nuclear DNA point mutations performed worse in functional measures than patients harboring single deletion, even if the latter had an earlier age at onset but similar disease duration. Both the biomarkers FGF21 and GDF15 were significantly higher in the patients compared with a matched control population; however, there was no relation with severity of disease.

Conclusions

We characterized a large cohort of PMM by evaluating baseline mitochondrial biomarkers and functional scales that represent potential outcome measures to monitor the efficacy of treatment in clinical trials; these outcome measures will be further reinvestigated longitudinally to define the natural history of PMM.

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Long-term follow-up of patients with type 2 and non-ambulant type 3 spinal muscular atrophy (SMA) treated with olesoxime in the OLEOS trial

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Abstract

In a previous Phase 2 study, olesoxime had a favorable safety profile. Although the primary endpoint was not met, analyses suggested that olesoxime might help in the maintenance of motor function in patients with Types 2/3 SMA. This open-label extension study (OLEOS) further characterizes the safety, tolerability and efficacy of olesoxime over longer therapy durations. In OLEOS, no new safety risks were identified. Compared to matched natural history data, patients treated with olesoxime demonstrated small, non-significant changes in motor function over 52 weeks. Motor function scores were stable for 52 weeks but declined over the remainder of the study. The greatest decline in motor function was seen in patients ≤15 years old, and those with Type 2 SMA had faster motor function decline versus those with Type 3 SMA. Previous treatment with olesoxime in the Phase 2 study was not protective of motor function in OLEOS. Respiratory outcomes were stable in patients with Type 3 SMA >15 years old but declined in patients with Type 2 SMA and in patients with Type 3 SMA ≤15 years

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The Genetic Landscape of Dystrophin Mutations in Italy: A Nationwide Study

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Dystrophinopathies are inherited diseases caused by mutations in the dystrophin (*DMD*) gene for which testing is mandatory for genetic diagnosis, reproductive choices and eligibility for personalized trials. We genotyped the *DMD* gene in our Italian cohort of 1902 patients (BMD n = 740, 39%; DMD n =1162, 61%) within a nationwide study involving 11 diagnostic centers in a 10-year window (2008–2017). In DMD patients, we found deletions

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Sodium Channel Myotonia Due to Novel Mutations in Domain I of $Na_V1.4$

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Sodium channel myotonia is a form of muscle channelopathy due to mutations that affect the $Na_V1.4$ channel. We describe seven families with a series of symptoms ranging from asymptomatic to clearly myotonic signs that have in common two novel mutations, p.Ile215Thr and p.Gly241Val, in the first domain of the $Na_V1.4$ channel. The families described have been clinically and genetically evaluated. p.Ile215Thr and p.Gly241Val lie, respectively, on extracellular and intracellular loops of the first domain of the $Na_V1.4$ channel. We assessed that the p.Ile215Thr mutation can be related to a founder effect in people from Southern Italy. Electrophysiological evaluation of the channel function showed that the voltage dependence of the activation for both the mutant channels was significantly shifted toward hyperpolarized potentials (Ile215Thr: -28.6 ± 1.5 mV and Gly241Val: -30.2 ± 1.3 mV vs. WT: -18.5 ± 1.3 mV). The slow inactivation was also significantly affected, whereas fast inactivation showed a different behavior in the two mutants. We characterized two novel mutations of the *SCN4A* gene expanding the knowledge about genetics of mild forms of myotonia, and we present, to our knowledge, the first homozygous patient with sodium channel myotonia.

Keywords: myotonia, sodium channel myotonia, founder effect, channelopathy, Na_v 1.4, mexiletine

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INTRODUCTION

Myotonia is an impaired muscle relaxation after a voluntary muscle contraction and is the main feature of a group of heterogeneous skeletal muscle channelopathies named non-dystrophic myotonias (NDMs). NDMs are caused by mutations in *CLCN1* and *SCN4A* genes, coding, respectively, for the chloride (ClC-1) and sodium ($Na_V1.4$) muscle channels (1).

$Na_V1.4$, the α-subunit of the sodium channel complex, mainly expressed in skeletal muscle, is formed by 1836 amino acids and displays a tetrameric structure composed of 4 domains (DI-DIV), each including six transmembrane α-helices (S1–S6). The inner part of the channel contains a pore (S5–S6 from each domain) where sodium ions flow through thanks to four voltage sensors

ANNALS of Clinical and Translational Neurology
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BRIEF COMMUNICATION

Dystonia-ataxia syndrome with permanent torsional nystagmus caused by ECHS1 deficiency

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Abstract

Biallelic mutations in *ECHS1*, encoding the mitochondrial enoyl-CoA hydratase, have been associated with mitochondrial encephalopathies with basal ganglia involvement. Here, we describe a novel clinical presentation consisting of dystonia-ataxia syndrome with hearing loss and a peculiar torsional nystagmus observed in two adult siblings. The presence of a 0.9-ppm peak at MR spectroscopy analysis suggested the accumulation of branched-chain amino acids. Exome sequencing in index probands identified two *ECHS1* mutations, one of which was novel (p.V82L). ECHS1 protein levels and residual activities were reduced in patients' fibroblasts. This paper expands the phenotypic spectrum observed in patients with impaired valine catabolism.

Introduction

ECHS1 encodes the mitochondrial enoyl-CoA hydratase which catalyzes the fourth degradation step of the branched-chain amino acid (BCAA) valine but also takes part in the beta-oxidation of short-chain fatty acids, contributing to energy metabolism.[1]

Biallelic mutations in *ECHS1* (OMIM 612677) have been mainly associated with early-onset Leigh-like syndrome (LLS) presenting severe progressive encephalopathy and signs of mitochondrial dysfunction including epilepsy, optic atrophy and hearing loss.[2] Cardiac abnormalities were also observed.[3] Prognosis is poor, with the mean age at death of 69 months although patients reaching adulthood have been described.[4] Additional clinical symptoms might include: *cutis laxa*,[5] paroxysmal exercise-induced dyskinesia[6] and dystonia.[7]

Increased serum lactate levels and elevated urinary concentrations of 2-methyl-2,3-dihydroxybutyrate and N-acetyl-S-(2-carboxypropyl)cysteine have been reported.[8] Reduced activities of pyruvate dehydrogenase and respiratory chain complexes have been linked with the accumulation of acryloyl-CoA and methacrylyl-CoA, originating from incomplete valine catabolism.[9] However, these abnormalities are detectable only in a subset of severe clinical courses.

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BRIEF RESEARCH REPORT
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TYMP Variants Result in Late-Onset Mitochondrial Myopathy With Altered Muscle Mitochondrial DNA Homeostasis

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Biallelic *TYMP* variants result in the mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), a juvenile-onset disorder with progressive course and fatal outcome. Milder late-onset (>40 years) form has been rarely described. Gene panel sequencing in a cohort of 60 patients featuring muscle accumulation of mitochondrial DNA (mtDNA) deletions detected *TYMP* defects in three subjects (5%), two of them with symptom onset in the fifth decade. One of the patients only displayed ptosis and ophthalmoparesis. Biochemical and molecular studies supported the diagnosis. Screening of *TYMP* is recommended in adult patients with muscle mtDNA instability, even in the absence of cardinal MNGIE features.

Keywords: mitochondrial myopathy, mitochondrial DNA instability, *TYMP*, mitochondrial neurogatrointestinal encephalomyopathy, mitochondrial DNA replication

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INTRODUCTION

Thymidine phosphorylase (TP) is a cytosolic enzyme that catalyzes the conversion of pyrimidine nucleosides thymidine and deoxyuridine into the corresponding bases by releasing 2-deoxy-1-phosphate ribose. Loss of TP activity results in the marked accumulation of its substrates, reaching toxic levels in plasma and other tissues (Spinazzola et al., 2002). Mitochondrial DNA (mtDNA) lacks an effective mismatch repair system (Bohr and Anson, 1999) and is particularly susceptible to dNTP imbalance due to TP deficiency (Hirano et al., 2012). As a consequence, quantitative and qualitative mtDNA changes might be found in affected tissues, including muscle (Papadimitriou et al., 1998).

Loss of function changes in *TYMP*, the gene ending TP, have been associated with a peculiar clinical presentation known as mitochondrial neurogastrointestinal encephalomyopathy (MNGIE, MIM 603041) featuring the following clinical hallmarks: extraocular muscle weakness, gastrointestinal (GI) dysmotility, cachexia, sensorimotor peripheral neuropathy, and leukoencephalopathy (Nishino et al., 1999). The vast majority of cases (>95%) exhibit an onset before the age of 20 years with a progressive course leading to a fatal outcome within the fourth decade (classical form) (Garone et al., 2011). Four late-onset (beyond 40 years of age) MNGIE cases have been described: they presented mild symptoms (including GI disturbances) and slow

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CLINICAL REPORT

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MYH2 myopathy, a new case expands the clinical and pathological spectrum of the recessive form

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Abstract

Background: Hereditary myosin myopathies are a group of rare muscle disorders, caused by mutations in genes encoding for skeletal myosin heavy chains (MyHCs). MyHCIIa is encoded by *MYH2* and is expressed in fast type 2A and 2B muscle fibers. *MYH2* mutations are responsible for an autosomal dominant (AD) progressive myopathy, characterized by the presence of rimmed vacuoles and by a reduction in the number and size of type 2A fibers, and a recessive early onset myopathy characterized by complete loss of type 2A fibers. Recently, a patient with a homozygous mutation but presenting a dominant phenotype has been reported.

Methods: The patient was examined thoroughly and two muscle biopsies were performed through the years. NGS followed by confirmation in Sanger sequencing was used to identify the genetic cause.

Results: We describe the second case presenting with late-onset ophthalmoparesis, ptosis, diffuse muscle weakness, and histopathological features typical for AD forms but with a recessive *MYH2* genotype.

Conclusion: This report contributes to expand the clinical and genetic spectrum of *MYH2* myopathies and to increase the awareness of these very rare diseases.

KEYWORDS

MYH2, myosin heavy chain myopathy, ophthalmoplegia, rimmed vacuoles

1 | INTRODUCTION

Hereditary myosin myopathies are a group of muscle disorders with variable age of onset and heterogeneous clinical features, caused by mutations in the skeletal muscle myosin heavy chain (MyHC) genes which are organized in a multigene cluster on chromosome 17 (Oldfors, 2007). In adult human skeletal muscle, there are three major MyHC isoforms: MyHC I (slow/ß-cardiac MyHC) encoded by *MYH7* (OMIM *160760) and expressed in slow type 1 muscle fibers and in heart ventricles; MyHC IIa, encoded by *MYH2* (OMIM *160740) and expressed in fast type 2A and 2B muscle fibers, and MyHC IIx, encoded

Roberta Telese and Serena Pagliarani have equally contributed to this work.

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LETTER TO THE EDITOR

Pediatric anti-HMGCR necrotizing myopathy: diagnostic challenges and literature review

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Dear Editor-in-Chief,

A 7-year-old boy presented with a 2-month subtle onset, progressive lower limb weakness with marked difficulty in getting up from the ground. Creatine kinase (CK) level at presentation was 10,000 U/L. No previous infection was reported, and family history was apparently negative for neuromuscular disorders. Neurological exam revealed proximal weakness of all limbs (Medical Research Council [MRC] grade 4, more pronounced at the level of iliopsoas muscles, MRC grade 3) and need for one-hand support to get up from the ground. Multiplex Ligation-Dependent Probe Amplification (MLPA) analysis of Duchenne Muscular Dystrophy (DMD) gene showed no deletion or duplication. Therefore, the patient underwent muscle biopsy (left biceps brachii), which showed a dystrophic pattern with numerous necrotizing and rare regenerating fibers (Fig. 1). In immunohistochemistry analyses, dystrophin and beta-sarcoglycan binding alterations were observed in rare fibers, but western blot analyses highlighted dystrophin, alpha-dystroglycan, calpain-3, dysferlin, and α- to δ-sarcoglycans normal molecular weights and amounts. Fukutin-related protein and acid alpha-glucosidase genetic investigation also resulted normal. Limb-girdle muscular dystrophy (LGMD) next generation sequencing (NGS) panel did not reveal any known pathogenic mutations. Lower limb muscle magnetic resonance imaging (MRI) showed relative hypotrophy of proximal muscles (i.e., gluteus maximus, thigh adductors, and posterior compartment of thigh) with only mild fibro-adipose substitution but without significant signs of edema (Fig. 2). Echocardiography was normal.

During the next 4 months, while work-up results were pending, no therapy was administered. However, the patient complained of rapidly progressive fatigue, weight reduction with mild swallowing dysfunction, difficulty in climbing stairs, and loss of running ability. Simultaneously, the patient developed dermatologic manifestations characterized by small bald patches of alopecia on the scalp, self-limiting recurring roughening, and cracking of hand fingertips and erythematous papular lesions on ears and extensor surface of elbows and knees (Fig. 1).

Four months after the first assessment, neurological examination evidenced four limb muscles weakness (MRC grade between 3 and 4) and diffuse loss of muscular tone involving axial muscles of trunk and head, along with complete Gowers' sign. Spirometry showed restrictive ventilatory defect of moderate severity (FVC 65%, FEV1 76%, FEV1/FVC 100% of predicted). The patient scored 18/34 at the North Star Ambulatory Assessment (NSAA) and reached 375 m at the 6 Minutes Walking Test (6MWT). CK level was only slightly reduced (8501 U/L, reference values 38-247). Re-evaluation of muscle biopsy highlighted sarcolemmal and sarcoplasmic positivity of anti-major histocompatibility complex (MHC)-I antibodies in some fibers and complement deposition on muscle fiber membranes and capillaries (Fig. 1). Autoimmune serologic screening revealed anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) antibody positivity (268 arbitrary units [AU], reference level < 20), while all the other myositis-specific and myositis-associated autoantibodies tested were negative (i.e., anti-Jo1, anti-PL-7, anti-PL-12, anti-Mi-2, anti-SRP-S4, anti-Scl70, anti-Ro/SSA, anti-La/SSB, anti-PM/Scl, and anti-Ku). Four limbs MRI showed the appearance of short-tau inversion-recovery (STIR) signal hyperintensity, particularly at the level of distal leg muscles (i.e., triceps surae, tibial posterior, and peroneus

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BMC Neurology

CASE REPORT Open Access

Hereditary hemorrhagic telangiectasia associated with cortical development malformation due to a start loss mutation in ENG

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Abstract

Background: Hereditary hemorrhagic telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is a rare disorder characterized by recurrent epistaxis, telangiectasias and systemic arteriovenous malformations (AVMs). HHT is associated with mutations in genes encoding for proteins involved in endothelial homeostasis such as *ENG* (endoglin) and *ACVRL1* (activin receptor-like kinase-1).

Case presentation: Here we describe a 22-year-old male presenting with a transient episode of slurred speech and left arm paresis. Brain MRI displayed polymicrogyria. A right-to-left shunt in absence of an atrial septum defect was noted. Chest CT revealed multiple pulmonary AVMs, likely causing paradoxical embolism manifesting as a transient ischemic attack. The heterozygous *ENG* variant, c.3G > A (p.Met1Ile), was detected in the patient. This variant was also found in patient's mother and in his younger brother who displayed cortical dysplasia type 2.

Conclusions: The detection of cortical development malformations in multiple subjects from the same pedigree may expand the phenotypic features of ENG-related HHT patients. We suggest considering HHT in young patients presenting with acute cerebral ischemic events of unknown origin.

Keywords: Cerebrovascular disorders, ENG, Hereditary hemorrhagic telangiectasia, Stroke, Case report

Background

Hereditary hemorrhagic telangiectasia (HHT, ORPHA774) or Rendu–Osler–Weber syndrome, is a rare vascular disorder characterized by telangiectasias and arteriovenous malformations (AVMs) of skin, mucosae and internal organs [1]. The incidence, 1:5000–1:8000 worldwide, is likely underestimated because of the reduced age-related penetrance and variable clinical expression. Pulmonary and cerebral AVMs have been detected in 24–40% [2, 3] and 10–20% [4] of HHT patients, respectively. Although AVMs often remain clinically silent, cerebral AVMs rupture may cause intracerebral hemorrhage resulting in increased morbidity and mortality. Moreover, HHT patients may experience ischemic stroke or cerebral abscess because of paradoxical embolism due to the right-to-left shunting associated with pulmonary AVMs.

Clinical diagnosis can be achieved according to Curaçao criteria: 1) spontaneous and recurrent epistaxis; 2) multiple telangiectasia affecting lips, fingers, and nose; 3)

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REVIEW

Management of patients with neuromuscular disorders at the time of the SARS-CoV-2 pandemic

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Abstract

The novel Coronavirus disease-19 (COVID-19) pandemic has posed several challenges for neuromuscular disorder (NMD) patients. The risk of a severe course of SARS-CoV-2 infection is increased in all but the mildest forms of NMDs. High-risk conditions include reduced airway clearance due to oropharyngeal weakness and risk of worsening with fever, fasting or infection Isolation requirements may have an impact on treatment regimens administered in hospital settings, such as nusinersen, glucosidase alfa, intravenous immunoglobulin, and rituximab infusions. In addition, specific drugs for SARS-CoV2 infection under investigation impair neuromuscular function significantly; chloroquine and azithromycin are not recommended in myasthenia gravis without available ventilatory support and prolonged prone positioning may influence options for treatment. Other therapeutics may affect specific NMDs (metabolic, mitochondrial, myotonic diseases) and experimental approaches for Coronavirus disease 2019 may be offered "compassionately" only after consulting the patient's NMD specialist. In parallel, the reorganization of hospital and outpatient services may change the management of non-infected NMD patients and their caregivers, favouring at-distance approaches. However, the literature on the validation of telehealth in this subgroup of patients is scant. Thus, as the first wave of the pandemic is progressing, clinicians and researchers should address these crucial open issues to ensure adequate caring for NMD patients. This manuscript summarizes available evidence so far and provides guidance for both general neurologists and NMD specialists dealing with NMD patients in the time of COVID-19.

Keywords Neuromuscular disorders · COVID-19 · Telemedicine · Vaccine · Pandemic · Disease-modifying therapies · Neuromuscular disorder centres · Ventilatory support

Introduction

Since the end of December 2019, the severe acute respiratory syndrome virus 2 (SARS-CoV-2) pandemic has claimed the lives of more than 400,000 individuals worldwide (https://coronavirus.jhu.edu/map.html). Symptomatic SARS-CoV-2 infection causes a wide spectrum of symptoms (referred to as "Coronavirus Disease 2019", COVID-19), such as fever, dry cough, and fatigue in milder cases and systemic manifestations in severe disease courses (Fig. 1). In parallel, SARS-CoV-2 infection poses a greater risk for old, oncologic, and immunosuppressed patients, which also include many individuals with hereditary and acquired neuromuscular disorders (NMD) that may already present increased risks due to the underlying disease (see "Risk assessment and stratification" section). As already reported in other papers, the first phase of the SARS-CoV-2 pandemic has seen the overwhelming access of COVID-19 patients to the Emergency Departments prompting an urgent reorganization of personnel and facilities in worst-hit areas, such as Wuhan [1], New York [2, 3] and Lombardy [4, 5]; this reallocation of resources has also imposed changes in the short- and mid-term management of NMD outpatients and non-urgent cases, favouring the use of at-distance approaches

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Review

Neural Stem Cell Transplantation for Neurodegenerative Diseases

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Abstract: Neurodegenerative diseases are disabling and fatal neurological disorders that currently lack effective treatment. Neural stem cell (NSC) transplantation has been studied as a potential therapeutic approach and appears to exert a beneficial effect against neurodegeneration via different mechanisms, such as the production of neurotrophic factors, decreased neuroinflammation, enhanced neuronal plasticity and cell replacement. Thus, NSC transplantation may represent an effective therapeutic strategy. To exploit NSCs' potential, some of their essential biological characteristics must be thoroughly investigated, including the specific markers for NSC subpopulations, to allow profiling and selection. Another key feature is their secretome, which is responsible for the regulation of intercellular communication, neuroprotection, and immunomodulation. In addition, NSCs must properly migrate into the central nervous system (CNS) and integrate into host neuronal circuits, enhancing neuroplasticity. Understanding and modulating these aspects can allow us to further exploit the therapeutic potential of NSCs. Recent progress in gene editing and cellular engineering techniques has opened up the possibility of modifying NSCs to express select candidate molecules to further enhance their therapeutic effects. This review summarizes current knowledge regarding these aspects, promoting the development of stem cell therapies that could be applied safely and effectively in clinical settings.

Keywords: neuronal stem cells; neural subpopulation; neurodegenerative disease; cell therapy

1. Introduction

Neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), are highly disabling and ultimately fatal disorders affecting millions of individuals worldwide, with increasing incidence and prevalence [1]. These diseases greatly impact both patients and their caregivers, but no curative therapies are currently available to arrest or reverse their progression. Thus, effective therapeutic approaches are urgently needed.

In neurodegenerative diseases, specific subsets of neurons, such as dopaminergic and cholinergic neurons or motor neurons (MNs), progressively degenerate, resulting in a specific pattern of nervous

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ORIGINAL ARTICLE

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Nusinersen treatment and cerebrospinal fluid neurofilaments: An explorative study on Spinal Muscular Atrophy type 3 patients

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Abstract

The antisense oligonucleotide Nusinersen has been recently licensed to treat spinal muscular atrophy (SMA). Since SMA type 3 is characterized by variable phenotype and milder progression, biomarkers of early treatment response are urgently needed. We investigated the cerebrospinal fluid (CSF) concentration of neurofilaments in SMA type 3 patients treated with Nusinersen as a potential biomarker of treatment efficacy. The concentration of phosphorylated neurofilaments heavy chain (pNfH) and light chain (NfL) in the CSF of SMA type 3 patients was evaluated before and after six months since the first Nusinersen administration, performed with commercially available enzyme-linked immunosorbent assay (ELISA) kits. Clinical evaluation of SMA patients was performed with standardized motor function scales. Baseline neurofilament levels in patients were comparable to controls, but significantly decreased after six months of treatment, while motor functions were only marginally ameliorated. No significant correlation was observed between the change in motor functions and that of neurofilaments over time. The reduction of neurofilament levels suggests a possible early biochemical effect of treatment on axonal degeneration, which may precede changes in motor performance. Our study mandates further investigations to assess neurofilaments as a marker of treatment response.

KEYWORDS

neurofilaments, Nusinersen, pharmacodynamics biomarker, spinal muscular atrophy

ORIGINAL ARTICLE

Value of insoluble PABPN1 accumulation in the diagnosis of oculopharyngeal muscular dystrophy

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Keywords:
oculopharyngeal musclular dystrophy, PABPN1 accumulations, PABPN1 immunofluorescence, rimmed vacuoles, tubulofilamentous intranuclear inclusions

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Background and purpose: The aim was to assess the value of insoluble PABPN1 muscle fibre nuclei accumulation in the diagnosis of atypical cases of oculopharyngeal muscular dystrophy (OPMD).

Methods: Muscle biopsies from a selected cohort of 423 adult patients from several Italian neuromuscular centres were analysed by immunofluorescence: 30 muscle biopsies of genetically proven OPMD, 30 biopsies from patients not affected by neuromuscular disorders, 220 from genetically undiagnosed patients presenting ptosis or swallowing disturbances, progressive lower proximal weakness and/or isolated rimmed vacuoles at muscle biopsy and 143 muscle biopsies of patients affected by other neuromuscular diseases.

Results: The detection of insoluble nuclear PABPN1 accumulation is rapid, sensitive (100%) and specific (96%). The revision of our cohort allowed us to discover 23 new OPMD cases out of 220 patients affected with nonspecific muscle diseases.

Conclusions: Oculopharyngeal muscular dystrophy is often misdiagnosed leading to diagnosis delay, causing waste of time and resources. A great number of these cases present symptoms and histological findings frequently overlapping with other muscle diseases, i.e. inclusion body myositis and progressive external ophthalmoplegia. PABPN1 nuclear accumulation is a reliable method for diagnostic purposes and it is safe and useful in helping pathologists and clinicians to direct genetic analysis in the case of suspected OPMD, even when clinical and histological clues are deceptive.

Introduction

Oculopharyngeal muscular dystrophy (OPMD) is a late-onset muscle disease, clinically characterized by ptosis, with or without ophthalmoparesis, dysphagia and proximal weakness. Inheritance can be autosomal dominant or recessive [1–3]. OPMD is caused by a GCN repeat expansion in the poly-A binding protein nuclear 1 gene (*PABPN1*) (14q11.2–q13), which leads to an expanded polyalanine tract in the N-terminal of the PABPN1 protein. Normal repeat size is (GCN)10, and (GCN)11–18 is considered pathological. Poly-A expansion in PABPN1 favours the accumulation of toxic, insoluble protein deposits in the nuclei of muscle cells [4]. The point mutation c.35G>C in *PABPN1* can also affect disease phenotype [1].

Oculopharyngeal muscular dystrophy is often overlooked and diagnosed after considerable delay, prolonging discomfort for patients and their relatives and wasting time and resources [6,7]. Histopathological findings of muscle biopsies from affected patients are overall nonspecific; however, rimmed vacuoles and oxidative stain alterations along with scattered

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Limb girdle muscular dystrophy due to *LAMA2* gene mutations: new mutations expand the clinical spectrum of a still challenging diagnosis

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Mutations in *LAMA2* gene, encoding merosin, are generally responsible of a severe congenital-onset muscular dystrophy (CMD type 1A) characterized by severe weakness, merosin absence at muscle analysis and white matter alterations at brain Magnetic Resonance Imaging (MRI). Recently, *LAMA2* mutations have been acknowledged as responsible of LGMD R23, despite only few cases with slowly progressive adult-onset and partial merosin deficiency have been reported. We describe 5 independent Italian subjects presenting with progressive limb girdle muscular weakness, brain white matter abnormalities, merosin deficiency and *LAMA2* gene mutations. We detected 7 different mutations, 6 of which are new. All patients showed normal psicomotor development and slowly progressive weakness with onset spanning from childhood to forties. Creatin-kinase levels were moderately elevated. One patient showed dilated cardiomyopathy. Muscle MRI allowed to evaluate the degree and pattern of muscular involvement in all patients. Brain MRI was fundamental in order to address and/or support the molecular diagnosis, showing typical widespread white matter hyperintensity in T2-weighted sequences. Interestingly these alterations were associated with central nervous system involvement in 3 patients who presented epilepsy and migraine. Muscle biopsy commonly but not necessarily revealed dystrophic features. Western-blot was usually more accurate than immunohystochemical analysis in detecting merosin deficiency. The description of these cases further enlarges the clinical spectrum of *LAMA2*-related disorders. Moreover, it supports the inclusion of LGMD R23 in the new classification of LGMD. The central nervous system involvement was fundamental to address the diagnosis and should be always included in the diagnostic work-up of undiagnosed LGMD.

Key words: limb girdle muscular dystrophy, merosin, *LAMA2* gene, brain MRI, muscle MRI, leukoencephalopathy

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ORIGINAL ARTICLES

Estimating the impact of COVID-19 pandemic on services provided by Italian Neuromuscular Centers: an Italian Association of Myology survey of the acute phase

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The Authors declare no conflict of interest

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ORIGINAL PAPER

Synaptotagmin 13 is neuroprotective across motor neuron diseases

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Abstract

In amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), spinal and lower brainstem motor neurons degenerate, but some motor neuron subtypes are spared, including oculomotor neurons (OMNs). The mechanisms responsible for this selective degeneration are largely unknown, but the molecular signatures of resistant and vulnerable motor neurons are distinct and offer clues to neuronal resilience and susceptibility. Here, we demonstrate that healthy OMNs preferentially express *Synaptotagmin 13 (SYT13)* compared to spinal motor neurons. In end-stage ALS patients, *SYT13* is enriched in both OMNs and the remaining relatively resilient spinal motor neurons compared to controls. Overexpression of SYT13 in ALS and SMA patient motor neurons in vitro improves their survival and increases axon lengths. Gene therapy with *Syt13* prolongs the lifespan of ALS mice by 14% and SMA mice by 50% by preserving motor neurons and delaying muscle denervation. SYT13 decreases endoplasmic reticulum stress and apoptosis of motor neurons, both in vitro and in vivo. Thus, SYT13 is a resilience factor that can protect motor neurons and a candidate therapeutic target across motor neuron diseases.

Introduction

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are lethal neurodegenerative diseases characterized by a progressive loss of motor neurons in the spinal cord, brainstem, and cortex [9, 21]. However, some motor neurons are preserved throughout late stages of these diseases, including oculomotor neurons (OMNs), trochlear neurons and neurons in the abducens, which regulate eye movement as well as Onuf's nuclei, which controls sphincter function. This has been demonstrated in both mouse models [11, 24, 29, 32, 55] and in *post-mortem* tissues from patients [31, 35, 39, 60]. Notably, both the familial (*f*) and sporadic (*s*) forms of ALS and SMA share this pattern of selective motor neuron resistance [35, 61]. Consequently, eye movement and sphincter function remain relatively preserved, even in the advanced stages of these diseases and ocular tracking have high utility for communication [35, 64]. This preservation across diseases indicates that differential vulnerability between motor neuron groups is largely independent on the cause of disease, and that mechanisms of vulnerability and resilience could be shared across diseases [9–11, 20]. Elucidation of the molecular basis of selective resistance may lead to the development of new therapies to prevent the relentless motor neuron loss. Previous studies of ALS and SMA [52] suggest that elements intrinsic to motor

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REVIEW

Current understanding of and emerging treatment options for spinal muscular atrophy with respiratory distress type 1 (SMARD1)

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Abstract

Spinal muscular atrophy (SMA) with respiratory distress type 1 (SMARD1) is an autosomal recessive motor neuron disease that is characterized by distal and proximal muscle weakness and diaphragmatic palsy that leads to respiratory distress. Without intervention, infants with the severe form of the disease die before 2 years of age. SMARD1 is caused by mutations in the *IGHMBP2* gene that determine a deficiency in the encoded IGHMBP2 protein, which plays a critical role in motor neuron survival because of its functions in mRNA processing and maturation. Although it is rare, SMARD1 is the second most common motor neuron disease of infancy, and currently, treatment is primarily supportive. No effective therapy is available for this devastating disease, although multidisciplinary care has been an essential element of the improved quality of life and life span extension in these patients in recent years. The objectives of this review are to discuss the current understanding of SMARD1 through a summary of the presently known information regarding its clinical presentation and pathogenesis and to discuss emerging therapeutic approaches. Advances in clinical care management have significantly extended the lives of individuals affected by SMARD1 and research into the molecular mechanisms that lead to the disease has identified potential strategies for intervention that target the underlying causes of SMARD1. Gene therapy via gene replacement or gene correction provides the potential for transformative therapies to halt or possibly prevent neurodegenerative disease in SMARD1 patients. The recent approval of the first gene therapy approach for SMA associated with mutations in the *SMN1* gene may be a turning point for the application of this strategy for SMARD1 and other genetic neurological diseases.

Keywords Distal hereditary motor neuropathy type 6 · SMARD1 · Motor neuron disease · IGHMBP2 · Gene therapy · Oligonucleotides

Introduction

Spinal muscular atrophy with respiratory distress type 1 (SMARD1, OMIM # 604320) is an early onset genetic degenerative motor neuron disease caused by autosomal recessive mutation in the *IGHMBP2* gene, mainly characterized by progressive distal muscular atrophy and respiratory failure due to diaphragmatic palsy [35, 37]. It is also known as distal spinal muscular atrophy 1 (DMSA1) and distal hereditary motor neuropathy type 6 (dHMN6) [55]. Recessive mutations in *IGHMBP2* cause a disease continuum with a neonatal onset and severe distal motor neuropathy with diaphragmatic weakness at one end (SMARD1) and a later onset of milder CMT2 at the other end (CMT2S). Both are thought to be due to a loss of *IGHMBP2* function.

The first description of SMARD1 dates back to 1974, when Mellins et al. described two infants with a disease that resembled an atypical form of Werdnig–Hoffmann disease (SMA1) [69]. In 1989, Bertini et al. defined this disorder as a spinal muscular atrophy (SMA) variant mainly characterized by diaphragm involvement [7]. In 1996, Rudnik-Schoneborn et al. recognized SMARD1 as a separate disease from SMA

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Original Investigation | Neurology

Phenotypic Variability Among Patients With D4Z4 Reduced Allele Facioscapulohumeral Muscular Dystrophy

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Abstract

IMPORTANCE Facioscapulohumeral muscular dystrophy (FSHD) is considered an autosomal dominant disorder, associated with the deletion of tandemly arrayed D4Z4 repetitive elements. The extensive use of molecular analysis of the D4Z4 locus for FSHD diagnosis has revealed wide clinical variability, suggesting that subgroups of patients exist among carriers of the D4Z4 reduced allele (DRA).

OBJECTIVE To investigate the clinical expression of FSHD in the genetic subgroup of carriers of a DRA with 7 to 8 repeat units (RUs).

DESIGN, SETTING, AND PARTICIPANTS This multicenter cross-sectional study included 422 carriers of DRA with 7 to 8 RUs (187 unrelated probands and 235 relatives) from a consecutive sample of 280 probands and 306 relatives from the Italian National Registry for FSHD collected between 2008 and 2016. Participants were evaluated by the Italian Clinical Network for FSHD, and all clinical and molecular data were collected in the Italian National Registry for FSHD database. Data analysis was conducted from January 2017 to June 2018.

MAIN OUTCOMES AND MEASURES The phenotypic classification of probands and relatives was obtained by applying the Comprehensive Clinical Evaluation Form which classifies patients in the 4 following categories: (1) participants presenting facial and scapular girdle muscle weakness typical of FSHD (category A, subcategories A1-A3), (2) participants with muscle weakness limited to scapular girdle or facial muscles (category B, subcategories B1 and B2), (3) asymptomatic or healthy participants (category C, subcategories C1 and C2), and (4) participants with myopathic phenotypes presenting clinical features not consistent with FSHD canonical phenotype (category D, subcategories D1 and D2).

RESULTS A total of 187 probands (mean [SD] age at last neurological examination, 53.5 [15.2] years; 103 [55.1%] men) and 235 relatives (mean [SD] age at last neurologic examination, 45.1 [17.0] years; 104 [44.7%] men) with a DRA with 7 to 8 RUs and a molecular diagnosis of FSHD were evaluated. Of 187 probands, 99 (52.9%; 95% CI, 45.7%-60.1%) displayed the classic FSHD phenotype, whereas 86 (47.1%; 95% CI, 39.8%-54.3%) presented incomplete or atypical phenotypes. Of 235 carrier relatives from 106 unrelated families, 124 (52.8%; 95% CI, 46.4%-59.7%) had no motor impairment, whereas a small number (38 [16.2%; 95% CI, 9.8%-23.1%]) displayed the classic FSHD phenotype, and 73 (31.0%; 95% CI, 24.7%-38.0%) presented with incomplete or atypical phenotypes. In 37 of 106 families (34.9%; 95% CI, 25.9%-44.8%), the proband was the only participant presenting with a myopathic phenotype, while only 20 families (18.9%; 95% CI, 11.9%-27.6%) had a member with autosomal dominant FSHD.

(continued)

Key Points

Question What are the phenotypes expressed among patients with facioscapulohumeral muscular dystrophy (FHSD) who are carriers of D4Z4 reduced allele with 7 to 8 repeat units?

Findings In this cross-sectional study of 187 probands and 235 relatives who carry a D4Z4 reduced allele with 7 to 8 repeat units, 47.1% of probands did not have the classic FSHD phenotype, and 52.8% of the carrier relatives were nonpenetrant. In 106 families, 18.9% had a member with autosomal dominant FSHD, whereas in 34.9%, the proband was the only participant expressing a myopathic phenotype.

Meaning The findings of this study suggest that knowledge of phenotypic variation in the expression of D4Z4 reduced allele with 7 to 8 repeat units in individuals with FSHD could be informative for clinical management and genetic counseling.

+ Invited Commentary

+ Supplemental content

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suggest the role of sex-specific factors that delay disease onset in women or accelerate or facilitate disease appearance in men. Considering the mean age at onset in woman, we can hypothesize a crucial role of hormonal factors related to fertile age, but this hypothesis should be confirmed by dedicated studies. It is also possible that factors expressed by men (eg, testosterone is a potent anabolic factor promoting muscle protein synthesis and muscular regeneration) create a major sensitivity to the alterations caused by the FSHD pathogenic mechanism among men.[54] Moreover, men and women may respond differently to catabolic conditions because of their hormonal profiles.[55,56]

Limitations

This study has some limitations. The CCEF is an extensive clinical tool, which takes about 20 minutes to apply. Only a physician with expertise in neuromuscular disease can use the tool correctly. Thus, it is preferable they be properly trained. Second, a long follow-up period may be necessary to evaluate whether some symptomatic patients will be assigned to a different clinical category or if some nonpenetrant relatives will develop any sign of muscle impairment.

Third, most nonpenetrant relatives were older than 20 years, and the mean (SD) of age at last neurological examination (ie, 41.1 [15.3] years) was older than that of symptomatic relatives (ie, 33.4 [17.3] years). Thus, it is likely that they will never develop disease or that they might develop some symptoms at older age. In our cohort we had several patients with atypical phenotypes who developed the disease when older than 40 years. The clinical follow-up of nonpenetrant subjects will provide relevant clinical information on this matter.

Conclusions

The findings of this study indicate that in the case of probands who carry a DRA with 7 to 8 RUs and do not present the classic FSHD phenotype, it is necessary to consider alternative myopathies. In sporadic cases presenting with atypical phenotypes, the random association of a myopathic phenotype with a contracted D4Z4 allele has to be considered, given that there is a 1.7% frequency of DRA with 7 to 8 RUs in the general population. This study showed that the genetic background can influence the penetrance and phenotypic expression of disease in relatives carrying the same molecular signature. Based on the results of our study, the precise phenotypic characterization of patients and families should support molecular testing and could advance the management of diagnosis, genetic counseling, and selection procedures for randomized clinical trials.

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CASE REPORT Open Access

A case report of late-onset cerebellar ataxia associated with a rare p.R342W *TGM6* (SCA35) mutation

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Abstract

Background: Mutations in *TGM6* gene, encoding for transglutaminase 6 (TG6), have been implicated in the pathogenesis of spinocerebellar ataxia type 35 (SCA35), a rare autosomal dominant disease marked by cerebellar degeneration and characterized by postural instability, incoordination of gait, features of cerebellar dysfunction and pyramidal signs.

Case presentation: Here we report the case of an Italian patient with late-onset, slowly progressive cerebellar features, including gait ataxia, scanning speech and ocular dysmetria and pyramidal tract signs. Whole exome sequencing revealed the rare heterozygous c.1024C > T (p.R342W) variant of *TGM6*, located at a highly evolutionary conserved position and predicted as pathogenic by in silico tools. Expression of TG6-R342W mutant in HEK293T cells led to a significant reduction of transamidase activity compared to wild-type TG6.

Conclusion: This finding extends SCA35 genetic landscape, highlighting the importance of *TGM6* screening in undiagnosed late-onset and slowly progressive cerebellar ataxias.

Keywords: Spinocerebellar ataxias, SCA35, *TGM6*, Transglutaminase, Case report

Background

Spinocerebellar ataxias (SCAs) embody a clinically and genetically heterogeneous group of disorders, characterized by cerebellar degeneration. A broad range of signs and symptoms, from retinopathy to neuropathy, pyramidal signs and epilepsy may be associated with the clinical core picture of cerebellar syndrome. The autosomal dominant inheritance represents a distinctive hallmark. Although pathological repeat expansions are responsible for the majority of presentations (including SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA12, SCA17, SCA31, SCA36, SCA37 and DRPLA), an increasing number of SCAs is progressively being associated with conventional mutations (e.g., SCA5 – *SPTBN2*; SCA11 – *TTBK2*; SCA14 – *PRKCG*; SCA28 – *AFG3L2*) [1]. In line with this last group, spinocerebellar ataxia type 35 (SCA35) results from missense mutations in *TGM6*, as found by Wang and colleagues by combining exome sequencing and linkage analysis in four probands of a Chinese family [2]. Since then, several *TGM6* mutations have been described. Some of them, sharing a common reduction in transamidase activity, are thought to be pathogenic, although the specific molecular pattern involved remains

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Clinical Case Report

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Hyperacute extensive spinal cord infarction and negative spine magnetic resonance imaging: a case report and review of the literature

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Abstract

Rationale: Spinal cord infarction (SCI) accounts for only 1% to 2% of all ischemic strokes and 5% to 8% of acute myelopathies. Magnetic resonance imaging (MRI) holds a role in ruling out non-ischemic etiologies, but the diagnostic accuracy of this procedure may be low in confirming the diagnosis, even when extensive cord lesions are present. Indeed, T2 changes on MRI can develop over hours to days, thus accounting for the low sensitivity in the hyperacute setting (ie, within 6 hours from symptom onset). For these reasons, SCI remains a clinical diagnosis. Despite extensive diagnostic work-up, up to 20% to 40% of SCI cases are classified as cryptogenic. Here, we describe a case of cryptogenic longitudinally extensive transverse myelopathy due to SCI, with negative MRI and diffusion-weighted imaging at 9 hours after symptom onset.

Patient concerns: A 51-year-old woman presented to our Emergency Department with acute severe abdominal pain, nausea, vomiting, sudden-onset of bilateral leg weakness with diffuse sensory loss, and paresthesias on the trunk and legs.

Diagnoses: On neurological examination, she showed severe paraparesis and a D6 sensory level. A 3T spinal cord MRI with gadolinium performed at 9 hours after symptom onset did not detect spinal cord alterations. Due to the persistence of a clinical picture suggestive of an acute myelopathy, a 3T MRI of the spine was repeated after 72 hours showing a hyperintense "pencil-like" signal mainly involving the grey matter from T1 to T6 on T2 sequence, mildly hypointense on T1 and with restricted diffusion.

Interventions: The patient was given salicylic acid (100 mg/d), prophylactic low-molecular-weight heparin, and began neuromotor rehabilitation.

Outcomes: Two months later, a follow-up neurological examination revealed a severe spastic paraparesis, no evident sensory level, and poor sphincteric control with distended bladder.

Lessons: Regardless of its relatively low frequency in the general population, SCI should be suspected in every patient presenting with acute and progressive myelopathic symptoms, even in the absence of vascular risk factors. Thus, a clinical presentation consistent with a potential vascular syndrome involving the spinal cord overrides an initially negative MRI and should not delay timely and appropriate management.

Abbreviations: ASA = anterior spinal artery, ASAS = anterior spinal artery syndrome, CSF = cerebrospinal fluid, DWI = diffusion-weighted imaging, LETM = longitudinally extensive transverse myelopathy, MRI = magnetic resonance imaging, SCI = spinal cord infarction.

Keywords: case report, longitudinally extensive transverse myelopathy, magnetic resonance imaging, spinal cord infarction

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SC and IF contributed equally to the manuscript.

The patient has provided informed consent for publication of the case.

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The authors of this work have nothing to disclose

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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CLINICAL/SCIENTIFIC NOTES OPEN ACCESS

Expanding the clinical spectrum of the mitochondrial mutation A13084T in the *ND5* gene

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Our group previously published about a patient with a LS/MELAS (Leigh syndrome/mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes) overlap phenotype associated with a novel mitochondrial mutation in the *ND5* gene.[1] At that time, his 38-year-old mother presented only migraine and asymptomatic bilateral optic atrophy, without other neurologic signs or symptoms. Headache attacks, occurring about twice a month, were localized mainly in the right frontoparietal region, sometimes associated with nausea or vomit or phonophotophobia, and were responsive to nonsteroidal anti-inflammatory drugs. She carried lower levels of heteroplasmy of the same A13084T mutation (57% in lymphocytes and 48% in fibroblasts) compared with her son (82% in blood and 72% in fibroblasts).

At 53 years of age, she was admitted to our hospital to treat the recent worsening of migraine attacks in terms of frequency (up to 12 a month) and severity. Neurologic examination was unremarkable, including manual visual field by confrontation. Ophthalmologic examination revealed visual acuity loss, which onset was not exactly datable. Visual acuity was 20/32 in the right eye (oculus dexter [OD]) and 20/40 in the left eye (oculus sinister [OS]). Fundoscopy confirmed known bilateral optic disc pallor and excavation, with major involvement of the temporal sectors (figure, A). Intraocular pressure was 16 mm Hg bilaterally. Computerized visual field analysis showed a moderate-severe defect in the superotemporal sector in the OD and centrocecal scotoma in the OS (figure, B). No retinal abnormalities were detected at infrared and autofluorescence retinoscopy (figure, C), whereas optical coherence tomography showed a diffused macular ganglion cell layer thinning and a retinal nerve fiber layer atrophy of the temporal sectors bilaterally (figure, D). Visual evoked response to flash stimulation was reduced in amplitude in the OS, with a markedly increased latency, and normal in the OD. Serum lactate was slightly elevated (1.6 mmol/L; normal values <1.3 mmol/mol), and folate levels were mildly reduced (3.5 μg/L; normal range 4.6–18.7 μg/L). The remaining blood tests, including thyroid and liver functions, were otherwise normal. We performed a brain MRI with gadolinium that also included studies of orbits and cerebral vessels. Subcortical white matter carried nonspecific hyperintensities in T2 sequences, but no signs of previous stroke-like lesions were detected. No optic nerve or chiasm abnormalities were seen on scans. Arteries of the circle of Willis were normally represented.

Our findings pointed out to a phenotype similar to Leber hereditary optic neuropathy (LHON), not previously diagnosed because the patient did not perform further evaluations in the past years nor reported ocular symptoms so far. Idebenone (dosage 90 mg trice a day) was added to the treatment with coenzyme Q10 (dosage 50 mg twice a day).[2] Because LHON

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NEUROMUSCULAR DISEASE

Spinal muscular atrophy — challenges in the therapeutic era

Irene Faravelli and Stefania Corti

With the advent of disease-modifying therapies for spinal muscular atrophy, prenatal and extra-neural alterations associated with the condition have garnered increasing attention as potential determinants of the therapeutic window and efficacy of novel drugs. Two recent studies highlight the impact of spinal muscular atrophy on prenatal bone and organ development.

Refers to Hensel, N. et al. Altered bone development with impaired cartilage formation precedes neuromuscular symptoms in Spinal Muscular Atrophy (SMA). *Hum. Mol. Genet.* https://doi.org/10.1093/hmg/ddaa145 (2020) | Motyl, A. A. L. et al. Pre-natal manifestation of systemic developmental abnormalities in spinal muscular atrophy. *Hum. Mol. Genet.* https://doi.org/10.1093/hmg/ddaa146 (2020).

Spinal muscular atrophy (SMA) is a severe neuromuscular disorder caused by a reduction in the expression of survival motor neuron protein (SMN) that results from mutations in the survival motor neuron 1 gene (*SMN1*). Without treatment, individuals with the most common form of the disorder, SMA type 1, never gain the ability to sit or stand and usually die or require permanent ventilation within the first 2 years of life[1]. However, in the past 10 years, the development of therapeutic strategies that increase SMN levels, including antisense oligonucleotides (ASOs) and gene therapy, has changed SMA from a frequently fatal progressive disorder to a chronic condition in which motor disability can be halted and even prevented[2,3].

> “therapies often fall short of complete phenotypic rescue”

Despite prolonging survival, these therapies often fall short of complete phenotypic rescue, especially when treatment is initiated after the onset of symptoms. The requirement of SMN for prenatal development might account for this narrow therapeutic window, especially in individuals with the most severe forms of SMA[4]. Moreover, SMN is ubiquitously expressed and has a major role in RNA metabolism, suggesting that the SMA phenotype might extend beyond the neuromuscular system.

Whether or not the approved SMA therapies can address extra-neural SMA pathology is unclear. ASO therapy for SMA is delivered intrathecally and shows limited distribution outside of the CNS[3,4]. Intravenous injection of adeno-associated virus-based SMA gene therapy can induce SMN expression in peripheral tissues, but further studies are needed to establish how long this expression persists for[2]. A recently approved, orally available, small molecule (Risdiplam) has the potential to overcome these delivery challenges, but the effect of this drug on different organs over time still has to be defined. Thus, understanding the extent and long-term clinical relevance of extra-neural SMA pathology is important.

A study by Motyl at al.[5] recently published in *Human Molecular Genetics* showed that the Taiwanese mouse model of severe SMA displays pathological features during early embryonic development. At embryonic day 14.5, SMA embryos were smaller than their healthy littermates and had a reduced heart size. Interestingly, these features preceded symptomatic motor impairment by about 13 days. At the same developmental time point, the authors identified widespread alterations in the proteomic profile of the brain, spinal cord, liver and skeletal muscle of SMA embryos compared with healthy littermates. Interestingly, the extent of overlap between the proteomic changes in different tissues was extremely low, and the degree of proteome downregulation in SMA tissues did not seem to correlate with SMN levels. These observations suggest a tissue-specific effect of SMN deficiency early in development, which would require the early administration of systemic treatments, taking into account the concentration thresholds for efficacy and toxicity in different organs.

> “These observations suggest a tissue-specific effect of SMN deficiency early in development”

In a second study[6] published in the same issue of *Human Molecular Genetics*, Hensel et al. identified systemic changes, including impaired bone development and cartilage formation, in children with SMA and in a mouse model of the disease. Interestingly, in mice these changes began before neuromuscular alterations became apparent. The authors reported that bone mineral density and the size of lumbar vertebral bodies were significantly lower in a cohort of children with SMA than in healthy age-matched controls. Similarly, the Taiwanese mouse model of SMA showed impaired growth compared with wild-type controls, and these alterations occurred before the onset of neuromuscular symptoms. However, an SMA-associated reduction in bone mineral density was not observed in the mice. These results suggest that bone alterations in individuals with SMA might be at least partially independent of neuromuscular degeneration, which was previously considered to be the underlying pathogenic mechanism.

In the study by Hensel et al.[6] the number of chondroblasts in the hypertrophic zone of the growth plate was greatly reduced in SMA mice compared with wild-type mice, resulting in decreased longitudinal bone growth, smaller vertebral bodies and shortened femora. Consistent with this observation, RNA-sequencing data obtained from the vertebral bodies at postnatal day 1 showed transcriptional changes associated with cell division and cartilage remodelling in the SMA mice. The authors speculated that alterations in chondrogenesis could be related to the disruption of the RhoA–ROCK pathway, which is involved in actin cytoskeleton metabolism and chondrocyte differentiation. Interestingly,

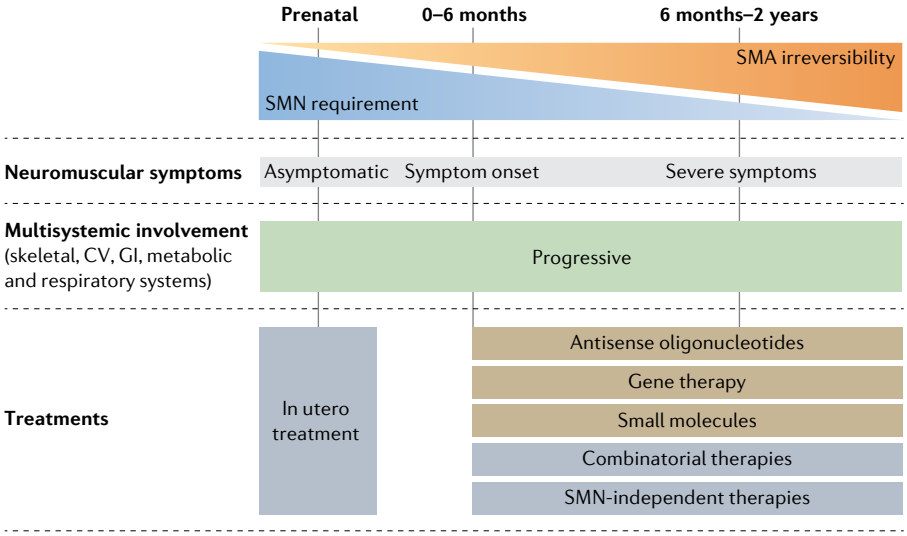

Fig. 1 | **Timeline of SMA type 1 pathology and therapeutic options.** Overview of survival motor neuron protein (SMN) requirement and irreversibility of the symptoms caused by SMN deficiency. The multisystemic effects and prenatal manifestations of spinal muscular atrophy (SMA) might limit the efficacy of the current approved treatments (yellow). Future therapeutic options (grey) could include SMN-independent therapies, combinatorial approaches and in utero treatment. CV, cardiovascular; GI, gastrointestinal.

in the study by Motyl et al.[5] proteins belonging to the RhoA–ROCK pathway were significantly downregulated in the liver of SMA mice compared with wild-type mice.

The systemic SMA phenotype described by both of these new studies poses a substantial challenge to the treatment of this disorder. Although intrathecally administered ASOs and gene therapy have both shown a certain degree of leakage outside the CNS[7,8], the extent to which these treatments can rescue the systemic SMA phenotype still needs to be established. Moreover, the biodistribution and stability of approved therapies in poorly vascularized tissues and highly replicative cells, for example, cartilage and chondroblasts, is still largely unknown. Thus, different combinations of therapies targeting both the CNS and peripheral tissues might be required to fully treat SMA. Nevertheless, evidence from previous studies suggests that the bone defects identified by Hensel et al.[6] might be improved by existing SMA treatments. For example, in the Taiwanese mouse model of SMA, systemic administration of an ASO treatment was associated with an increase in both size and weight of the animals[9]. However, size varied among individual animals, and a specific analysis of the bone was not performed. In a clinical trial of a therapy that increases SMN levels, intrathecal treatment administered at the pre-symptomatic stage, was associated with increased growth[10]. However, the number of participants included in this study was too small to enable definitive conclusions to be drawn. Thus, although growth impairment is not often prominent at the point of SMA diagnosis, it is likely to occur and is relevant to the treatment of patients with SMA. Systemic SMN therapy or other SMN-independent therapeutic strategies should be optimized to address this aspect of the disease phenotype.

The era of effective therapies for SMA has certainly brought with it a major change for patients who previously faced an untreatable progressive disorder. However, the findings of Motyl at al.[5] and Hensel et al.[6] suggest that the outcome for patients with SMA could be improved if therapies were specifically tailored to target the systemic aspects of the disease (FIG. 1). Previous studies have shown that SMN is highly expressed in the fetal CNS and that, after birth, levels of SMN decrease markedly in the majority of tissues[4]. This suggests that SMN is particularly important during prenatal development. The results of the two new studies suggest that effective delivery of SMA therapeutics would involve administration during the pre-symptomatic phases of the disorder, that is, during the perinatal, or even prenatal, period. Indeed, further studies that investigate the multisystemic aspects of SMA from early in development could be pivotal in identifying the optimal therapeutic window. However, the efficacy of approved therapies on SMA-associated early developmental alterations remains to be fully elucidated. Furthermore, technical and ethical challenges regarding the use of human in utero therapeutic strategies will need to be overcome before this new knowledge can be applied to clinical practice. Regardless, the evidence of early developmental and systemic alterations in SMA should be comprehensively discussed during prenatal counselling and whenever treatment is started.

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Competing interests

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REVIEW

Extracellular vesicles and amyotrophic lateral sclerosis: from misfolded protein vehicles to promising clinical biomarkers

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Abstract

Extracellular vesicles (EVs) are small reservoirs of different molecules and important mediators of cell-to-cell communication. As putative vehicles of misfolded protein propagation between cells, they have drawn substantial attention in the field of amyotrophic lateral sclerosis (ALS) and other neurodegenerative disorders. Moreover, exosome-mediated non-coding RNA delivery may play a crucial role in ALS, given the relevance of RNA homeostasis in disease pathogenesis. Since EVs can enter the systemic circulation and are easily detectable in patients' biological fluids, they have generated broad interest both as diagnostic and prognostic biomarkers and as valuable tools in understanding disease pathogenesis. Here, after a brief introduction on biogenesis and functions of EVs, we aim to investigate their role in neurodegenerative disorders, especially ALS. Specifically, we focus on the main findings supporting EV-mediated protein and RNA transmission in ALS in vitro and in vivo models. Then, we provide an overview of clinical applications of EVs, summarizing the most relevant studies able to detect EVs in blood and cerebrospinal fluid (CSF) of ALS patients, underlying their potential use in aiding diagnosis and prognosis. Finally, we explore the therapeutic applications of EVs in ALS, either as targets or as vehicles of proteins, nucleic acids and molecular drugs.

Keywords Extracellular vesicles · Amyotrophic lateral sclerosis · Prion-like properties · Biomarkers · Neurodegenerative disorders · Therapeutics

Abbreviations

ALS	Amyotrophic Lateral sclerosis
ASCs	Adipose stem cells
BBB	Blood brain barrier
C9-ALS	C9orf72-related ALS
C9orf72	Chromosome 9 open reading frame 72
CNS	Central nervous system
CSF	Cerebrospinal fluid
CTF	C-terminal fragment
DPRs	Dipeptide repeat proteins
EVs	Extracellular vesicles
FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FUS	Fused-in-sarcoma
HRE	Hexanucleotide repeat expansion
IL-6	Interleukin-6
iPSC	Induced pluripotent stem cell
lnRNAs	Long non-coding RNAs
miRNAs	Micro-RNAs
MN	Motor neuron
mRNAs	Messenger RNAs
MVBs	Multivesicular bodies
MVs	Microvesicles
NIR	Novel INHAT repressor
PrLD	Prion-like domain
pTDP-43	Phosphorylated-TDP-43
RBP	RNA-binding protein
ROS	Reactive oxygen species
sALS	Sporadic ALS
SOD1	Superoxide dismutase
TDP-43	TAR DNA-binding protein 43

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Safety and efficacy of rt-PA treatment for acute stroke in pseudoxanthoma elasticum: the first report

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Abstract
Pseudoxanthoma elasticum is a rare cause for ischaemic stroke. Little is known about acute and secondary prevention strategies in these subjects given the increased risk of gastrointestinal and urinary bleedings. Here we present the case of a 62 years old man affected by pseudoxanthoma elasticum who presented with acute ischaemic stroke and was successfully treated with intravenous thrombolysis. Neurological signs improved after intravenous thrombolysis without bleeding complication. To our knowledge, this is the first case of pseudoxanthoma elasticum—related stroke undergoing intravenous thrombolysis.

Keywords Pseudoxanthoma elasticum · Thrombolysis · rt-PA · Ischaemic stroke

Highlights

- Pseudoxanthoma elasticum is a rare disease.
- A patient with pseudoxanthoma elasticum and acute ischaemic stroke was successfully treated with intravenous thrombolysis.
- This case makes stroke acute treatment and secondary prevention strategies challenging.

Introduction

Pseudoxanthoma elasticum (PXE, OMIM 264800) is an autosomal recessive monogenic disease caused by mutations in the ABCC6 gene (ATP binding cassette family C member 6) gene, which encodes the Multidrug resistance-associated protein 6 (MRP6). It is an inherited multisystem disorder in which circulating levels of an anti-mineralization factor are low. The lack of functional ABCC6 protein leads to reduced plasma levels of inorganic pyrophosphate, with an increased risk for progressive calcification of medium and small sized arteries [1–5]. Genetics studies highlighted the role of ABCC6 mutations with a variable detection rate ranging from 66 to 87.7% in different settings. A wide clinical phenotypic variability has been reported, probably reflecting the role of genetic modifiers. A clear genotype–phenotype correlation is lacking even if Schultz et al. reported that the patients with predicted non-functional protein are more likely to present earlier age at onset and multisystem disease as compared to subjects with some potentially functional protein [6, 7].

The clinical prevalence of PXE has been estimated at between 1 per 100,000 and 1 per 25,000 [8].

Pseudoxanthoma elasticum is characterized by skin yellowish papules and plaques (pseudoxanthomas) and ocular complications (angioid streaks, haemorrhage and progressive loss of visual acuity). Moreover, lesions in artery walls are typical, resulting in defective vasoconstriction of affected arteries (with subsequently gastrointestinal bleeding), narrowing and occlusion of arteries, leading to peripheral arterial disease, increased prevalence of hypertension, coronary artery disease and ischaemic stroke.

Intravenous thrombolysis with recombinant tissue plasminogen activator (rt-PA) has been considered the most

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Cellular and Molecular Life Sciences

REVIEW

Noncoding RNAs in Duchenne and Becker muscular dystrophies: role in pathogenesis and future prognostic and therapeutic perspectives

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Abstract

Noncoding RNAs (ncRNAs), such as miRNAs and long noncoding RNAs, are key regulators of gene expression at the post-transcriptional level and represent promising therapeutic targets and biomarkers for several human diseases, including Duchenne and Becker muscular dystrophies (DMD/BMD). A role for ncRNAs in the pathogenesis of muscular dystrophies has been suggested, even if it is still incompletely understood. Here, we discuss current progress leading towards the clinical utility of ncRNAs for DMD/BMD. Long and short noncoding RNAs are differentially expressed in DMD/BMD and have a mechanism of action via targeting mRNAs. A subset of muscle-enriched miRNAs, the so-called myomiRs (miR-1, miR-133, and miR-206), are increased in the serum of patients with DMD and in dystrophin-defective animal models. Interestingly, myomiRs might be used as biomarkers, given that their levels can be corrected after dystrophin restoration in dystrophic mice. Remarkably, further evidence demonstrates that ncRNAs also play a role in dystrophin expression; thus, their modulations might represent a potential therapeutic strategy with the aim of upregulating the dystrophin protein in combination with other oligonucleotides/gene therapy approaches.

Keywords Duchenne muscular dystrophy · Becker muscular dystrophy · lncRNA · miRNA · Biomarkers · Antisense oligonucleotides

Introduction

Duchenne muscular dystrophy (DMD) is one of the most common neuromuscular disorders in childhood, involving around 1 in 3500 male births [1] with an incidence from 10.71 to 27.78 per 100.000 males [2]. It is an X-linked disease, usually caused by out-of-frame mutations in the dystrophin gene *DMD*, which leads to the absence of protein expression. On the contrary, mutations retaining the reading frame are generally related to partial residual expression of dystrophin and a milder phenotype called Becker Muscular Dystrophy (BMD) [3].

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Dystrophin is a cytoplasmatic protein that plays a major structural role in skeletal muscles, linking the cytoskeleton to the extracellular matrix via a complex (dystrophin-associated protein complex or DAPC) formed with dystroglycans, sarcoglycans, sarcospan, dystrobrevins and syntrophin [4]. The disruption of this structure, especially the linking with actin and beta-dystroglycan, destabilizes the sarcolemma during the muscle contraction [5]. Membrane instability allows the entrance of calcium ions with subsequent oxidative stress, activation of Ca^{2+}-dependent calpain and protein degradation. Activation of complement and inflammation causes necrosis and generation of pro-fibrotic factors. While muscle regeneration initially increases to replace muscle damage, over time, muscle regeneration fails and fibro-fatty substitution takes place, impairing the muscle function [6]. In fact, the prolonged exposure to an adverse environment prevents myogenesis, despite satellite cells retaining regenerative capacity [7].

Moreover, dystrophin has an important scaffolding role, participating in the cell signaling pathways [8] and enabling the correct localization of nitric oxide synthetase (nNOS) [9]. Other components of DAPC are also involved

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Review

Glial cells involvement in spinal muscular atrophy: Could SMA be a neuroinflammatory disease?

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ABSTRACT

Spinal muscular atrophy (SMA) is a severe, inherited disease characterized by the progressive degeneration and death of motor neurons of the anterior horns of the spinal cord, which results in muscular atrophy and weakness of variable severity. Its early-onset form is invariably fatal in early childhood, while milder forms lead to permanent disability, physical deformities and respiratory complications. Recently, two novel revolutionary therapies, antisense oligonucleotides and gene therapy, have been approved, and might prove successful in making long-term survival of these patients likely. In this perspective, a deep understanding of the pathogenic mechanisms and of their impact on the interactions between motor neurons and other cell types within the central nervous system (CNS) is crucial. Studies using SMA animal and cellular models have taught us that the survival and functionality of motor neurons is highly dependent on a whole range of other cell types, namely glial cells, which are responsible for a variety of different functions, such as neuronal trophic support, synaptic remodeling, and immune surveillance. Thus, it emerges that SMA is likely a non-cell autonomous, multifactorial disease in which the interaction of different cell types and disease mechanisms leads to motor neurons failure and loss. This review will introduce the different glial cell types in the CNS and provide an overview of the role of glial cells in motor neuron degeneration in SMA. Furthermore, we will discuss the relevance of these findings so far and the potential impact on the success of available therapies and on the development of novel ones.

1. Introduction

Spinal Muscular Atrophy (SMA), also termed 5q-SMA to distinguish from less common forms, is a hereditary neuromuscular disease, caused by loss-of-function mutations in the gene Survival Motor Neuron 1 (*SMN1*), causing the reduction of SMN protein, and characterized by hypotonia and weakness, which might prove fatal in early childhood (Faravelli et al., 2015; Pearn, 1978; Sumner et al., 2016). SMA has been considered for a long time an incurable deadly disease, until recent therapeutic advances, namely antisense oligonucleotides and gene therapy, have been successful in modifying its natural history from a rapidly fatal disease to a condition in which long-term survival is likely (Parente and Corti, 2018). At a pathological level, SMA is defined by the degeneration of anterior horn cells in the spinal cord and motor nuclei in the lower brainstem, which results in a progressive, diffuse and symmetric muscle weakness and atrophy (Pearn, 1978). However, despite the traditional belief that SMA is a motoneuronal-restricted disease, recent evidence advocates the involvement of many different cells and systems alongside with motor neurons in the pathogenesis of this disease. Glial cells are the most abundant cell type in the central nervous system (CNS), surrounding neurons and providing nutritional and trophic support for them (Papadimitriou et al., 2010). These cells also play an important role in neuronal communication and neuroinflammation (Papadimitriou et al., 2010). Although the presence of cell-autonomous motoneuronal toxicity in this disease has been established, growing evidence supports the possibility that, in addition to that, glial dysfunction and glial-mediated inflammation might be able to compromise neuronal survival and promote progression and propagation of the degenerative process (Philips and Rothstein, 2014). Arguably, it can be hypothesized that, under specific circumstances, neuroinflammatory cells - astrocytes and microglia – could directly start the process of neurodegeneration. In this review, we will briefly recapitulate the different glial cell types and their role within the CNS, and we will provide an overview of the different mechanisms implicated in glial-mediated

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CASE REPORT AND REVIEW OF THE LITERATURE

WILEY

Herpes Simplex virus type 2 myeloradiculitis with a pure motor presentation in a liver transplant recipient

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Abstract

In this case report, we describe the first PCR-confirmed case of HSV2 myeloradiculitis with a purely motor presentation, occurring in a 68-year-old liver transplant recipient. The patient reported ascending weakness with no sensory nor sphincteric symptoms, thereby resembling acute demyelinating inflammatory neuropathy, or Guillain-Barré syndrome. HSV2 was detected in cerebrospinal fluid by PCR, and the patient was successfully treated with intravenous Acyclovir.

KEYWORDS

ascending weakness, Elsberg syndrome, Herpes simplex virus type 2, HSV2 infection, liver transplant, myeloradiculitis

1 | INTRODUCTION

Elsberg syndrome is an extremely rare lumbosacral infectious disorder characterized by involvement of the *cauda equina* and associated with Herpes simplex virus type 2 (HSV2) reactivation or, infrequently, primary infection.[1,2] Only 12 PCR-confirmed cases have been reported to date,[2-6] and they all featured sphincterial and/or sensorimotor symptoms, while a purely motor presentation is not typical and occurs rarely. HSV2 disseminated infection is known to occur in transplant recipients as a result of reactivation of dormant virus or, rarely, in a donor-derived fashion, although a neurologic involvement has never been described.[7,8] We hereby describe the case of a 68-year-old immunocompromised man with progressive motor impairment due to HSV2 central nervous system (CNS) infection.

2 | CLINICAL CASE

The patient was admitted to our Emergency Department for a six-day history of progressive limb weakness. He reported a recent gastrointestinal illness with watery diarrhea, which lasted 5 days and disappeared after treatment with metronidazole. Six days after the resolution of the diarrhea, he started to experience progressive weakness of the lower limbs, with impairment in walking and climbing stairs, associated with lumbar pain and fatigue. He denied both sensory and autonomic or sphincteric deficits. Four days later, weakness of the hands appeared.

His medical history was relevant for a liver transplant following acute HBV hepatitis at the age of 60. Seven years later, he was diagnosed with post-transplant large B-cell intestinal lymphoma and treated with jejunoileal resection. Since then, the patient had been receiving immunosuppressive treatment with tacrolimus and mofetil mycophenolate, and antiviral treatment with anti-HbS immunoglobulins and entecavir.

Upon admittance, physical examination was unremarkable while neurological examination showed symmetric upper and lower limb weakness with proximal predominance, steppage gait, normal sensory testing, and no sphincterial involvement. The remaining neurological examination, including cranial nerves, muscle tone, deep tendon reflexes (DTRs), and plantar cutaneous response, was normal. Blood tests were normal, except for leukocytosis (12.000 wbc/mm3) and elevated C-reactive protein (6 mg/dL).

Abati and Gagliardi equally contributed to the work.

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Review

Back to the origins: Human brain organoids to investigate neurodegeneration

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HIGHLIGHTS

- Human organoids allow researchers to investigate brain development and pathology.
- Late disease onset could be linked to alterations during brain development.
- Brain organoids can be used to model neurodegenerative disorders.
- Organoid maturation can be enhanced by *in vivo* transplantation or *in vitro* patterning.

ARTICLE INFO

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Disease modelling

ABSTRACT

Neurodegenerative disorders represent a high burden in terms of individual, social and economical resources. No ultimate therapy has been established so far; human brain morphology and development can not be entirely reproduced by animal models, and genomic, metabolic and biochemical differences might contribute to a limited predictive power for human translation. Thus, the development of human brain organoid models holds a wide potential to investigate the range of physiological and pathological features that characterise the early onset of the degeneration. Moreover, central nervous system development has gained a crucial role in the study of the pathogenesis of neurodegenerative disorders. Premature alterations during brain maturation have been related to late disease manifestations; genetic mutations responsible for neurodegeneration have been found in genes highly expressed during neural development. Elucidating the mechanisms triggering neuronal susceptibility to degeneration is crucial for pathogenetic studies and therapeutic discoveries. In the present work, we provide an overview on the current applications of human brain organoids towards studies of neurodegenerative diseases, with a survey on the recent discoveries and a closing discussion on the present challenges and future perspectives.

1. Introduction

Neurodegenerative diseases are a clinically and pathologically heterogeneous group of disorders that affects a specific subset of neurons and whose progression is nowadays inevitable (Przedborski et al., 2003). They can be classified according to the clinical phenotype or to the area most predominantly affected. Alzheimer's disease (AD), frontotemporal dementia (FTD), Parkinson's disease (PD) and Huntington's disease (HD) have a devastating impact on patients and families, representing a high burden in terms of individual, social and economical resources. No ultimate therapy has been established so far, although some of these conditions benefit the availability of drugs slightly able to modify the natural history. These disorders share some common features and pathogenetic mechanisms, which have been identified in protein misfolding and aggregation, altered RNA homeostasis, inflammation and involvement of non-neuronal cells and hindered lysosomal functioning (Katsnelson et al., 2016). No unique mechanism seems to be primarily causative of neurodegeneration, suggesting that the complex synergy of different pathways could play a role. Reliable human *in vitro* models are precious tools for the discovery of specific pathogenic mechanisms and potential therapeutic approaches.

Brain organoid cultures were implemented in 2013 by Lancaster

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Molecular Approaches for the Treatment of Pompe Disease

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Abstract

Glycogen storage disease type II (GSDII, Pompe disease) is a rare metabolic disorder caused by a deficiency of acid alpha-glucosidase (GAA), an enzyme localized within lysosomes that is solely responsible for glycogen degradation in this compartment. The manifestations of GSDII are heterogeneous but are classified as early or late onset. The natural course of early-onset Pompe disease (EOPD) is severe and rapidly fatal if left untreated. Currently, one therapeutic approach, namely, enzyme replacement therapy, is available, but advances in molecular medicine approaches hold promise for even more effective therapeutic strategies. These approaches, which we review here, comprise splicing modification by antisense oligonucleotides, chaperone therapy, stop codon readthrough therapy, and the use of viral vectors to introduce wild-type genes. Considering the high rate at which innovations are translated from bench to bedside, it is reasonable to expect substantial improvements in the treatment of this illness in the foreseeable future.

Keywords GSDII · Pompe disease · Alpha-glucosidase (GAA) · Therapy · Gene therapy · Molecular therapy · Antisense oligonucleotides

Introduction

Pompe Disease

Glycogenosis type II (GSDII), or Pompe disease, is a rare autosomal recessive disease caused by a deficiency of the enzyme solely responsible for glycogen degradation within lysosomes: acid maltase or acid alpha-glucosidase (GAA). Over time, the progressive accumulation of glycogen alters cellular architecture, causing a loss of function and eventually necrosis. Although it has long been considered a disease that mainly affects striated muscular tissue with a disproportionate involvement of respiratory muscles, GSDII is multisystemic: glycogen accumulates in all tissues and organs, particularly in the skeletal muscle, central nervous system, heart and brain (the latter are almost exclusively affected by the early-onset form of the disease), causing not only a reduction in motor function and important respiratory deficits, the main cause of death in patients with Pompe disease, but also arrhythmias, dysphagia, incontinence, gastrointestinal symptoms, and several other problems [1–3].

Pompe disease has been documented in most ethnicities, with an incidence ranging from 1:14,000 (in African populations and African-Americans) [4] to 1:238,000 in Europe [5]. The advent of newborn screening (NBS) and the increasing availability of genetic testing for at-risk patients contribute to more precocious diagnosis and are uncovering the real incidence of the disease; for example, in Taiwan, the global incidence of GSDII based on NBS programs is estimated to be 1:17,000 [6].

Patients are divided into two main groups based on the age at disease onset: early-onset Pompe disease (EOPD) and late-onset Pompe disease (LOPD); however, presentation is extremely varied and correlates only partially with residual enzyme activity levels and the mutations carried by patients [7]. Early-onset Pompe disease (EOPD) is defined as GSDII arising before 12 months of age and more typically manifests during the first two months of life; patients present with severe muscular hypotonia and hypertrophic cardiomyopathy, which

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Expert Opinion on Therapeutic Targets

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Silence superoxide dismutase 1 (SOD1): a promising therapeutic target for amyotrophic lateral sclerosis (ALS)

Elena Abati, Nereo Bresolin, Giacomo Comi & Stefania Corti

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Silence superoxide dismutase 1 (SOD1): a promising therapeutic target for amyotrophic lateral sclerosis (ALS)

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Animal Models of CMT2A: State-of-art and Therapeutic Implications

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Abstract

Charcot–Marie–Tooth disease type 2A (CMT2A), arising from mitofusin 2 (*MFN2*) gene mutations, is the most common inherited axonal neuropathy affecting motor and sensory neurons. The cellular and molecular mechanisms by which *MFN2* mutations determine neuronal degeneration are largely unclear. No effective treatment exists for CMT2A, which has a high degree of genetic/phenotypic heterogeneity. The identification of mutations in *MFN2* has allowed the generation of diverse transgenic animal models, but to date, their ability to recapitulate the CMT2A phenotype is limited, precluding elucidation of its pathogenesis and discovery of therapeutic strategies. This review will critically present recent progress in in vivo CMT2A disease modeling, discoveries, drawbacks and limitations, current challenges, and key reflections to advance the field towards developing effective therapies for these patients.

Keywords CMT2A · MFN2 · Animal model · Strengths and weaknesses

Introduction

Charcot–Marie–Tooth type 2A (CMT2A; OMIM 609260) is a dominant inherited sensory motor neuropathy that affects peripheral nerve axons and is characterized by a heterogeneous phenotype including not only neuropathy-related features but also systemic impairment of the central nervous system (CNS) [1, 2]. Motor symptoms not only are predominant in the distal lower limbs, but they may also involve distal upper limbs in half of the diagnosed cases [3], leading to progressive muscle weakness, foot deformities (*pes cavus*), gait disturbances, and areflexia. Other typical features are sensory impairment, mainly affecting vibratory sensation and proprioception and neuropathic pain [4]. CMT2A may also lead to sensory neural impairment, such as hearing loss [5, 6] and other clinical features, such as hoarse voice, vocal cord paresis, and signs of respiratory insufficiency [2].

CMT2A is caused by missense mutations in the *mitofusin 2* (*MFN2*) gene, which encodes a GTPase dynamin-like protein localizing to the outer mitochondrial membrane that is mainly involved in the regulation of mitochondria-related processes, such as mitochondrial fusion, mitochondrial transport along axons, and mitophagy [7–11]. Furthermore, MFN2 participates in mitochondrial metabolism and intracellular signaling [12].

Although few recessive forms have been described [13, 14], CMT2A is generally associated with dominant mutations distributed along the *MFN2* sequence [7, 15–19]. Dominant mutations may lead either to a gain or to a loss of function according to the position of the mutation within MFN2 domains [20]. In particular, *MFN2* mutations seem to induce disease with a "dominant-negative" mechanism, where the expression of the wild-type *MFN2* allele is negatively regulated by the mutant protein. To date, no *MFN2* mutations have been associated with haploinsufficiency. The cellular and molecular mechanisms by which *MFN2* mutations determine neuronal degeneration are not fully understood. However, recent progress revealed that multiple mechanisms contribute to pathogenic *MFN2*–related axonal degeneration including alteration of mitochondria transport and localization [10, 21–25] and mitochondrial–endoplasmic reticulum crosstalk impairment [25, 26].

Considering the high degree of genetic and phenotypic heterogeneity of CMT2A and the involvement of motor and sensory components in disease pathogenesis, the generation of reliable models is very tricky but essential for studying disease pathogenesis and finding resolute therapeutic approaches,

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Neuron

Article

Pathogenic Huntingtin Repeat Expansions in Patients with Frontotemporal Dementia and Amyotrophic Lateral Sclerosis

Graphical Abstract

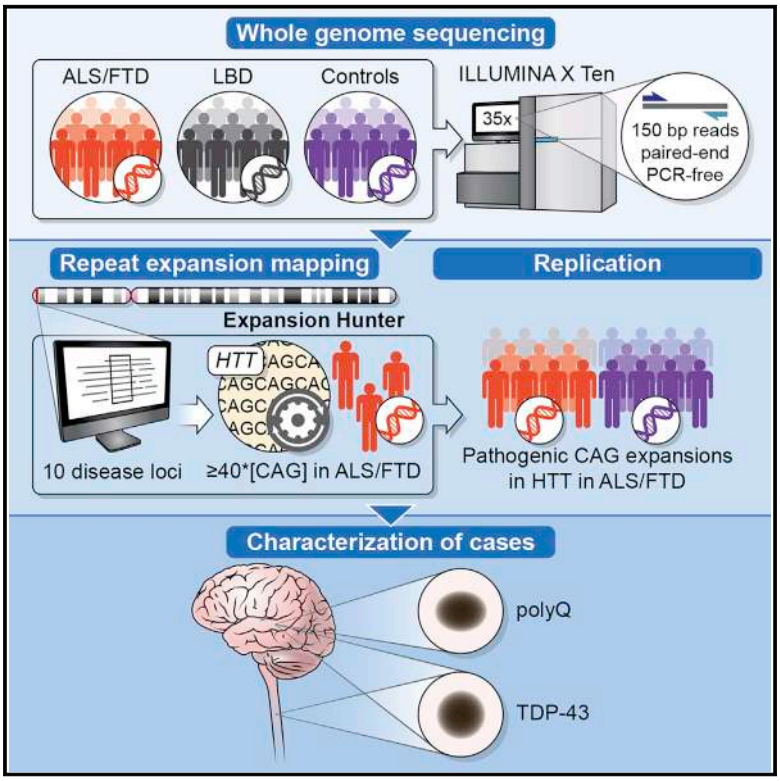

Figure360▷ For a Figure360 author presentation of this figure, see https://doi.org/10.1016/j.neuron.2020.11.005.

Highlights

- Pathogenic expansions in the *HTT* gene are a rare cause of FTD/ALS spectrum diseases
- Autopsies showed both the expected TDP-43 pathology of FTD/ALS and polyQ inclusions
- *HTT* repeat expansions were not seen in healthy subjects or Lewy body dementia cases
- Clinicians should screen FTD/ALS patients for *HTT* repeat expansions

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In Brief

Using large-scale whole-genome sequencing, Dewan et al. identify pathogenic *HTT* repeat expansions in patients diagnosed with FTD/ALS neurodegenerative disorders. Autopsies confirm the TDP-43 pathology expected in FTD/ALS and show polyglutamine inclusions within the frontal cortices but no striatal degeneration. These data broaden the phenotype resulting from *HTT* repeat expansions.

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years, leading to death within 3–8 years of symptom onset (Chiò et al., 2013; Neary et al., 2005). Approximately 15,000 individuals die of FTD or ALS in the United States annually (Arthur et al., 2016), and there are no treatments that halt the degenerative process. Clinical, genetic, and neuropathologic data demonstrate that FTD and ALS are closely related conditions that exist along a spectrum of neurological disease (Lillo and Hodges, 2009).

Though progress has been made, much remains unclear about the genetic etiology of the FTD/ALS spectrum. Approximately 40% of FTD cases are familial, and causative mutations have been identified, most notably in *MAPT*, *GRN*, *C9orf72*, and *VCP* (Ferrari et al., 2019). In ALS, 10% of patients report a family history of the disease. The genetic etiology is known for two-thirds of these familial cases, whereas the underlying gene is recognized in 10% of sporadic cases (Chia et al., 2018; Renton et al., 2014). The intronic repeat expansion of the *C9orf72* gene is the most common cause of FTD and ALS (Majounie et al., 2012). Other repeat expansions have been implicated in neurological diseases. These include polyglutamine repeats observed in Huntington's disease (MacDonald et al., 1993) and spinobulbar muscular atrophy (La Spada et al., 1991) and more complex expansions in the *RFC1* gene that were recently associated with autosomal recessive cerebellar ataxia (Cortese et al., 2019). Together, these data suggest that repeat expansions play a critical role in the pathogenesis of neurodegenerative diseases. This type of mutation may be amenable to antisense oligonucleotide therapy, adding further incentive to their identification (Tabrizi et al., 2019).

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Review

Insights into disease mechanisms and potential therapeutics for *C9orf72*-related amyotrophic lateral sclerosis/frontotemporal dementia

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ABSTRACT

In 2011, a hexanucleotide repeat expansion (HRE) in the noncoding region of *C9orf72* was associated with the most frequent genetic cause of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). The main pathogenic mechanisms in C9-ALS/FTD are haploinsufficiency of the C9orf72 protein and gain of function toxicity from bidirectionally-transcribed repeat-containing RNAs and dipeptide repeat proteins (DPRs) resulting from non-canonical RNA translation. Additionally, abnormalities in different downstream cellular mechanisms, such as nucleocytoplasmic transport and autophagy, play a role in pathogenesis. Substantial research efforts using *in vitro* and *in vivo* models have provided valuable insights into the contribution of each mechanism in disease pathogenesis. However, conflicting evidence exists, and a unifying theory still lacks.

Here, we provide an overview of the recently published literature on clinical, neuropathological and molecular features of C9-ALS/FTD. We highlight the supposed neuronal role of C9orf72 and the HRE pathogenic cascade, mainly focusing on the contribution of RNA foci and DPRs to neurodegeneration and discussing the several downstream mechanisms. We summarize the emerging biochemical and neuroimaging biomarkers, as well as the potential therapeutic approaches. Despite promising results, a specific disease-modifying treatment is still not available to date and greater insights into disease mechanisms may help in this direction.

Abbreviations: AAV, adeno-associated virus; AD, Alzheimer's disease; ADAR2, adenosine deaminase acting on RNA 2; ALS, amyotrophic lateral sclerosis; ASOs, antisense oligonucleotides; BAC, bacterial artificial chromosome; C9-ALS, *C9orf72*-related ALS; C9-FTD, *C9orf72*-related FTD; CARM1, coactivator-associated arginine methyltransferase 1; Cas, CRISPR associated protein; CBD, corticobasal degeneration; CHIT-1, chitotriosidase-1; CNS, central nervous system; CRISPR, clustered regularly interspaced short palindromic repeats; CSF, cerebrospinal fluid; DSBs, double-strand breaks; DENN, differentially expressed in normal and neoplasia; DPRs, dipeptide repeat proteins; DSIF, DRB sensitivity-inducing factor; DTI, diffusion tensor imaging; ER, endoplasmic reticulum; fALS, familial ALS; FTD, frontotemporal dementia; G_4C_2, GGGGCC; GA, Gly-Ala; GP, Gly-Pro; GR, Gly-Arg; HD, Huntington's Disease; HRE, hexanucleotide repeat expansion; HSPs, heat shock proteins; iPSCs, induced pluripotent stem cells; iPSNs, induced pluripotent stem cell derived neurons; KO, knock-out; LCDs, low complexity sequence domains; LDH, lactate dehydrogenase; LLPS, liquid-liquid phase separation; miRNA, micro RNA; MND, motor neuron disease; MNs, motor neurons; MQC, mitochondrial quality control; MRI, magnetic resonance imaging; mRNA, messenger RNA; MSD, mesoscale discovery; mTOR, mammalian target of rapamycin; N/C, nucleo/cytoplasmic; NCT, nucleo-cytoplasmic transport; NEFM, neurofilament medium polypeptide; NES, nuclear export signal; NfL, light chain neurofilament; Nfs, neurofilaments; NLS, nuclear localization sequence; NMD, nonsense-mediated mRNA decay; NPCs, nuclear pore complexes; NPTXR, neuronal pentraxin receptor; Nups, Nucleoporins; P-bodies, processing bodies; p-NfH, phosphorylated neurofilament heavy chain; PA, Pro-Ala; PAF1C, polymerase II-associated factor 1 complex; PD, Parkinson's disease; PFN1, profilin-1; PMA, progressive muscular atrophy; PLS, primary lateral sclerosis; PR, Pro-Arg; PreSxC9, Presymptomatic C9 expansion carrier; PSP, progressive supranuclear palsy; Rab-GEF, Rab-Guanosine Exchange Factor; RAN, repeat-associated non-ATG translation; RanGAP1, Ran GTPase-activating protein 1; RBP, RNA binding protein; RNAi, RNA interference; RNP, ribonucleoprotein; ROS, reactive species of oxygen; sALS, sporadic ALS; SGs, stress granules; shRNAs, short hairpin RNAs; SINEs, selective inhibitors of nuclear export; siRNA, short interfering RNA; SMA, spinal muscular atrophy; SOD1, superoxide dismutase; SRSF1, serine/arginine-rich splicing factor 1; TDP-43, TAR-DNA binding protein 43; TMS, transcranial magnetic stimulation; TMX2, thioredoxin-related transmembrane protein 2; UCHL1, ubiquitin carboxyl-terminal hydrolase isozyme L1; UPS, ubiquitin-proteasome system.

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Systematic elucidation of neuron-astrocyte interaction in models of amyotrophic lateral sclerosis using multi-modal integrated bioinformatics workflow

Vartika Mishra et al.[#]

Cell-to-cell communications are critical determinants of pathophysiological phenotypes, but methodologies for their systematic elucidation are lacking. Herein, we propose an approach for the Systematic Elucidation and Assessment of Regulatory Cell-to-cell Interaction Networks (SEARCHIN) to identify ligand-mediated interactions between distinct cellular compartments. To test this approach, we selected a model of amyotrophic lateral sclerosis (ALS), in which astrocytes expressing mutant superoxide dismutase-1 (mutSOD1) kill wild-type motor neurons (MNs) by an unknown mechanism. Our integrative analysis that combines proteomics and regulatory network analysis infers the interaction between astrocyte-released amyloid precursor protein (APP) and death receptor-6 (DR6) on MNs as the top predicted ligand-receptor pair. The inferred deleterious role of APP and DR6 is confirmed in vitro in models of ALS. Moreover, the DR6 knockdown in MNs of transgenic mutSOD1 mice attenuates the ALS-like phenotype. Our results support the usefulness of integrative, systems biology approach to gain insights into complex neurobiological disease processes as in ALS and posit that the proposed methodology is not restricted to this biological context and could be used in a variety of other non-cell-autonomous communication mechanisms.

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REVIEW

WILEY

Spinal muscular atrophy with respiratory distress type 1: Clinical phenotypes, molecular pathogenesis and therapeutic insights

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Abstract

Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is a rare autosomal recessive neuromuscular disorder caused by mutations in the *IGHMBP2* gene, which encodes immunoglobulin μ-binding protein 2, leading to progressive spinal motor neuron degeneration. We review the data available in the literature about SMARD1. The vast majority of patients show an onset of typical symptoms in the first year of life. The main clinical features are distal muscular atrophy and diaphragmatic palsy, for which permanent supportive ventilation is required. No effective treatment is available yet, but novel therapeutic approaches, such as gene therapy, have shown encouraging results in preclinical settings and thus represent possible methods for treating SMARD1. Significant advancements in the understanding of both the SMARD1 clinical spectrum and its molecular mechanisms have allowed the rapid translation of preclinical therapeutic strategies to human patients to improve the poor prognosis of this devastating disease.

KEYWORDS

gene therapy, IGHMBP2, motor neuron disease, Spinal muscular atrophy with respiratory distress type 1

1 | INTRODUCTION

Autosomal recessive spinal muscular atrophy with respiratory distress type 1 (SMARD1) is a form of spinal muscular atrophy with severe diaphragmatic involvement that causes respiratory distress. This condition is due to autosomal recessive mutations in the *IGHMBP2* gene, which is located on chromosome 11q13.2-q13.4.[1,2] Mellins, considering this mutation a variant of spinal muscular atrophy (SMA) 5q with respiratory onset, provided the first description of this condition in 1974, and it was not recognized as a separate clinical entity until 1996.[3,4] The actual prevalence of SMARD1 is unknown, but diaphragmatic paralysis is observed in approximately 1% of patients with an early onset of the clinical features of spinal muscle atrophy and an estimated incidence of 1/100 000.[5] The main clinical feature is the onset of respiratory distress requiring mechanical ventilation between the ages of 6 weeks and 6 months. The clinical symptoms rapidly progress in the first years of life, with distal limb muscular atrophy extending to proximal regions. The overall prognosis is poor, and progressive autonomic nervous system dysfunction also develops in association with the progressive worsening of motor functions in affected children. In fact, there are no approved treatments for SMARD1.[6]

the histological features of cardiomyocytes and in cardiac fibrosis, with a significant delay in the onset of dilated cardiomyopathy compared with that of the untreated controls. However, the ICV-treated mice gained more weight, and these mice showed significantly better rescue of hindlimb motor function, with a significant reduction in gastrocnemius contracture and a great improvement in the rotarod test performance compared to that of both the untreated controls and the IV-treated mice. The authors concluded that the two methods of administration can be considered equally effective in the rescue of the disease phenotype of *nmd* mice due to the equivalent lifespan prolongation and cardiac improvement. ICV injection was instead correlated with a better improvement in hindlimb function.[48] However, although the ICV and IV doses were the same, the quantity of lentiviral particles received by the CNS may have been higher after ICV delivery compared to IV delivery as ICV injections are delivered locally while IV injections are delivered systemically.

In conclusion, gene therapy seems to show encouraging results in laboratory and in vivo tests and thus requires great effort to reach the knowledge necessary to treat SMARD1.

9 | DISCUSSION

Due to the great achievements provided by preclinical research, the field of neuromuscular diseases has quickly evolved in recent years, and some devastating diseases, such as SMA,[44,45] may finally be removed from the list of untreatable diseases. Unfortunately, SMARD1 remains an unsolved burden, probably due to its lower incidence and its complex and poorly understood pathogenetic mechanisms.

The main clinical features of this disease include neonatal onset (within the year of life), diaphragmatic paralysis and the wasting of distal limb muscles, which leads affected individuals to be completely dependent on ventilatory support and the daily supportive care of parents or caregivers.[6,8-10]

The prognosis of affected patients is currently very poor,[10,17] but the advances made in the treatment of similar diseases have made it possible to study the therapeutic approaches for SMARD1. The benefits obtained by the in vitro use of neurotrophic factors and pharmacological agents are not encouraging,[38,39] but better results have been obtained with the injection of rAAV in *nmd* mice.[32,48]

The pathogenetic mechanisms underlying this disease are complex and not yet completely deciphered; in fact, mutations in the same IGHMBP2 gene can determine very serious (SMARD1) or mild phenotypes (CMT). Moreover, SMARD1 itself demonstrated substantial variability in terms of age of presentation, severity of the symptoms and survival. The role of the protein in the CNS and in other systems and how the different mutations impact the pathogenesis need to be better characterized because the behaviour of the wild-type and mutated proteins affects the strategy of application and the rate of success of the potential therapy. For example, the type of administration adopted is crucial; in fact, although ICV administration would make it possible to reach the central nervous system directly, systemic administration would have the advantage of reaching other tissues that are known to be affected by the pathogenesis. Nevertheless, the published data and the results obtained also by our group show that gene therapy represents a real potential for the treatment of SMARD1. To verify the amplitude of this potential, further studies will be required, aiming to increase the number of analysed cases, thus permitting a wider statistical analysis of the correlation between genotype, IGHMBP2 mRNA and protein levels in animal models and human iPSCs, and the clinical phenotypes observed. Future advances in the knowledge of the pathogenetic mechanisms of this disease and in gene therapy administration will also permit the development of new experimental trials to better clarify its applicability in human SMARD1 patients.

There are still many unsolved questions, such as the best route of administration, the immunogenicity of these therapies in humans, the possible side effects and the long-term efficacy.

For the transition into the clinic, it is necessary to proceed with the validation of the method in the preclinical phase and invest in this research pathology, which, although rare, shares pathogenetic aspects with SMA and therefore also the susceptibility of treatment for SMARD1 patients.

The SMARD1 therefore represents an excellent candidate for the application of this innovative method, which has been demonstrated to be increasingly promising in the treatment of both neuromuscular and other diseases.

In conclusion, treatments for SMARD1 are not currently available, but recent preclinical therapeutic advances have laid the foundation for future solutions to this health issue, and the combination of various therapeutic possibilities that have been studied may lead to an effective therapy for SMARD1 patients.

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We thank Association Centro Dino Ferrari for its support. The image was generated using images from Servier Medical Art, under a Creative Commons Attribution 3.0 Generic License. http://smart.servier.com/

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

MS performed the search and wrote the manuscript; MN, AG, MT, NB and GPC contributed to manuscript writing; and SC supervised the work and wrote the manuscript. All authors read and approved the final manuscript.

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Short communication

SLC25A46 mutations in patients with Parkinson's Disease and optic atrophy

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A B S T R A C T

Mutations in the gene encoding the mitochondrial carrier protein SLC25A46 are known to cause optic atrophy associated with peripheral neuropathy and congenital pontocerebellar hypoplasia.

We found novel biallelic *SLC25A46* mutations (p.H137R, p.A401Sfs*17) in a patient with Parkinson's disease and optic atrophy. Screening of six unrelated patients with parkinsonism and optic atrophy allowed us to identify two additional mutations (p.A176V, p.K256R) in a second patient. All identified variants are predicted likely pathogenic and affect very conserved protein residues.

These findings suggest for the first time a possible link between Parkinson's Disease and *SLC25A46* mutations. Replication in additional studies is needed to conclusively prove this link.

1. Introduction

Several monogenic forms of Parkinson's Disease (PD) are linked to genes encoding for mitochondrial proteins [1]. Mutations in *PINK1* and *Parkin*, two crucial players of the mitophagic machinery, cause recessive forms of PD, mainly characterized by early-onset PD and dystonia [2]. More complex phenotypes are linked to mutations in *OPA1*, *POLG* and *Twinkle* including: optic atrophy, ophthalmoplegia, neurosensorial hearing loss, neuropathy, cerebellar ataxia, and myopathy [3–5].

SLC25A46 encodes for a mitochondrial carrier protein located on the outer membrane that interacts with MFN2, OPA1, the mitochondrial contact site and cristae organizing system complex [6–8]. Mutations in this gene lead to a broad spectrum of neurodegenerative disorders. Congenital lethal pontocerebellar hypoplasia, optic atrophy, peripheral neuropathy, cerebellar ataxia, and spasticity are the clinical features described so far as consequences of loss of SLC25A46 function [9–11]. Nevertheless, parkinsonism has been reported neither as predominant feature, nor in association with a more complex phenotype linked to *SLC25A46* gene mutations.

Here we report, for the first time, the finding of biallelic likely pathogenetic *SLC25A46* mutations in patients affected by PD and optic atrophy.

2. Methods

A Caucasian family of Italian origin, including a single affected patient (Tn-1), was studied. There was no history of parkinsonism or other neurological disorders in previous generations, and there was no evidence of parental consanguinity. A screening of six unrelated patients displaying parkinsonism plus optic atrophy was performed detecting a second subject (Ps-1) with *SLC25A46* mutations.

The relevant ethical authorities approved the study and written informed consent was obtained from all participants to the publication of their images and videotapes, in both the print and online modalities. Neurological examination was performed by movement disorders specialists and included the Hoehn-Yahr scale, and Mini-Mental State Examination. Clinical details of the mutated subjects are reported in

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Short communication

A rapid and low-cost test for screening the most common Parkinson's disease-related *GBA* variants

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ABSTRACT

Introduction: Deleterious variants in the *GBA* gene confer a 2- to 20-fold increased risk of Parkinson's disease (PD) and are associated with a more severe disease course. The presence of a highly-similar pseudogene complicates genetic screening, both by Sanger and next-generation sequencing (NGS). Among PD-associated *GBA* variants, four missense substitutions (p.L444P, p.N370S, p.T369M, p.E326K) account for the majority of cases. Here, we aimed at developing an allele-specific PCR (AS-PCR) able to concomitantly detect the most common PD-related *GBA* variants.

Methods: A multiplex PCR assay was designed using allele-specific oligonucleotides that distinguish the gene from the pseudogene and can exclusively amplify the variant alleles. Primer sequences and molarity, and thermal profiles were empirically optimized. The assay was validated on 4016 DNAs extracted by standard salting-out and previously analyzed by whole-exome sequencing (WES) followed by Sanger validation.

Results: AS-PCRs performed on 4016 DNAs detected 103 variants; among them, 97 were true positives and 6 false positives. When comparing the results with the original WES data, for the "difficult" p.L444P, the number of false positives was 2/9 and 18/24 for multiplex-AS-PCR and WES, respectively. As we could have missed some p.L444P alleles by NGS, we verified the test performance on 50 DNAs from Sanger-validated p.L444P heterozygotes. All samples tested correctly.

Conclusion: We set up and validated a rapid and inexpensive test for screening large cohorts of individuals for variants conferring a significant PD risk. This screening method is particularly interesting to identify patients who could benefit most from early access to personalized therapies.

1. Introduction

Heterozygous mutations in the *GBA* gene (OMIM*606,463), encoding the lysosomal enzyme beta-glucocerebrosidase (GCase), are the most frequent genetic risk factor for Parkinson's disease (PD). Biallelic mutations in this gene, dramatically reducing GCase activity, cause the recessive lysosomal storage disorder Gaucher disease (GD). Many studies have shown an increased frequency of *GBA* mutations in PD compared to controls. Heterozygous *GBA* variants were shown to confer a 2- to 20-fold increased risk of PD (according to the severity of the mutation) [1,2] and to modify PD manifestations, causing earlier age of onset, more cognitive dysfunction, and accelerated disease progression [3–5]. Unfortunately, the screening for mutations in the *GBA* gene is not trivial. This is mainly due to the presence of a highly similar pseudogene, *GBAP1*, located in close proximity to *GBA*, whose exonic sequence is 96% identical to the *GBA*'s sequence. The main difference between *GBA* and *GBAP1* is the presence of three additional Alu insertions in *GBA* introns, while a 55-bp deletion in exon 9 is a unique hallmark of *GBAP1* and can be used to distinguish the gene from the pseudogene [6]. To further complicate the screening, *GBAP1* naturally contains some of the

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Supplementary Information

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Additional file 1: Sympathetic Skin Responses (SSR). SSR were simultaneously recorded both from hands and feet, following electrical stimulation delivered over the median nerve at the wrist: stimulation intensity was set at 30 mA for 0.2 milliseconds and three stimuli were delivered at random intervals of more than 1 min to avoid habituation, in accordance with previously described methods [11]. Note that onset and peak-latencies were within normal limits (O: onset-latency; P: peak-latency).

Additional file 2: In silico pathogenicity prediction. Assessment of the deleterious impact of the *TGM6* p.R342W variant by the in silico prediction tools CADD, Mutation Taster, SIFT, PolyPhen2, FATHMM, Mutation Assessor and MutPred2.

Additional file 3: Original full-length blots for Fig. 3. Original uncropped blots showing the three independent experiments performed to analyse the transamidase activity of TG6-R342W compared to wild-type TG6 (TG6-WT) and TG6-R111C. Red arrow indicates overexpressed TG6. Replicate number 1 was chosen as representative blot for Fig. 3. Each single blot (labelled from A to F) was added on separate pages below.

Abbreviations

SCAs: Spinocerebellar ataxias; NGS: Next generation sequencing; PD: Parkinson's disease; MRI: Magnetic resonance imaging; 2-[18F]FDG: 2-[18F]fluoro-2-deoxy-D-glucose; PET: Positron emission tomography; SRR: Sympathetic skin responses; WT: Wild-type

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Authors' contributions

A.M., A.D.F.: design of the study, writing of the manuscript. A.M., A.D.F., E.M., D.R.: genetic analysis. A.M., M.B.: in vitro functional analysis. T.B., A.D.R., F.S., G.F., A.P., S.C., G.P.C., N.B.: clinical evaluation and collection of samples. All the authors have read and approved the manuscript.

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Availability of data and materials

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics approval and consent to participate

The "Comitato Etico Milano Area 2 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico" (Milan, Italy) approved the study. The patient and his cousins provided written informed consent to participate to the study.

Consent for publication

Written informed consent was obtained from the patient and his cousins included in the study.

Competing interests

A.D.F. declares to be a member of the editorial board (Associate Editor) of *BMC Neurology*. The other authors declare that they have no conflict of interest.

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Parkinson's disease in Gaucher disease patients: what's changing in the counseling and management of patients and their relatives?

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Abstract

Background: How to address the counseling of lifetime risk of developing Parkinson's disease in patients with Gaucher disease and their family members carrying a single variant of the *GBA1* gene is not yet clearly defined. In addition, there is no set way of managing Gaucher disease patients, taking into account the possibility that they may show features of Parkinson's disease.

Methods: Starting from an overview on what has recently changed in our knowledge on this issue and grouping the experiences of healthcare providers of Gaucher disease patients, we outline a path of counseling and management of Parkinson's disease risk in Gaucher disease patients and their relatives.

Conclusion: The approach proposed here will help healthcare providers to communicate Parkinson's disease risk to their patients and will reduce the possibility of patients receiving inaccurate information from inadequate sources. Furthermore, this resource will help to empower healthcare providers to identify early signs and/or symptoms of Parkinson's disease and decide when to refer these patients to the neurologist for appropriate specific therapy and follow-up.

Keywords: Gaucher disease, Risk of Parkinson's disease, Counseling, Management

Background

Gaucher disease (GD) is an inherited metabolic disorder caused by biallelic mutations in the *GBA1* gene. *GBA1* encodes the glucocerebrosidase (GCase) enzyme, which catalyses the hydrolysis of glucosylceramide into ceramide and glucose. Macrophages engorged with aberrant lysosomes, as a result of the GCase-impaired activity (Gaucher cells), infiltrate into the reticuloendothelial system of the affected organs [1].

GD type 1, which accounts for up to 95% of patients with GD in Europe and America, is typically considered a systemic disorder, without neurological involvement. Anaemia, leukopenia, thrombocytopenia with frequent bleeding, hepatosplenomegaly, osteopenia with bone pain, easy fractures, failure to grow and delayed puberty, bone marrow infiltration with bone medullary infarcts and osteonecrosis are the main features of this disease.

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Translational Neurodegeneration

REVIEW Open Access

Nucleo–cytoplasmic transport defects and protein aggregates in neurodegeneration

Giacomo Bitetto and Alessio Di Fonzo*

Abstract

In the ongoing process of uncovering molecular abnormalities in neurodegenerative diseases characterized by toxic protein aggregates, nucleo-cytoplasmic transport defects have an emerging role. Several pieces of evidence suggest a link between neuronal protein inclusions and nuclear pore complex (NPC) damage. These processes lead to oxidative stress, inefficient transcription, and aberrant DNA/RNA maintenance. The clinical and neuropathological spectrum of NPC defects is broad, ranging from physiological aging to a suite of neurodegenerative diseases. A better understanding of the shared pathways among these conditions may represent a significant step toward dissecting their underlying molecular mechanisms, opening the way to a real possibility of identifying common therapeutic targets.

Keywords: Aging, Neurodegeneration, Neurodegenerative disease, Nucleo–cytoplasmic transport, Nuclear pore complex, Protein aggregate

Background

Neuronal protein aggregates are a characteristic neuropathological hallmark of neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Huntington's disease (HD), polyglutamine expansion related ataxias, and Parkinson's disease (PD) [1]. One possible consequence of these aggregates is interference with the proper functioning of the highly conserved nucleo–cytoplasmic transport (NCT) mechanism in the cell, which ensures the transport of nucleic acids and proteins across the nuclear membrane. This pathway is fundamental for long-lived cells such as neurons [2, 3], and emerging evidence links damage to the nuclear membrane and nuclear pores to neurodegenerative diseases and physiological neuronal aging [4].

A properly functional NCT is fundamental for neurons to allow transcription factors to enter into the nucleus [5]. One essential NCT role is to activate regulatory elements necessary for adaptation and response to external stimuli, especially those implicated in neuronal plasticity [6]. Moreover, in non-dividing post-mitotic cells such as neurons, proteins involved in DNA maintenance and repair reach the nucleus through this mechanism [7, 8]. In addition, all ribonucleic proteins involved in processes of RNA maturation, stability, splicing, and export require this transport for localization to the nucleus [9].

The cell relies on an essential, conserved, and dynamic structure called Nuclear Pore Complex (NPC) to accomplish a correct NCT [5]. Small molecules (molecular weight < 40 kDa, diameter < 5 nm) can relatively freely diffuse through the NPC [5]. In contrast, higher molecular-weight proteins depend on the highly regulated and very specific mechanisms of the NCT machinery [5].

NPCs are large cellular structures that span the nuclear envelope [10] and control exchanges between the cytoplasm and nucleus. The NPC is constituted by proteins collectively referred to as nucleoporins (Nups) [11] that have different roles in regulating NPC functions and transport of matter, energy, and information

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Pharmacological antagonism of kainate receptor rescues dysfunction and loss of dopamine neurons in a mouse model of human parkin-induced toxicity

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Abstract

Mutations in the *PARK2* gene encoding the protein parkin cause autosomal recessive juvenile Parkinsonism (ARJP), a neurodegenerative disease characterized by dysfunction and death of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Since a neuroprotective therapy for ARJP does not exist, research efforts aimed at discovering targets for neuroprotection are critically needed. A previous study demonstrated that loss of parkin function or expression of parkin mutants associated with ARJP causes an accumulation of glutamate kainate receptors (KARs) in human brain tissues and an increase of KAR-mediated currents in neurons in vitro. Based on the hypothesis that such KAR hyperactivation may contribute to the death of nigral DA neurons, we investigated the effect of KAR antagonism on the DA neuron dysfunction and death that occur in the parkinQ311X mouse, a model of human parkin-induced toxicity. We found that early accumulation of KARs occurs in the DA neurons of the parkinQ311X mouse, and that chronic administration of the KAR antagonist UBP310 prevents DA neuron loss. This neuroprotective effect is associated with the rescue of the abnormal firing rate of nigral DA neurons and downregulation of GluK2, the key KAR subunit. This study provides novel evidence of a causal role of glutamate KARs in the DA neuron dysfunction and loss occurring in a mouse model of human parkin-induced toxicity. Our results support KAR as a potential target in the development of neuroprotective therapy for ARJP.

Introduction

Mutations in the *PARK2* gene (OMIM 600116) cause the most common form of autosomal recessive juvenile Parkinsonism (ARJP), a neurodegenerative disease characterized by the loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc)[1]. The *PARK2* gene encodes parkin, a ubiquitin-ligase enzyme that catalyzes the transfer of ubiquitin to protein substrates, thus regulating their trafficking and turnover[2]. Many parkin substrates have been identified to date, indicating that parkin is a multifunctional protein involved in many intracellular processes, including the control of mitochondrial integrity and the regulation of apoptosis and transcription[3,4]. These studies notwithstanding how *PARK2* mutations lead to DA neuron death are still unclear. More importantly, a therapy that prevents or slows down the neurodegeneration in ARJP patients has not been developed yet.

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Conception and design of the study: A.C. and J.S. Acquisition of data: M.R., S.C., D.M., S.N., A.P., R.B., E.D., L.C., F.A., L.Z., G.M.S., and S.T. Analysis of data: M.R., S.C., S.T., M.M., and J.S. Drafting the paper: A.C., M.M., and J.S. Revision for important intellectual content: R.B., E.D., G.G.C., M.P., A.D.F., F.V., A.C., S.T., and M.M. All authors read and approved the final paper.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethics approval

Mice were maintained and bred at the animal house of Ospedale San Raffaele in compliance with institutional guidelines and international laws (EU Directive 2010/63/EU EEC Council Directive 86/609, OJL 358, 1, December 12, 1987, NIH Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996). All experiments were conducted with the aim of minimizing the number of sacrificed animals.

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ORIGINAL ARTICLE

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Leukoencephalopathy with calcifications and cysts: Genetic and phenotypic spectrum

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A novel homozygous *VPS11* variant may cause generalized dystonia

Running head: *VPS11* variant associated with dystonia

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LETTER TO THE EDITOR

Expanding the genotypic and phenotypic spectrum of Beta-propeller potein-associated neurodegeneration

Beta-propeller protein-associated neurodegeneration (BPAN) is a very rare early-onset neurodevelopmental-neurodegenerative disorder due to X-linked dominant mutations of the *WDR45* gene [1,2]. One hundred and twenty-eight BPAN patients have been described so far [3]. BPAN, also known as neurodegeneration with brain iron accumulation 5 (NBIA5) or "static encephalopathy of childhood with neurodegeneration in adulthood" (SENDA), is characterized by global early psychomotor delay and epilepsy, followed, in young adulthood, by progressive dystonia, parkinsonism and cognitive deterioration [4]. Brain magnetic resonance imaging (MRI) of affected subjects shows iron accumulation in the globus pallidus and substantia nigra. The pathognomonic MRI finding is the T1-weighted mesencephalic hyperintense signal surrounding the substantia nigra. Cerebral and cerebellar atrophy are also frequently observed [5]. Most affected subjects are female. The rare finding of *WDR45* mutations in males was initially attributed to the poor viability of hemizygotes. However, recent reports suggest that males carrying a hemizygous *WDR45* mutation can present a different phenotype predominated by epileptic encephalopathy [6]. To our knowledge, 97 *WDR45* mutations have been reported in the literature to date. The spectrum of variant types comprises 35 frameshift variants, 21 nonsense variants, 20 splice-site variants, 15 missense variants, three in-frame deletions and three large deletions. Most of the identified mutations occurred de novo, with very few exceptions [3].

Here we present two novel BPAN cases (subjects 1 and 2) each harboring a deleterious *WDR45* variant. The IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) Ethics Committee approved the study. Written informed consent was obtained from all involved subjects.

Subject 1 is a 30-year-old female, the only daughter of healthy unrelated parents. Pregnancy and delivery were unremarkable. Familial history was negative for neurological disorders. At 2 years of age, intellectual disability was diagnosed. She started walking at 5 years of age and maintained the ability to walk until the age of 12 when she underwent knee surgery for post-traumatic ligament rupture. She never acquired a functional language, although comprehension was partially preserved. Her motor and cognitive abilities remained rather stable until the age of 24 when a progressive deterioration of these functions appeared. At 27, the neurological examination showed severe hypertonia of the limbs with hyperreflexia, bilateral Babinski sign, severe cognitive deterioration and, remarkably, complete ophthalmoplegia without ptosis, which was not reported in the previous examination. Whether this oculomotor abnormality was attributable to oculomotor apraxia, supranuclear gaze palsy or oculomotor nuclear impairment was difficult to assess due to disease severity and the poor collaboration of the patient. Brain MRI revealed a significant symmetrical hypointensity of pallidal nuclei and substantia nigra in T2-weighted sequences. T1-weighted imaging revealed the typical hyperintense signal surrounding substantia nigra. Cerebellar and supratentorial cortico-subcortical atrophy was also observed (Figure 1a).

Subject 2 is a 44-year-old female, the second daughter of healthy unrelated parents. No familial history of neurological disorders was reported. Pregnancy and delivery were normal. Psychomotor development was reportedly delayed. She started walking at 18 months. She attended primary school with a dedicated support teacher. At 8 years of age, she developed absence-like seizures, which were effectively treated with sodium valproate. At 10 years of age, she was diagnosed with mild intellectual disability showing a prominent involvement of expressive language. The clinical picture remained stable until the age of 36 when she developed an extrapyramidal syndrome on the right side, characterized by hemiparkinsonism and an abnormal dystonic posture of the foot. Brain MRI showed the typical MRI pattern of BPAN (Figure 1b). Single-photon emission computed tomography showed reduced ioflupane (^{123}I) uptake in the left striatum. She started levodopa therapy with major clinical benefit on parkinsonism; however, after 2 years, dyskinetic movements appeared on the right side at levodopa dose peak.

The genetic analysis of subject 1, performed by Sanger sequencing, revealed a de novo heterozygous *WDR45* splice-site mutation c.519+1_3delGTG (NM_007075) (Figure 1c,d). This mutation was previously reported in a single subject from Japan, displaying a classical BPAN phenotype [7] Transcript analysis on cDNA from blood RNA showed the retention of intron 8 in the proband, probably due to the loss of splice donor site caused by the micro-deletion (Figure 1c).

Genetic analysis of the *WDR45* gene by Sanger sequencing in subject 2 displayed a novel heterozygous frameshift mutation c.968_969delCT → p.323Cfs*18 (NM_007075) (Figure 1c,d). The parents were not available for blood sampling.

In this report, two pathogenic *WDR45* mutations carried by two subjects affected by BPAN are presented. Original relevant findings of this work are the presence of complete ophthalmoplegia in

subject 1 and the identification of a novel pathogenic *WDR45* mutation in subject 2. Therefore, this report expands the genotypic and phenotypic spectrum of this very rare neurogenetic disorder.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author ADF.

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Design and Operation of the Lombardy Parkinson's Disease Network

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Background: Parkinson's disease (PD) is one of the most common chronic neurological conditions leading to disability and social burden. According to the 2016 Italian National Plan on Chronic Diseases, regional health authorities are implementing dedicated networks to manage neurological diseases, including PD.

Methods: A panel of experts representing health-care providers in Lombardy reached consensus on the organization of a patient-centered regional PD healthcare network.

Results: The panel proposed a structure and organization implementing a hub-and-spoke PD network model. Three levels of neurological services were identified: General Neurologist, PD Clinic, PD Center. This model was applied to health service providers currently accredited in Lombardy, yielding 12 candidate PD Centers, each serving an area of ~1,000–2,000 km^2, and not less than 27 PD Clinics. The panel agreed on uniform diagnostic and staging criteria for PD, and on a minimum common clinical data set, on PD patient management by the network at initial and follow-up assessments, on the cadence of follow-up visits, on patient referrals, and on outcome measures for the assessment of network activities.

Conclusions: The implementation of disease-centered networks for chronic neurological diseases provides an innovative opportunity to improve patient management, facilitate research and education.

Keywords: Parkinson disease, health maintenance organizations, disease management, managed care programs, consensus

genes

MDPI

Article

Comprehensive Genomic Analysis Reveals the Prognostic Role of *LRRK2* Copy-Number Variations in Human Malignancies

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Abstract: Genetic alterations of leucine-rich repeat kinase 2 (*LRRK2*), one of the most important contributors to familial Parkinson's disease (PD), have been hypothesized to play a role in cancer development due to demographical and preclinical data. Here, we sought to define the prevalence and prognostic significance of *LRRK2* somatic mutations across all types of human malignancies by querying the publicly available online genomic database cBioPortal. Ninety-six different studies with 14,041 cases were included in the analysis, and 761/14,041 (5.4%) showed genetic alterations in *LRRK2*. Among these, 585 (76.9%) were point mutations, indels or fusions, 168 (22.1%) were copy number variations (CNVs), and 8 (1.0%) showed both types of alterations. One case showed the somatic mutation R1441C. A significant difference in terms of overall survival (OS) was noted between cases harboring somatic *LRRK2* whole deletions, amplifications, and CNV-unaltered cases (median OS: 20.09, 57.40, and 106.57 months, respectively; $p = 0.0008$). These results suggest that both *LRRK2* amplifications and whole gene deletions could play a role in cancer development, paving the way for future research in terms of potential treatment with LRRK2 small molecule inhibitors for *LRRK2*-amplified cases.

Keywords: LRRK2; cancer; mutations; CNV; prognostic

1. Introduction

Identification of the PARK8 locus [1] and mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene [2,3], located on chromosome 12 (12q12) in familial cases of Parkinson's disease (PD), more than fifteen years ago is considered a game-changing discovery in our knowledge of this yet incurable

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

GBA-Related Parkinson's Disease: Dissection of Genotype–Phenotype Correlates in a Large Italian Cohort

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ABSTRACT: **Background:** Variants in *GBA* are the most common genetic risk factor for Parkinson's disease (PD). The impact of different variants on the PD clinical spectrum is still unclear.

Objectives: We determined the frequency of *GBA*-related PD in Italy and correlated *GBA* variants with motor and nonmotor features and their occurrence over time.

Methods: Sanger sequencing of the whole *GBA* gene was performed. Variants were classified as mild, severe, complex, and risk. β-glucocerebrosidase activity was measured. The Kaplan-Meier method and Cox proportional hazard regression models were performed.

Results: Among 874 patients with PD, 36 variants were detected in 14.3%, including 20.4% early onset. Patients with GBA-PD had earlier and more frequent occurrence of several nonmotor symptoms. Patients with severe and complex GBA-PD had the highest burden of symptoms and a higher risk of hallucinations and cognitive impairment. Complex GBA-PD had the lowest β-glucocerebrosidase activity.

Conclusions: GBA-PD is highly prevalent in Italy. Different types of mutations underlie distinct phenotypic profiles. © 2020 International Parkinson and Movement Disorder Society

Key Words: dementia; GBA; genotype–phenotype correlates; impulsive–compulsive behavior; Parkinson's disease

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The Role of Mitochondria in Neurodegenerative Diseases: the Lesson from Alzheimer's Disease and Parkinson's Disease

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Abstract

Although the pathogenesis of neurodegenerative diseases is still widely unclear, various mechanisms have been proposed and several pieces of evidence are supportive for an important role of mitochondrial dysfunction. The present review provides a comprehensive and up-to-date overview about the role of mitochondria in the two most common neurodegenerative disorders: Alzheimer's disease (AD) and Parkinson's disease (PD). Mitochondrial involvement in AD is supported by clinical features like reduced glucose and oxygen brain metabolism and by numerous microscopic and molecular findings, including altered mitochondrial morphology, impaired respiratory chain function, and altered mitochondrial DNA. Furthermore, amyloid pathology and mitochondrial dysfunction seem to be bi-directionally correlated. Mitochondria have an even more remarkable role in PD. Several hints show that respiratory chain activity, in particular complex I, is impaired in the disease. Mitochondrial DNA alterations, involving deletions, point mutations, depletion, and altered maintenance, have been described. Mutations in genes directly implicated in mitochondrial functioning (like Parkin and PINK1) are responsible for rare genetic forms of the disease. A close connection between alpha-synuclein accumulation and mitochondrial dysfunction has been observed. Finally, mitochondria are involved also in atypical parkinsonisms, in particular multiple system atrophy. The available knowledge is still not sufficient to clearly state whether mitochondrial dysfunction plays a primary role in the very initial stages of these diseases or is secondary to other phenomena. However, the presented data strongly support the hypothesis that whatever the initial cause of neurodegeneration is, mitochondrial impairment has a critical role in maintaining and fostering the neurodegenerative process.

Keywords Neurodegeneration · Mitochondria · Alzheimer's disease · Parkinson's disease · Pathogenesis

Background

Neurodegenerative diseases represent one of the most important challenges which have to be faced by modern societies, with millions of patients affected worldwide [1].

Despite the devastating effects that neurodegenerative diseases cause to patients and despite the substantial rebound on families and on the entire community, the pathogenic mechanisms of these diseases remain widely unclear and only few therapeutic options are available.

Several molecular mechanisms have been proposed to be involved in the pathogenesis of these diseases, and mitochondria have often been considered as potential candidates implicated in the degenerative process.

Mitochondria are intracellular organelles which contribute to several metabolic pathways. They are complex structures composed of two membranes, an intermembrane space and a matrix. They contain their own DNA which encodes part of

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CLINICAL/SCIENTIFIC NOTES OPEN ACCESS

Late-onset leukoencephalopathy in a patient with recessive *EARS2* mutations

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Mitochondrial aminoacyl-transfer RNA (tRNA) synthetases catalyze the attachment of specific amino acids to their cognate tRNA, enabling intramitochondrial protein synthesis. Recessive mutations in their coding nuclear genes are associated with heterogeneous clinical presentations, often displaying leukoencephalopathy.[1]

Biallelic mutations in *EARS2*, encoding the mitochondrial glutamyl-tRNA synthetase, result in an infantile-onset neurologic disorder hallmarked by extensive symmetrical white matter abnormalities sparing the periventricular zone, symmetrical signal abnormalities of the thalami and brainstem and thin corpus callosum (leukoencephalopathy with thalamus and brainstem involvement and high lactate [LTBL], OMIM#614924). High blood lactate and mitochondrial dysfunction in muscle and fibroblasts can be observed, especially in more severe cases.[2]

Clinical spectrum of patients with LTBL ranges from severe neonatal or early infantile disease with delayed psychomotor development, seizures, hypotonia, and persistent lactate increase to a more favorable form, characterized by a transient psychomotor regression in the first year of life followed by stabilization and, in some cases, partial recovery of lost skills by age 2 years. Of interest, some cases display an even milder phenotype characterized only by minor clinical regression and abnormalities on brain MRI, suggesting that some mutation carriers can escape the genetic diagnosis.[2] Long-term clinical follow-up of *EARS2*-mutated patients has never been reported.[3]

Here, we describe a male patient with late-onset multisystemic neurodegenerative disorder presenting with behavioral abnormalities, pyramidal, and extrapyramidal signs and progressive cognitive decline. Whole-exome sequencing (WES) analysis in the proband allowed the identification of 2 novel *EARS2* mutations. The Ethics Committee of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) approved the study. Written informed consent was obtained from the patient.

The patient was born at term after an uneventful pregnancy. Reportedly, developmental psychomotor milestones were reached with some delay. He completed high school and attended military service. He got married and had a son. He worked as an office worker until retirement. At age 63 years, he presented mood deflection for which he was examined by a psychiatrist who diagnosed an atypical form of depression. The following year, the patient developed postural and intention tremor of the right upper limb, which initially responded favorably to beta-blocking therapy. At age 68 years, the tremor got significantly worse involving the left limb and presenting at rest. Neurologic examination revealed the presence of upper limb dysmetria, plastic rigidity of the 4 limbs, and diffuse hyperreflexia. The Mini-Mental State Examination score was 25/30. The serum lactate level was within normal limits. Brain MRI showed extensive confluent almost symmetrical white matter abnormalities and callosal atrophy, without thalamus and brainstem involvement (figure, A). Head CT did not show pathologic calcifications. Brain ^{18}F-fluorodeoxyglucose PET displayed

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The SPID-GBA study

Sex distribution, Penetrance, Incidence, and Dementia in GBA-PD

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Abstract

Objective

To provide a variant-specific estimate of incidence, penetrance, sex distribution, and association with dementia of the 4 most common Parkinson disease (PD)-associated *GBA* variants, we analyzed a large cohort of 4,923 Italian unrelated patients with primary degenerative parkinsonism (including 3,832 PD) enrolled in a single tertiary care center and 7,757 ethnically matched controls.

Methods

The p.E326K, p.T369M, p.N370S, and p.L444P variants were screened using an allele-specific multiplexed PCR approach. All statistical procedures were performed using R or Plink v1.07.

Results

Among the 4 analyzed variants, the p.L444P confirmed to be the most strongly associated with disease risk for PD, PD dementia (PDD), and dementia with Lewy bodies (DLB) (odds ratio [OR] for PD 15.63, 95% confidence interval [CI] = 8.04–30.37, $p = 4.97*10^{-16}$; OR for PDD 29.57, 95% CI = 14.07–62.13, $p = 3.86*10^{-19}$; OR for DLB 102.7, 95% CI = 31.38–336.1, $p = 1.91*10^{-14}$). However, an unexpectedly high risk for dementia was conferred by p.E326K (OR for PDD 4.80, 95% CI = 2.87–8.02, $p = 2.12*10^{-9}$; OR for DLB 12.24, 95% CI = 4.95–30.24, $p = 5.71*10^{-8}$), which, on the basis of the impact on glucocerebrosidase activity, would be expected to be mild. The 1.5–2:1 male sex bias described in sporadic PD was lost in p.T369M carriers. We also showed that PD penetrance for p.L444P could reach the 15% at age 75 years.

Conclusions

We report a large monocentric study on *GBA*-PD assessing mutation-specific data on the sex distribution, penetrance, incidence, and association with dementia of the 4 most frequent deleterious variants in *GBA*.

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MULTIPLE SCLEROSIS JOURNAL MSJ

Original Research Paper

Kappa free light chains is a valid tool in the diagnostics of MS: A large multicenter study

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Multiple Sclerosis Journal

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Abstract

Objective: To validate kappa free light chain (KFLC) and lambda free light chain (LFLC) indices as a diagnostic biomarker in multiple sclerosis (MS).

Methods: We performed a multicenter study including 745 patients from 18 centers (219 controls and 526 clinically isolated syndrome (CIS)/MS patients) with a known oligoclonal IgG band (OCB) status. KFLC and LFLC were measured in paired cerebrospinal fluid (CSF) and serum samples. Gaussian mixture modeling was used to define a cut-off for KFLC and LFLC indexes.

Results: The cut-off for the KFLC index was 6.6 (95% confidence interval (CI) = 5.2–138.1). The cut-off for the LFLC index was 6.9 (95% CI = 4.5–22.2). For CIS/MS patients, sensitivity of the KFLC index (0.88; 95% CI = 0.85–0.90) was higher than OCB (0.82; 95%CI = 0.79–0.85; $p < 0.001$), but specificity (0.83; 95% CI = 0.78–0.88) was lower (OCB = 0.92; 95% CI = 0.89–0.96; $p < 0.001$). Both sensitivity and specificity for the LFLC index were lower than OCB.

Conclusion: Compared with OCB, the KFLC index is more sensitive but less specific for diagnosing CIS/MS. Lacking an elevated KFLC index is more powerful for excluding MS compared with OCB but the latter is more important for ruling in a diagnosis of CIS/MS.

Keywords: Multiple sclerosis, KFLC, OCB, CSF, biomarkers

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Introduction

Cerebrospinal fluid (CSF) assessment is often part of the diagnostic workup for multiple sclerosis (MS), and its value is supported by the latest 2017 revisions of the McDonalds criteria.[1] CSF examination is frequently performed in diagnosing MS for excluding alternative diagnoses, although, in the current MS criteria,[2] the assessment of oligoclonal IgG bands (OCB) plays a limited role. However a recent study showed the added value of OCB in the MS diagnostic criteria.[3] In the 2017 revisions, the OCB have a prominent role in patients with a clinically isolated syndrome (CIS). The presence of both magnetic resonance imaging (MRI) criteria for dissemination in space (DIS) and CSF-specific OCB will enable to establish the MS diagnosis in patients with a single clinical episode suggestive of central nervous system (CNS) inflammatory demyelinating disease. The OCB assessment also has important prognostic value in CIS and MS.[4,5] However, the assessment of OCB is labor-intensive, requires trained personnel, and is in some cases examiner- and method-dependent, which may affect its reliability.

Alongside intact immunoglobulins, which are composed of two heavy and two light chains, plasma cells produce and secrete immunoglobulin free light chains (FLC) of either kappa (KFLC) or lambda (LFLC) chains. KFLC and LFLC can be detected in both CSF and serum.[6–10] Since the late 1970s,

multiple studies have reported increased CSF levels of KFLC in MS.[6–14] The analytical specificity of the earlier methods (e.g. radioimmunoassay,[15,16] quantitative enzyme-linked immunosorbent assay)[8,17] was insufficient, but with the recent emergence of the more sensitive nephelometric and turbidimetric FLC assays, research in this field has been revived. Nephelometric (and turbidimetric) FLC level determination has the additional advantage compared to OCB of being assessed by an automated procedure and being quantifiable.[18]

Using the FLC assay,[19] recent studies showed that both CSF KFLC levels and the KFLC index are increased in patients with CIS or relapsing-remitting MS (RRMS) compared with controls.[8,14,18,20–22] The use of an index measure is necessary, for example [CSF KFLC/serum KFLC]/[CSF albumin/serum albumin], to include blood-CSF barrier permeability.[10,14] The KFLC index has comparable sensitivity and specificity to OCB for diagnosis of MS and CIS.[14,21,23] However, large-scale studies comparing diagnostic performance of the two methods and to define the cut-off of FLC are lacking. The main aim of this study was to validate KFLC and LFLC indices as a diagnostic biomarker in MS compared with OCB in a large multicenter study including samples from 18 MS centers across Europe.

Methods

Patients and controls

Eighteen MS centers participated, located in the Netherlands, Spain, France, Belgium, Hungary, Italy, Poland, Turkey, Denmark, Serbia, Austria, and Switzerland. We selected 745 paired CSF/serum samples from patients with known OCB status, diagnosed as CIS ($n=242$), RRMS ($n=235$), primary-progressive MS (PPMS) ($n=41$), and secondary-progressive MS (SPMS) ($n=8$). We also included inflammatory neurological disease controls (INDC) ($n=67$), non-inflammatory neurological disease controls (NINDC) ($n=76$), symptomatic controls (SC) ($n=49$), and healthy controls (HC) ($n=27$) as defined previously.[24] The different control groups were pooled into one control group ($n=219$). The CIS and MS patients were also pooled (CIS/MS) ($n=526$).

The large majority (84%) of the CIS/MS patients fulfilled the 2010 McDonald criteria[2] but, in some cases, the patients were diagnosed according to the 2005 McDonald criteria[25] (16%). Table 1 presents the demographic and clinical characteristics of the patients and controls.

CSF and serum samples

Only CSF samples that were immediately centrifuged and stored in polypropylene tubes within 2 hours at −80°C, at the local center, were included. The assessment of OCB had been performed by isoelectric focusing (on agarose or polyacrylamide gel), followed by immunofixation by the centers as part of the diagnostic workup.

Samples were taken between 2005 and 2016, with a median age of 2.9 years (IQR = 1.7–5.7).

We used fresh aliquots, and in our lab, we did not freeze and thaw the samples during the analyses. As far as we know, no effects of freezing and thawing have been reported.

KFLC, LFLC, and albumin analysis

KFLC, LFLC, and albumin concentrations in CSF and serum samples were analyzed using the turbidimetric analyzer SPAplus® (The Binding Site, Birmingham, UK) with the serum free light chain immunoassay (Freelite®, The Binding Site, Birmingham, UK) according to the manufacturer's instructions. All samples were measured centrally in the Neurochemistry Laboratory of the Department of Clinical Chemistry of the VU University Medical Center (VUmc), Amsterdam, the Netherlands. All samples were run blinded for the clinical data.

To verify the QC data supplied by the manufacturer, we calculated intra-assay coefficient of variation (CV) by taking the mean CVs of four replicates of five samples (CSF/serum) within one run. We calculated inter-assay CV based on $n=5$ samples (CSF/serum) measured in 5 different days. CV values for KFLC and LFLC were all found to be lower than those supplied by the manufacturer (Supplemental Table 1). CV values for albumin were comparable to those supplied by the manufacturer. Assay linearity was experimentally confirmed for albumin (serum and CSF) and the FLC assays in serum and showed that recalculated values varied by 25.7% (KFLC) and 14.2% (LFLC) from the original value.

For 29 samples, CSF albumin results were below detection. Here, we assigned a random uniform value between 35 mg/mL (lowest detected value in undiluted rerun) and 175 mg/mL (formal detection limit).

FLC indices

We determined the CSF/serum quotients (Q FLC) of KFLC and LFLC and calculated indices in order to

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Clinical trial

CSF β-amyloid predicts early cerebellar atrophy and is associated with a poor prognosis in multiple sclerosis

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ABSTRACT

Background: Neurodegeneration is present from the earliest stages of multiple sclerosis (MS) and is critically involved in MS related clinical disability. Aim of the present study was to assess the connection between amyloid burden and early cerebellar grey matter (GM) atrophy compared to early brain GM atrophy in MS patients.
Methods: Forty newly diagnosed relapsing-remitting (RR-) MS patients were recruited. β-amyloid1-42 (Aβ) levels were determined in cerebrospinal fluid (CSF) samples from all subjects. All participants underwent neurological examination and brain magnetic resonance imaging (MRI) at baseline. Twenty-nine out of 40 patients repeated a brain MRI at 1-year follow-up. T1-weighted scans were segmented using the Voxel-Based Morphometry (VBM) protocol and the Spatially Unbiased Infratentorial Toolbox (SUIT) from Statistical Parametric Mapping (SPM12).
Results: Between-group comparison of cerebellar parenchymal fraction (GM + WM/total cerebellar volume%) showed significant differences between $A\beta^{high}$ and $A\beta^{low}$ at baseline ($p < 0.0001$) and follow-up ($p = 0.02$). Similarly, a between-group comparison of cerebellar GM fraction (GMF) showed significant differences between $A\beta^{high}$ and $A\beta^{low}$ at baseline ($p = 0.002$) and follow-up ($p = 0.04$). The multiple regression analysis showed CSF Aβ concentration as the best predictor of GMF both at baseline and over time ($\beta = 0.505$, $\beta = 0.377$; $p < 0.05$). No significant results were found regarding global brain atrophy and CSF Aβ concentration.
Conclusions: Early cerebellar atrophy seems to be crucial in predicting a poor prognosis in MS, more than early global brain atrophy.

1. Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory demyelinating disease of the central nervous system (CNS) (Reich et al., 2018). Although its pathologic hallmark is myelin loss, the neurodegenerative component is now considered remarkably relevant, particularly as it is related to long-term disability (Filippi et al., 2013). Histological studies have demonstrated that neurodegeneration is reflected by imaging-derived grey matter (GM) atrophy, as assessed by magnetic resonance imaging (MRI) (Filippi et al., 2012). As expected, global GM atrophy develops at a faster rate in patients with MS than healthy control subjects. Interestingly, GM atrophy is not uniform across the brain, and some regions are more susceptible than others (Steenwijk et al., 2016; Preziosa et al., 2017), suggesting that it occurs largely in a nonrandom manner and develops according to distinct anatomical patterns. At present, however, the biological mechanisms underlying GM atrophy in MS are still largely unknown. Consequently, evaluating neurodegeneration using novel MRI techniques, and its possible link with predictive biomarkers, has become a crucial area of research in MS (Pietroboni et al., 2017, 2018).

Cerebellar impairment is frequent in MS and traditionally considered predictive of a negative outcome (Weinshenker et al., 1991).

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C9ORF72 hexanucleotide repeat expansion frequency in patients with Paget's disease of bone

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ABSTRACT

Paget's disease of bone (PDB) is a focal bone disorder affecting the skeleton segmentally. A strong genetic component has been shown in PDB, and variants in several genes, such as *SQSTM1, VCP,* and *OPTN,* have been associated with the disease. Mutations in the same genes have also been reported in patients with frontotemporal dementia and amyotrophic lateral sclerosis. Hexanucleotide repeat expansions in the *C9ORF72* gene have been shown to be responsible for both familial and sporadic frontotemporal dementia/amyotrophic lateral sclerosis. Thence, we evaluated the frequency of the *C9ORF72* hexanucleotide repeat expansions in a cohort of 191 Italian PDB patients and in 106 controls. The pathogenic repeat expansion was detected in 2 PDB patients (1.0%). During the follow-up period, both PDB patients did not develop any sign of mental decline and/or motor neuron disease. Our study suggests that repeat expansions in the *C9ORF72* gene are rare in patients with PDB.

1. Introduction

Paget's disease of bone (PDB) (OMIM 602080) is the second most common skeletal disorder after osteoporosis. PDB generally affects approximately 1% of adults in Western Europe, increasing significantly with age to affect about 5% of people aged 85 years. The disease is characterized by focal areas of abnormal bone metabolism because of increased activity of both osteoclasts and osteoblasts, resulting in formation of disorganized bone (Galson and Roodman, 2014). Additional abnormalities include bone marrow fibrosis and increased vascularity of affected bones. The most commonly affected bones are the pelvis, the spine, the femora, and the skull, but any bone of the skeleton may be affected. Thence, the disease may be diagnosed incidentally by skeletal radiographs or by biochemical finding of an unexplained elevation of serum alkaline phosphatase. About one-third of PDB patients have only one affected bone and are asymptomatic. The commonest symptoms are pain, due to pagetic lesions in bone itself or caused by pathological fractures, bone enlargement, deformity, and deafness.

The etiology of PDB has remained largely unknown for several decades. Recent studies suggested that the disease is due to a complex interaction between several genetic and environmental risk factors. Epidemiologic studies have indicated a strong genetic component in PDB: 15%–40% of patients have a familial form of the disease, which is transmitted in an autosomal-dominant model of inheritance with incomplete penetrance (Ralston and Albagha, 2014). To date, causative PDB mutations have been identified mainly in the *SQSTM1* gene, coding for the p62 protein, with a frequency of 25%–50% in familial and 5%–10% in sporadic patients (Laurin et al., 2002). In addition, mutations in the *VCP* gene have been associated with a complex phenotype that comprises inclusion body myopathy, Paget's disease of bone and frontotemporal dementia (IBMPFD) (Weihl et al., 2009). Finally, genetic variants in several genes, including *CSF1*, *TNFRSF11A*, *TNFRSF11B*, and *OPTN*, have also been implicated in the pathogenesis of the disease.

In the last few years, mutations in some of the genes involved in PDB have also been described in patients with frontotemporal dementia (FTD) and/or amyotrophic lateral sclerosis (ALS), suggesting the presence of overlapping pathogenetic mechanisms. *SQSTM1* gene mutations have been found in approximately 2.5% of patients with ALS and 3% of patients with FTD (Rainero et al., 2017). A UK kindred segregating the P392L mutation in the *SQSTM1* gene showed the coexistence of both ALS and PDB phenotypes (Kwok et al., 2014). Finally, mutations in the *OPTN* gene were also

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ORIGINAL RESEARCH

Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia

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ABSTRACT

Background There are few validated fluid biomarkers in frontotemporal dementia (FTD). Glial fibrillary acidic protein (GFAP) is a measure of astrogliosis, a known pathological process of FTD, but has yet to be explored as potential biomarker.

Methods Plasma GFAP and neurofilament light chain (NfL) concentration were measured in 469 individuals enrolled in the Genetic FTD Initiative: 114 *C9orf72* expansion carriers (74 presymptomatic, 40 symptomatic), 119 *GRN* mutation carriers (88 presymptomatic, 31 symptomatic), 53 *MAPT* mutation carriers (34 presymptomatic, 19 symptomatic) and 183 non-carrier controls. Biomarker measures were compared between groups using linear regression models adjusted for age and sex with family membership included as random effect. Participants underwent standardised clinical assessments including the Mini-Mental State Examination (MMSE), Frontotemporal Lobar Degeneration-Clinical Dementia Rating scale and MRI. Spearman's correlation coefficient was used to investigate the relationship of plasma GFAP to clinical and imaging measures.

Results Plasma GFAP concentration was significantly increased in symptomatic *GRN* mutation carriers (adjusted mean difference from controls 192.3 pg/mL, 95% CI 126.5 to 445.6), but not in those with *C9orf72* expansions (9.0, –61.3 to 54.6), *MAPT* mutations (12.7, –33.3 to 90.4) or the presymptomatic groups. GFAP concentration was significantly positively correlated with age in both controls and the majority of the disease groups, as well as with NfL concentration. In the presymptomatic period, higher GFAP concentrations were correlated with a lower cognitive score (MMSE) and lower brain volume, while in the symptomatic period, higher concentrations were associated with faster rates of atrophy in the temporal lobe.

Conclusions Raised GFAP concentrations appear to be unique to *GRN*-related FTD, with levels potentially increasing just prior to symptom onset, suggesting that GFAP may be an important marker of proximity to onset, and helpful for forthcoming therapeutic prevention trials.

INTRODUCTION

Frontotemporal dementia (FTD) is a progressive neurodegenerative condition with around a third of cases caused by an autosomal dominant gene mutation in progranulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*) or microtubule-associated protein tau (*MAPT*).[1] As clinical trials in genetic FTD are fast approaching, robust biomarkers that allow accurate measurement of disease onset and progression are becoming increasingly important. In particular, many trials will focus on the presymptomatic stage of disease where neuropathological alterations are already present[2] and yet few biomarkers have been shown to be abnormal in this phase.[3–5]

Cerebrospinal fluid (CSF) or plasma/serum progranulin levels in *GRN* mutation carriers[4 6] and CSF (poly)GP dipeptide repeat concentrations in *C9orf72* expansion carriers[5 7 8] are markers of specific protein abnormalities in genetic FTD, but both are abnormal from early in the presymptomatic period (and potentially from birth). In contrast, neurofilament light chain (NfL) is a marker of neuronal death and axonal degeneration (measurable in CSF[3 9 10] as well as both plasma[11] and serum[12 13]) that is not specific to FTD[14] and has only been shown to be abnormal in the very late presymptomatic period prior to conversion to the symptomatic phase.[3] Glial fibrillary acidic protein (GFAP) is a marker of astrogliosis, the abnormal proliferation of astrocytes due to neuronal damage[15] and has previously been shown to be increased in frontal cortical tissue in FTD,[16] and raised in both the CSF and serum of patients with symptomatic FTD.[17–19] However, it has yet to be

the lowest correlation (r=0.38) was in the symptomatic *GRN* mutation carriers, suggesting that in this group astrogliosis and neurodegeneration are not so closely related.

Correlation of GFAP concentration with cognitive and imaging measures revealed a negative correlation, that is, higher concentration with a lower cognitive score and lower cross-sectional brain volumes in FTD-related regions in presymptomatic *GRN* mutation carriers. This suggests that GFAP levels start to increase as the brain starts to decrease in volume, and as cognition starts to become affected thus in the later stages of the presymptomatic period in proximity to symptom onset. This would be an important biomarker for *GRN*-related FTD, as an increase in concentration from baseline during the presymptomatic period would identify a time around the onset of neurodegeneration, and potentially a time when therapeutic intervention may be optimal. Despite the lack of a significant increase in concentration in *C9orf72* mutation carriers, a similar pattern of negative correlation with cognition and brain imaging was seen in the presymptomatic period—it would be useful in future studies to investigate the subset of *C9orf72* expansion carriers that have increased GFAP concentrations, and how they differ from those with a lower concentration. In particular, it would be helpful to compare carriers with and without concomitant ALS. We also assessed whether GFAP correlated with the rate of brain atrophy measured with longitudinal brain imaging and found a significant positive correlation only in the symptomatic *GRN* carriers (in the temporal lobe), implicating an association of GFAP levels with the intensity of the disease process, that is, how fast the disease is progressing. With longitudinal follow-up of participants, it would therefore be hypothesised that higher GFAP concentration would be associated with shorter survival in *GRN*-related FTD.

While the multicentre nature of the GENFI study allows collection of samples from a large genetic cohort of FTD worldwide, there remains a relatively small number of cases in each group (leading to low statistical power to detect differences), particularly in the symptomatic carriers, and replication in a larger dataset would be helpful. Due to the nature of the disease process, the mean age of the controls overall is lower compared with the symptomatic mutation carriers, but nonetheless the same results are found whether performing an age-adjusted comparison (as presented above in **Plasma GFAP concentration**) or when symptomatic mutation carriers are compared with an age-matched and gender-matched subset of older controls (see online supplementary figure 2). The advantage of studying levels in plasma is that blood is more easily accessible and a relatively cost-efficient way to access bodily fluids in comparison to performing a lumbar puncture; in this study, the use of the ultrasensitive SIMOA assay allowed detection at a level in blood that other assays do not. However, it will be important to study CSF levels in more detail in this group, as concentrations can differ between blood and CSF.[18] Lastly, despite significant differences between the groups, there is a substantial overlap in concentrations between carriers and controls: longitudinal study of GFAP concentration over time, particularly in participants that convert from presymptomatic to symptomatic status, will therefore be important to truly evaluate whether changes do occur towards the end of the presymptomatic period and how levels change with progression of disease.

In summary, plasma GFAP levels appear to be uniquely increased in *GRN* mutation carriers in the current study, and importantly, concentrations may well be abnormal during the late presymptomatic period, suggesting that GFAP might act as marker of proximity to symptom onset.

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BIPOLAR DISORDERS WILEY

CLINICAL CURIOSITY

A case of bipolar disorder developing into atypical parkinsonism and presenting with frontotemporal asymmetrical brain degeneration. A TREDEM Registry Case Report

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1 | CASE PRESENTATION

A 67-year-old man with a diagnosis of bipolar disorder (BD) Type I since he was 35 years old, was admitted to our Memory Clinic in 2016 because, throughout the last year, his wife had noticed the onset of attentional deficits and a significant worsening of motor slowness. The patient is married and a father of two, he has worked as a naval carpenter and he was retired at the time of the first admission at our clinic. He has never smoked nor drunk alcohol. No family history for psychiatric or neurodegenerative disease was reported. The patient has been monitored by the local psychiatric services throughout his life and the psychiatric reports describe his periods of mania, lasting for 2-3 years, with the presence of: dangerous driving, sleep disorder during which the patient carried out domestic activities in the night, expansive behaviour towards women, periods of excessive prodigality for hobbies and holidays. These hypo-maniac periods were characterized by an increase of energy, impulsiveness and risky behaviour. His condition included periods of remission and periods of hypomania with extremely depressed mood during which made it hard for him to go to work. Long-acting injectable antipsychotics (Haloperidol) were initially dispensed by the psychiatric service. Subsequently, about 25 years ago, the patient was started on lithium, which was partially affective to control behavioural symptoms that remained stable in frequency for at least further 20 years. About ten years ago the patient was started with olanzapine. In the last four years, while the psychiatric complaints decreased in severity, the patient has started to develop motor slowness.

Four years ago the patient was visited by the local neurology department for signs of bradykinesia, the neurological examination performed in 2014 showed signs of akinetic-rigid parkinsonism that were believed secondary to the treatment with lithium 300 mg twice daily and olanzapine 5 mg. The patient was well-functioning and in a condition of good psychopathological balance.

In 2016, the patient was admitted to our Memory Clinic. Neurological examination at first admission showed severe parkinsonism with moderate-to-severe limb rigidity, marked impairment of finger and toe tapping with predominance on the right side. Axial symptoms were also present and included reduced postural reflexes, marked neck rigidity and limitation of vertical eye pursuit. Dystonia or phantom limb phenomenon was absent. Therapy at admission was: Carbolithium 300 mg 2/daily; Lorazepam 2.5 mg/daily and Olanzapine 5 mg/daily. Serum lithium was always within therapeutic range.

Treatment with levodopa was started, up to 800 mg/daily. The subsequent evaluations showed the stability of cognition but the worsening of motor symptoms. Throughout the last two years, postural instability worsened with frequent backwards falls and the

Age at symptom onset and death and disease duration in genetic frontotemporal dementia: an international retrospective cohort study

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Summary

Background Frontotemporal dementia is a heterogenous neurodegenerative disorder, with about a third of cases being genetic. Most of this genetic component is accounted for by mutations in *GRN*, *MAPT*, and *C9orf72*. In this study, we aimed to complement previous phenotypic studies by doing an international study of age at symptom onset, age at death, and disease duration in individuals with mutations in *GRN*, *MAPT*, and *C9orf72*.

Methods In this international, retrospective cohort study, we collected data on age at symptom onset, age at death, and disease duration for patients with pathogenic mutations in the *GRN* and *MAPT* genes and pathological expansions in the *C9orf72* gene through the Frontotemporal Dementia Prevention Initiative and from published papers. We used mixed effects models to explore differences in age at onset, age at death, and disease duration between genetic groups and individual mutations. We also assessed correlations between the age at onset and at death of each individual and the age at onset and at death of their parents and the mean age at onset and at death of their family members. Lastly, we used mixed effects models to investigate the extent to which variability in age at onset and at death could be accounted for by family membership and the specific mutation carried.

Findings Data were available from 3403 individuals from 1492 families: 1433 with *C9orf72* expansions (755 families), 1179 with *GRN* mutations (483 families, 130 different mutations), and 791 with *MAPT* mutations (254 families, 67 different mutations). Mean age at symptom onset and at death was 49·5 years (SD 10·0; onset) and 58·5 years (11·3; death) in the *MAPT* group, 58·2 years (9·8; onset) and 65·3 years (10·9; death) in the *C9orf72* group, and 61·3 years (8·8; onset) and 68·8 years (9·7; death) in the *GRN* group. Mean disease duration was 6·4 years (SD 4·9) in the *C9orf72* group, 7·1 years (3·9) in the *GRN* group, and 9·3 years (6·4) in the *MAPT* group. Individual age at onset and at death was significantly correlated with both parental age at onset and at death and with mean family age at onset and at death in all three groups, with a stronger correlation observed in the *MAPT* group (r=0·45 between individual and parental age at onset, r=0·63 between individual and mean family age at onset, r=0·58 between individual and parental age at death, and r=0·69 between individual and mean family age at death) than in either the *C9orf72* group (r=0·32 individual and parental age at onset, r=0·36 individual and mean family age at onset, r=0·38 individual and parental age at death, and r=0·40 individual and mean family age at death) or the *GRN* group (r=0·22 individual and parental age at onset, r=0·18 individual and mean family age at onset, r=0·22 individual and parental age at death, and r=0·32 individual and mean family age at death). Modelling showed that the variability in age at onset and at death in the *MAPT* group was explained partly by the specific mutation (48%, 95% CI 35–62, for age at onset; 61%, 47–73, for age at death), and even more by family membership (66%, 56–75, for age at onset; 74%, 65–82, for age at death). In the *GRN* group, only 2% (0–10) of the variability of age at onset and 9% (3–21) of that of age of death was explained by the specific mutation, whereas 14% (9–22) of the variability of age at onset and 20% (12–30) of that of age at death was explained by family membership. In the *C9orf72* group, family membership explained 17% (11–26) of the variability of age at onset and 19% (12–29) of that of age at death.

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See Comment page 106

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About a third of frontotemporal dementia cases are genetic,[3] with mutations in multiple genes shown to be causative of this disease. However, most of the heritability of frontotemporal dementia is accounted for by mutations in three genes: progranulin (*GRN*), microtubule-associated protein tau (*MAPT*), and chromosome 9 open reading frame 72 (*C9orf72*; also known as *C9orf72*-SMCR8 complex subunit). Although much has been learned over the past decade about the clinical features of these genetic forms of frontotemporal dementia, most studies exploring age at symptom onset and disease duration have been small and geographically restricted.[4–6] In particular, although individual case series have suggested that such phenotypic characteristics can be quite variable, no studies have systematically investigated these factors across all the different genetic groups and the different mutations found within these groups.

Therefore, in this large international study, we aimed to analyse phenotypic characteristics of the main three forms of genetic frontotemporal dementia, including ages at symptom onset and death and disease duration, as well as examining the effect of mutation type and family membership on these factors.

Methods

Study design and participants

In this international retrospective cohort study, we collected data from centres that are part of the Frontotemporal Dementia Prevention Initiative (FPI) and through a literature review of publications. The FPI is a group connecting natural history cohort studies of genetic frontotemporal dementia: the Genetic Frontotemporal Dementia Initiative (GENFI),[7] Advancing Research and Treatment for Frontotemporal Lobar Degeneration (ARTFL), Longitudinal Evaluation of Familial Frontotemporal Dementia Subjects (LEFFTDS), and the Dominantly Inherited Non-Alzheimer's Dementias (DINAD) studies. These research studies include most of the centres investigating genetic frontotemporal dementia in Europe and eastern Canada (GENFI), USA and western Canada (ARTFL and LEFFTDS), and Australia (DINAD). In total, 33 centres across the world (12 countries; appendix p 20) provided participant data for our study. We included all known pathogenic mutations in the *GRN*, *MAPT*, and *C9orf72* genes in our study. Families with intermediate length expansions of *C9orf72* were not included in the study. All mutations were reviewed by two geneticists (RG and JB) to examine pathogenicity and were only included if both agreed on their probable pathogenic nature (full inclusion and exclusion criteria are in the appendix, p 2). Local ethics committees at each of the sites approved the study and data from participants was provided through informed written consent.

Procedures

Participant data collected from FPI centres included genetic group, individual mutation (for participants with mutations in *GRN* and *MAPT*), sex, clinical phenotype, age at symptom onset (defined by the onset of progressive behavioural, cognitive, or motor symptoms reported either by an informant [usually a family member] or, for non-behavioural symptoms, by the patient themselves), age at death, and relationship to other affected family members.

For the literature review, we assessed publications cited in the Alzheimer Disease & Frontotemporal Dementia Mutation database, and supplemented this by a detailed search of PubMed (done between Jan 1, 2015, and July 1, 2017) for other publications with data for age at symptom onset, age at death, or disease duration in people with genetic frontotemporal dementia: this identified 308 journal articles. To avoid potential double reporting, centres were asked to provide a list of publications relevant to their dataset. These lists were then manually examined for possible duplicates, which were removed when identified.

Statistical analysis

We grouped participants into a *GRN*, *MAPT*, or *C9orf72* group according to the mutation present. We calculated the numbers and percentages of participants within each genetic group by geographic location and clinical phenotype. We used a χ^2 test to compare sex distribution in each of the genetic groups. We calculated means and SDs for age at symptom onset, age at death, and disease duration in each genetic group and in the most common mutations in the *MAPT* and *GRN* groups (defined as those identified in the greatest number of individuals in the study). We used mixed effects models to examine differences in age at symptom onset, age at death, and disease duration between genetic groups (*GRN*, *MAPT*, and *C9orf72*), between the most common mutations in the *GRN* and *MAPT* groups, between an earlier (first) and later (second) generation of family members in all genetic groups, between men and women within each genetic group, and between the main clinical phenotypes within each genetic group. Analyses accounted for relatedness by including family membership as a random effect. We calculated Pearson correlation coefficients to explore the relationship between an individual's age at symptom onset (or death) and the age at symptom onset (or death) of their affected parent and the association between an individual's age at symptom onset (or death) and the average age at symptom onset (or death) of other members of the same family. Lastly, we also used mixed effects models to explore the extent to which variability in age at symptom onset and at death were explained by family membership (exploring variability both within and between families) and the specific mutation carried (in *GRN* and *MAPT* groups). Detailed statistical methods are shown in the appendix (pp 15–19). All statistical analyses were done with Stata (v.14 or later).

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Role for *ATXN1*, *ATXN2*, and *HTT* intermediate repeats in frontotemporal dementia and Alzheimer's disease

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ABSTRACT

We analyzed the frequency of intermediate alleles (IAs) in the *ATXN1, ATXN2,* and *HTT* genes in several neurodegenerative diseases. The study included 1126 patients with Alzheimer's disease (AD), 440 patients with frontotemporal dementia (FTD), and 610 patients with Parkinson's disease. In all cohorts, we genotyped *ATXN1* and *ATXN2* CAG repeats. In addition, in the FTD cohort, we determined the number of *HTT* CAG repeats. The frequency of *HTT* IAs was higher in patients with FTD (6.9%) versus controls (2.9%) and in the *C9orf72* expansion noncarriers (7.2%) versus controls (2.9%), although the difference was nonsignificant after correction for multiple testing. Compared with controls, progressive nonfluent aphasia (PNFA) groups showed a significantly higher frequency of *HTT* IAs (13.6% vs. 2.9% controls). For the *ATXN2* gene, we observed an increase in IA frequency in AD cases (AD 4.1% vs. controls 1.8%) and in the behavioral FTD group (4.8% vs. 1.8%). For the *ATXN1* gene, we found a significant increase of IAs in

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Anti-Cholinergic Derangement of Cortical Metabolism on ^{18}F-FDG PET in a Patient with Frontotemporal Lobar Degeneration Dementia: A Case of the TREDEM Registry

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Abstract. We present the case of a patient with an atypical course of frontotemporal lobar degeneration (FTLD) complicated by the use of an anticholinergic drug. A 70-year-old patient, followed by psychiatrists for depression and behavioral disorders, received a diagnosis of dementia with Lewy bodies (DLB) at another Center due to auditory hallucinations, gait impairment, and tendency to fall. He was then admitted to our Memory Clinic Unit for behavioral disturbances, such as delusional thinking, auditory hallucinations, and memory complaints. At that time, the patient's therapy included Lorazepam, Quetiapine, Promazine, and Biperiden. The latter was immediately suspended for the absence of extrapyramidal signs and to avoid the anticholinergic cognitive side effects. A ^{18}F-FDG PET showed a derangement of cortical metabolism with diffusely reduced activity, and limited areas of hyperactivity involving lateral frontal and lateral temporal inferior regions bilaterally. The patient underwent a series of exams, including neuropsychological tests, ^{123}I-MIBG scintigraphy, cerebrospinal fluid examination, and genetic analysis. A second ^{18}F-FDG PET showed an extensive remodulation of metabolic activity: relative higher concentration of the tracer in the prefrontal and inferior temporal cortex was no more detectable. Similarly, the diffuse reduced metabolic activity could not be traced anymore. Nonetheless, the metabolic activity still appeared reduced in the frontal lobe, in the anterior cingulate bilaterally, and in the anterior part of the hemispheric fissure. Taken together,

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REVIEW ARTICLE

Recommendations to distinguish behavioural variant frontotemporal dementia from psychiatric disorders

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The behavioural variant of frontotemporal dementia (bvFTD) is a frequent cause of early-onset dementia. The diagnosis of bvFTD remains challenging because of the limited accuracy of neuroimaging in the early disease stages and the absence of molecular biomarkers, and therefore relies predominantly on clinical assessment. BvFTD shows significant symptomatic overlap with non-degenerative primary psychiatric disorders including major depressive disorder, bipolar disorder, schizophrenia, obsessive-compulsive disorder, autism spectrum disorders and even personality disorders. To date, ~50% of patients with bvFTD receive a prior psychiatric diagnosis, and average diagnostic delay is up to 5–6 years from symptom onset. It is also not uncommon for patients with primary psychiatric disorders to be wrongly diagnosed with bvFTD. The Neuropsychiatric International Consortium for Frontotemporal Dementia was recently established to determine the current best clinical practice and set up an international collaboration to share a common dataset for future research. The goal of the present paper was to review the existing literature on the diagnosis of bvFTD and its differential diagnosis with primary psychiatric disorders to provide consensus recommendations on the clinical assessment. A systematic literature search with a narrative review was performed to determine all bvFTD-related diagnostic evidence for the following topics: bvFTD history taking, psychiatric assessment, clinical scales, physical and neurological examination, bedside cognitive tests, neuropsychological assessment, social cognition, structural neuroimaging, functional neuroimaging, CSF and genetic testing. For each topic, responsible team members proposed a set of minimal requirements, optimal clinical recommendations, and tools requiring further research or those that should be developed. Recommendations were listed if they reached a $\geqslant 85\%$ expert consensus based on an online survey among all consortium participants. New recommendations include performing at least one formal social cognition test in the standard neuropsychological battery for bvFTD. We emphasize the importance of 3D-T_1 brain MRI with a standardized review protocol including validated visual atrophy rating scales, and to consider volumetric analyses if available. We clarify the role of ^{18}F-fluorodeoxyglucose PET for the exclusion of bvFTD when normal, whereas non-specific regional metabolism abnormalities should not be over-interpreted in the case of a psychiatric differential diagnosis. We highlight the potential role of serum or CSF neurofilament light chain to differentiate bvFTD from primary psychiatric disorders. Finally, based on the increasing literature and clinical experience, the consortium determined that screening

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for *C9orf72* mutation should be performed in all possible/probable bvFTD cases or suspected cases with strong psychiatric features.

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Keywords: frontotemporal dementia; psychiatry; differential diagnosis; guidelines; biomarkers

Abbreviations: ALS = amyotrophic lateral sclerosis; bvFTD = behavioural variant of frontotemporal dementia; FTLD = frontotemporal lobar degeneration; NfL = neurofilament light chain; PPD = primary psychiatric disorders

Introduction

Frontotemporal dementia (FTD) is one of the most common forms of early-onset dementia (Ratnavalli *et al.*, 2002; Onyike and Diehl-Schmid, 2013). Most cases are sporadic, with ~20% having an autosomal-dominant genetic mutation [hexanucleotide repeat expansions near the chromosome 9 open reading frame gene (*C9orf72*), progranulin (*GRN*), and microtubule-associated protein tau (*MAPT*), being the most common causative genes]

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C9orf72, age at onset, and ancestry help discriminate behavioral from language variants in FTLD cohorts

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Abstract

Objective

We sought to characterize *C9orf72* expansions in relation to genetic ancestry and age at onset (AAO) and to use these measures to discriminate the behavioral from the language variant syndrome in a large pan-European cohort of frontotemporal lobar degeneration (FTLD) cases.

Methods

We evaluated expansions frequency in the entire cohort (n = 1,396; behavioral variant frontotemporal dementia [bvFTD] [n = 800], primary progressive aphasia [PPA] [n = 495], and FTLD–motor neuron disease [MND] [n = 101]). We then focused on the bvFTD and PPA cases and tested for association between expansion status, syndromes, genetic ancestry, and AAO applying statistical tests comprising Fisher exact tests, analysis of variance with Tukey post hoc tests, and logistic and nonlinear mixed-effects model regressions.

Results

We found *C9orf72* pathogenic expansions in 4% of all cases (56/1,396). Expansion carriers differently distributed across syndromes: 12/101 FTLD-MND (11.9%), 40/800 bvFTD (5%), and 4/495 PPA (0.8%). While addressing population substructure through principal components analysis (PCA), we defined 2 patients groups with Central/Northern (n = 873) and Southern European (n = 523) ancestry. The proportion of expansion carriers was significantly higher in bvFTD compared to PPA (5% vs 0.8% [$p = 2.17 \times 10^{-5}$; odds

ratio (OR) 6.4; confidence interval (CI) 2.31–24.99]), as well as in individuals with Central/Northern European compared to Southern European ancestry (4.4% vs 1.8% [$p = 1.1 \times 10^{-2}$; OR 2.5; CI 1.17–5.99]). Pathogenic expansions and Central/Northern European ancestry independently and inversely correlated with AAO. Our prediction model (based on expansions status, genetic ancestry, and AAO) predicted a diagnosis of bvFTD with 64% accuracy.

Conclusions

Our results indicate correlation between pathogenic *C9orf72* expansions, AAO, PCA-based Central/Northern European ancestry, and a diagnosis of bvFTD, implying complex genetic risk architectures differently underpinning the behavioral and language variant syndromes.

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Review

The Role of Amyloid-β in White Matter Damage: Possible Common Pathogenetic Mechanisms in Neurodegenerative and Demyelinating Diseases

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Abstract.
Just as multiple sclerosis (MS) has long been primarily considered a white matter (WM) disease, Alzheimer's disease (AD) has for decades been regarded only as a grey matter disorder. However, convergent evidences have suggested that WM abnormalities are also important components of AD, at the same extent as axonal and neuronal loss is critically involved in MS pathophysiology since early clinical stages. These observations have motivated a more thorough investigation about the possible mechanisms that could link neuroinflammation and neurodegeneration, focusing on amyloid-β (Aβ). Neuroimaging studies have found that patients with AD have widespread WM abnormalities already at the earliest disease stages and prior to the presence of Aβ plaques. Moreover, a correlation between cerebrospinal fluid (CSF) Aβ levels and WM lesion load was found. On the other hand, recent studies suggest a predictive role for CSF Aβ levels in MS, possibly due in the first instance to the reduced capacity for remyelination, consequently to a higher risk of WM damage progression, and ultimately to neuronal loss. We undertook a review of the recent findings concerning the involvement of CSF Aβ levels in the MS disease course and of the latest evidence of AD related WM abnormalities, with the aim to discuss the potential causes that may connect WM damage and amyloid pathology.

Keywords: Amyloid-β, inflammation, neurodegeneration, white matter damage

BIOLOGY OF AMYLOID: STRUCTURE, FUNCTION, AND REGULATION

Amyloid-β protein precursor (AβPP) is a single-pass transmembrane protein which is expressed at high levels in the brain. Its main biological function is likely to promote cell growth. AβPP is cleaved by two pathways. In the case of the non-amyloidogenic pathway, AβPP is first cleaved by α-secretase, generating membrane-tethered α-C terminal fragments (CTFs). The cleavage of AβPP by α-secretase releases sAβPPα from the cell surface and leaves an 83-amino-acid C-terminal AβPP fragment (C83). Further processing involves the intramembrane cleavage of α-CTFs by γ-secretase, which

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SHORT COMMUNICATION

Late-onset presentation and phenotypic heterogeneity of the rare *R377W PSEN1* mutation

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Keywords:
Alzheimer's disease, frontotemporal dementia, late-onset Alzheimer disease, mutation, presenilin 1

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Background and purpose: Mutations in the *PSEN1* gene are the most common cause of autosomal-dominant Alzheimer's disease and have been associated with the earliest disease onset. We describe an unusual presentation of the rare *R377W PSEN1* mutation with a late age of onset, and we provide for the first time *in vivo* pathological evidence for this mutation.

Methods: A 71-year-old female patient with progressive cognitive decline in the past 3 years and positive family history for dementia underwent neurological evaluation, neuropsychological testing, lumbar puncture, conventional brain imaging, amyloid-positron emission tomography (PET) and extensive genetic screening with a next-generation sequencing technique.

Results: The diagnostic workup revealed mixed behavioural and amnestic disease features on neuropsychological tests, magnetic resonance imaging, and 18-fluorodeoxyglucose (FDG)-PET. Amyloid-PET detected amyloid deposition in the frontal areas, in the parietal lobes and the precunei. The genetic screening revealed the presence of the rare *R377W* mutation in the *PSEN1* gene.

Conclusions: Extensive genetic screening is also advisable for late-onset presentations of Alzheimer's disease, especially in the presence of a positive family history or atypical clinical features.

Introduction

Because cerebrospinal fluid (CSF) biomarkers and amyloid-PET allowed *in vivo* detection of amyloid pathology, atypical variants of Alzheimer's disease (AD) are increasingly recognized in clinical settings [1,2]. Autosomal-dominant mutations in the amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) genes also provide the highest level of diagnostic certainty ante-mortem [1]. Although mutations in the *PSEN1* gene are found in less than 1% of AD patients, they are the most common cause of early-onset AD (EOAD), and a unique opportunity to understand disease mechanisms and their relationship to clinical phenotype [3]. More than 140 *PSEN1* mutations have been identified so far [3]. *PSEN1* mutations have been associated with the earliest AD onset, with an average age of 43 [4], and a typical amnestic syndrome of the hippocampal type [5]. Nevertheless, some *PSEN1* mutations have been linked to atypical presentation, including behavioural and psychiatric symptoms [5]. In the present work, we describe an atypical late-onset presentation of the rare *R377W PSEN1* mutation, and for the first time provide *in vivo* visualization of amyloid pathology in the proband as well as extensive characterization of her family.

Patient consent

The patient and her relatives provided informed consent for genetic analysis and for the use of their anonymized data for research purposes.

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ORIGINAL RESEARCH

Early symptoms in symptomatic and preclinical genetic frontotemporal lobar degeneration

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ABSTRACT

Objectives The clinical heterogeneity of frontotemporal dementia (FTD) complicates identification of biomarkers for clinical trials that may be sensitive during the prediagnostic stage. It is not known whether cognitive or behavioural changes during the preclinical period are predictive of genetic status or conversion to clinical FTD. The first objective was to evaluate the most frequent initial symptoms in patients with genetic FTD. The second objective was to evaluate whether preclinical mutation carriers demonstrate unique FTD-related symptoms relative to familial mutation non-carriers.

Methods The current study used data from the Genetic Frontotemporal Dementia Initiative multicentre cohort study collected between 2012 and 2018. Participants included symptomatic carriers (n=185) of a pathogenic mutation in chromosome 9 open reading frame 72 (*C9orf72*), progranulin (*GRN*) or microtubule-associated protein tau (*MAPT*) and their first-degree biological family members (n=588). Symptom endorsement was documented using informant and clinician-rated scales.

Results The most frequently endorsed initial symptoms among symptomatic patients were apathy (23%), disinhibition (18%), memory impairments (12%), decreased fluency (8%) and impaired articulation (5%). Predominant first symptoms were usually discordant between family members. Relative to biologically related non-carriers, preclinical *MAPT* carriers endorsed worse mood and sleep symptoms, and *C9orf72* carriers endorsed marginally greater abnormal behaviours. Preclinical *GRN* carriers endorsed less mood symptoms compared with non-carriers, and worse everyday skills.

Conclusion Preclinical mutation carriers exhibited neuropsychiatric symptoms compared with non-carriers that may be considered as future clinical trial outcomes. Given the heterogeneity in symptoms, the detection of clinical transition to symptomatic FTD may be best captured by composite indices integrating the most common initial symptoms for each genetic group.

INTRODUCTION

Frontotemporal dementia (FTD) is a neurodegenerative disorder with approximately 30% of patients showing a strong family history, with mutations in the chromosome 9 open reading frame 72 (*C9orf72*), progranulin (*GRN*) or microtubule-associated protein tau (*MAPT*) genes each accounting for 5%–10% of patients with FTD.[1] While therapies targeting the underlying pathology are in development,[2] currently, no treatments are available to prevent or alter the course of disease progression.

Even during the early stages of disease, symptoms of FTD are quite impairing[3]; thus, treatments will likely need to intervene during the preclinical stage, before a patient meets the current international consensus criteria.[4 5] Consequently, there is a growing interest in identifying biomarkers and clinical end points that can best inform when to administer these interventions and how to track treatment efficacy. A major challenge in designing clinical trials and the designation of clinical end points is the heterogeneity of genetic FTD at the phenotypic[6] and pathological levels.[7 8] For instance, clinical symptoms in genetic FTD range from language disturbances[5] to behavioural and neuropsychiatric features,[4] which occur at various frequencies and ages even within families, and have different neuroanatomic associations.[9 10] Furthermore, at present, it is not yet known whether or when symptoms associated with genetic FTD may occur during the prodromal period, and whether such symptoms may be specific to the later development of clinical FTD.

To inform clinical end point selection for future clinical trials in at-risk cohorts, the first objective of the current study was to evaluate the most frequent initial symptoms in patients with symptomatic genetic FTD due to *C9orf72*, *GRN* or *MAPT* mutations. The second objective was to evaluate whether preclinical mutation carriers demonstrate greater or different symptoms relative to biologically related non-carriers during the preclinical period.

CONCLUSIONS

In conclusion, , we report the frequencies of the most common initial symptoms for the main genetic forms of FTD and suggest that given the heterogeneity between gene groups, family members and even specific mutations, composite measures of these symptoms may serve as clinical tools for detection of early conversion to symptomatic FTD. Of interest, we did not find differences between preclinical mutation carriers and non-carriers for the most common initial symptoms in affected patients. Future studies examining initial symptoms with additional longitudinal data points will aid in the understanding of the progression of these symptoms from the preclinical, to affected disease stages and further pinpoint the onset of initial symptoms heralding conversion to symptomatic FTD.

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SHORT REPORT

Abnormal pain perception is associated with thalamo-cortico-striatal atrophy in *C9orf72* expansion carriers in the GENFI cohort

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ABSTRACT

Objective Frontotemporal dementia (FTD) is typically associated with changes in behaviour, language and movement. However, recent studies have shown that patients can also develop an abnormal response to pain, either heightened or diminished. We aimed to investigate this symptom in mutation carriers within the Genetic FTD Initiative (GENFI).

Methods Abnormal responsiveness to pain was measured in 462 GENFI participants: 281 mutation carriers and 181 mutation-negative controls. Changes in responsiveness to pain were scored as absent (0), questionable or very mild (0.5), mild (1), moderate (2) or severe (3). Mutation carriers were classified into *C9orf72* (104), *GRN* (128) and *MAPT* (49) groups, and into presymptomatic and symptomatic stages. An ordinal logistic regression model was used to compare groups, adjusting for age and sex. Voxel-based morphometry was performed to identify neuroanatomical correlates of abnormal pain perception.

Results Altered responsiveness to pain was present to a significantly greater extent in symptomatic *C9orf72* expansion carriers than in controls: mean score 0.40 (SD 0.71) vs 0.00 (0.04), reported in 29% vs 1%. No significant differences were seen between the other symptomatic groups and controls, or any of the presymptomatic mutation carriers and controls. Neural correlates of altered pain perception in *C9orf72* expansion carriers were the bilateral thalamus and striatum as well as a predominantly right-sided network of regions involving the orbitofrontal cortex, inferomedial temporal lobe and cerebellum.

Conclusion Changes in pain perception are a feature of *C9orf72* expansion carriers, likely representing a disruption in somatosensory, homeostatic and semantic processing, underpinned by atrophy in a thalamo-cortico-striatal network.

INTRODUCTION

Frontotemporal dementia (FTD) is a complex neurodegenerative disease that encompasses a spectrum of symptoms. Whilst a combination of behavioural abnormalities, language dysfunction, cognitive deficits and motor impairments form the classical phenotype of FTD, a number of other symptoms have been reported that are often overlooked, including altered perception of pain.[1–5]

Descriptions of reduced response to pain in FTD have been intermittently reported over many years, although with variable frequency, for example, only 3% in one report,[4] but up to 45% in papers from another research group.[1 2] An exaggerated reaction to pain has also been reported, with one series finding its presence in up to 55% of people with FTD, particularly in those with the temporal variant.[1] A more recent study described altered responsiveness to pain in 8/15 (67%) people with behavioural variant FTD (bvFTD), 8/11 (72%) with semantic dementia (SD) and 2/5 (40%) with progressive non-fluent aphasia (PNFA), with decreased responsiveness more typical in bvFTD, and increased responsiveness in the language variants, SD and PNFA.[5] For the first time, this study found a particular association with mutations in the *C9orf72* gene, although only six patients were studied.[5] We therefore set out to explore the presence of this symptom in a larger cohort of patients with genetic FTD, through the Genetic FTD Initiative (GENFI), investigating the frequency of altered responsiveness to pain in both the symptomatic and presymptomatic period, and its neural correlates.

METHODS

Participants were recruited from the third data freeze of the GENFI study,[6] which incorporated 533 participants from 22 centres. Of these participants, 462 had data on abnormal pain perception from the GENFI core clinical assessment: 281 mutation carriers (104 *C9orf72*, 128 *GRN*, 49 *MAPT*), classified as either presymptomatic or symptomatic, and 181 mutation-negative controls. Of note, the

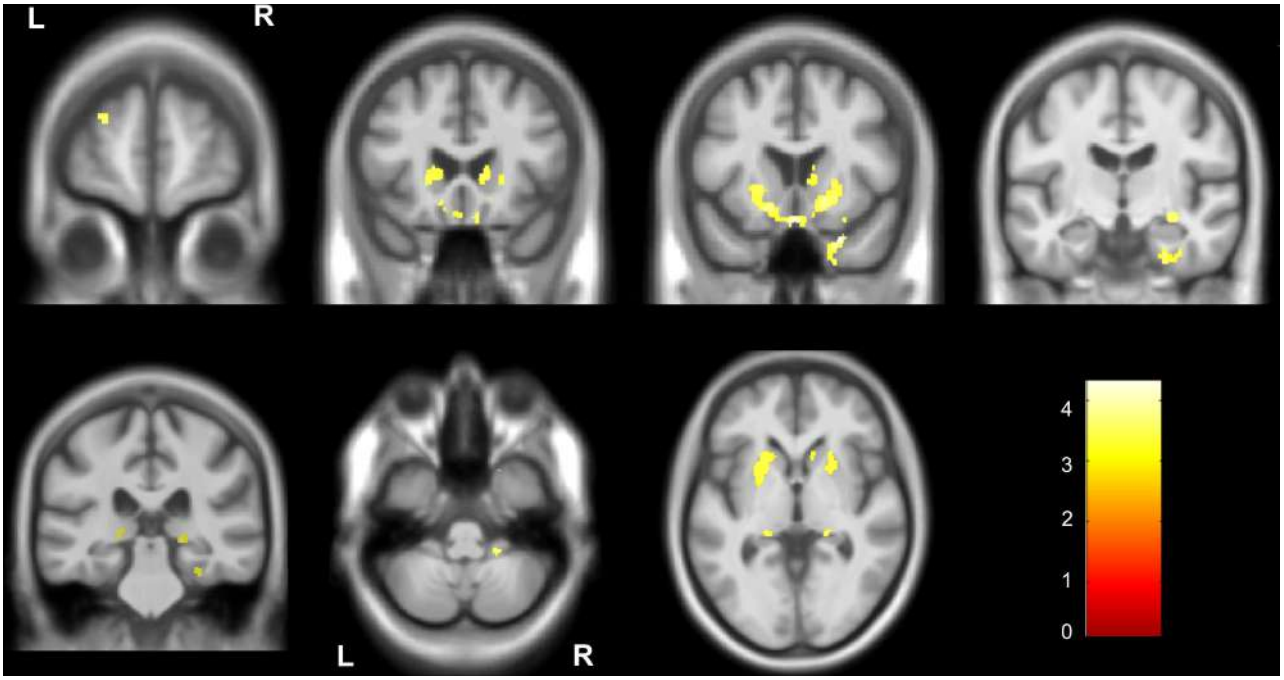

Figure 1 Neural correlates of abnormal pain perception in *C9orf72* mutation carriers. Statistical parametric maps are thresholded at p<0.001 uncorrected. Results are rendered on a study-specific T1-weighted MRI template in MNI space. The colour bar indicates the T-score.

somatosensory, homeostatic, semantic and reward processing underlies the altered perception of pain.

We did not separate out decreased and increased responsiveness in this study, but further studies of genetic FTD should do this, and attempt to understand whether there are specific correlates of these two features. Furthermore, future longitudinal studies that include those that convert from presymptomatic to symptomatic status will allow a clearer timeline of when altered pain perception starts in the disease process of *C9orf72*-associated FTD.

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cells

MDPI

Article

MiRNA Profiling in Plasma Neural-Derived Small Extracellular Vesicles from Patients with Alzheimer's Disease

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Abstract: Small extracellular vesicles (EVs) are able to pass from the central nervous system (CNS) into peripheral blood and contain molecule markers of their parental origin. The aim of our study was to isolate and characterize total and neural-derived small EVs (NDEVs) and their micro RNA (miRNA) cargo in Alzheimer's disease (AD) patients. Small NDEVs were isolated from plasma in a population consisting of 40 AD patients and 40 healthy subjects (CTRLs) using high throughput Advanced TaqMan miRNA OpenArrays®, which enables the simultaneous determination of 754 miRNAs. MiR-23a-3p, miR-223-3p, miR-100-3p and miR-190-5p showed a significant dysregulation in small NDEVs from AD patients as compared with controls (1.16 ± 0.49 versus 7.54 ± 2.5, $p = 0.026$; 9.32 ± 2.27 versus 0.66 ± 0.18, $p < 0.0001$; 0.069 ± 0.01 versus 0.5 ± 0.1, $p < 0.0001$ and 2.9 ± 1.2 versus 1.93 ± 0.9, $p < 0.05$, respectively). A further validation analysis confirmed that miR-23a-3p, miR-223-3p and miR-190a-5p levels in small NDEVs from AD patients were significantly upregulated as compared with controls ($p = 0.008$; $p = 0.016$; $p = 0.003$, respectively) whereas miR-100-3p levels were significantly downregulated ($p = 0.008$). This is the first study that carries out the comparison between total plasma small EV population and NDEVs, demonstrating the presence of a specific AD NDEV miRNA signature.

Keywords: Alzheimer's disease; extracellular vesicles (EVs); neural-derived extracellular vesicles (NDEVs); microRNA

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Journal of Neuroinflammation

RESEARCH Open Access

IL-33 and its decoy sST2 in patients with Alzheimer's disease and mild cognitive impairment

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Abstract

Background: Interleukin-33 is a cytokine endowed with pro- and anti-inflammatory properties that plays a still poorly defined role in the pathogenesis of a number of central nervous system (CNS) conditions including Alzheimer's disease (AD). We analyzed this cytokine and its decoy receptor sST2 in Alzheimer's disease (AD) and mild cognitive impairment (MCI).

Method: IL-33 and sST2 were analyzed in serum and CSF of AD and MCI patients, comparing the results to those obtained in age-matched healthy controls (HC). Because of the ambiguous role of IL-33 in inflammation, the concentration of both inflammatory (IL-1β and IL-6) and anti-inflammatory (IL-10) cytokines was analyzed as well in serum and cerebrospinal fluid (CSF) of the same individuals. Finally, the effect of IL-33 on in vitro $A\beta_{42}$-stimulated monocytes of AD, MCI, and HC individuals was examined.

Results: As compared to HC, (1) IL-33 was significantly decreased in serum and CSF of AD and MCI, (2) sST2 was increased in serum of AD and MCI but was undetectable in CSF, (3) serum and CSF IL-1β concentration was significantly increased and that of IL-10 was reduced in AD and MCI, whereas no differences were observed in IL-6. In vitro addition of IL-33 to LPS+$A\beta_{42}$-stimulated monocytes downregulated IL-1β generation in MCI and HC, but not in AD, and stimulated IL-10 production in HC alone. IL-33 addition also resulted in a significant reduction of NF-kB nuclear translocation in LPS+$A\beta_{42}$-stimulated monocytes of HC alone.

Conclusions: These data support the hypothesis that IL-33 plays a complex anti-inflammatory role that is lost in AD- and MCI-associated neuroinflammation; results herein also suggest a possible use of IL-33 as a novel therapeutic approach in AD and MCI.

Keywords: Alzheimer's disease, Inflammation, Interleukin 33, sST2 decoy receptor

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whom dementia is not present. Thus, IL-33 was shown to have a regulatory effect on the NF-kB pathway, which is mediated by its ability to binding the NF-kB p65 subunit [9]. The IL-33/NF-kB p65 complex impedes p65-mediated transactivation; this downregulates NF-kB activity, with a dampening effect on inflammation. The observation that this effect was lost in cells of MCI and AD individuals suggests that the role of IL-33 in the pathogenesis of AD- and MCI-associated inflammation is multifaceted, as this cytokine would exert its effect both at the intracellular and at the extracellular level. To summarize, (1) reduction of IL-33 production per se, (2) increased concentrations of the sST2 decoy receptor, and (3) an impairment of the ability of IL-33 to induce NF-kB nuclear translocation are different mechanism that can explain the proinflammatory role played by IL-33 in AD and MCI.

IL-33 was also reported to induce the polarization of monocytes toward an M2 anti-inflammatory phenotype; these monocytes produce IL-10, whose concentration, as indicated above, is greatly reduced in AD and MCI individuals [43, 44]. M2 polarization by IL-33 is possibly explained by the observation that the IL-33 binding to ST2 negatively regulates TLR signaling by competing with MyD88 [45, 46]. The IL-33/ST2 complex thus suppresses IL-1β generation and the down-stream activation of the TLR signaling pathway by sequestration of MyD88 with the consequence of further inhibiting NF-kB and activating MAP kinases [46]. IL-33 indeed activates ERK1\2 and STAT3, facilitating binding of STAT3 to the − 1954 to − 1936 bp sequence upstream of the IL-10 transcription start site, thereby promoting its transcription in macrophages [47]. Although we did not analyze M1 and M2 monocytes in this study, previously published data indicate that M2 cells are significantly reduced in AD. The reduction of M2 monocytes seen in AD could explain why IL-33 supplementation did not increase IL-10 production by monocytes of AD and MCI individuals in our in vitro system and could be a third way to justify why the lower quantities of IL-33 seen in AD results in inflammation in these patients.

IL-33 has repeatedly been suspected to be involved in the pathogenesis of CNS diseases. In particular, the administration of recombinant IL-33 was shown to promote recovery in a mouse model of autoimmune encephalomyelitis (EAE) [48, 49] and to provide neuroprotection in a mouse model of contusion spinal cord injury (SCI) [43]. In the APP/PS1 animal model of AD, IL-33 attenuated AD pathology and memory deficit and stimulated the polarization of microglia in an anti-inflammatory direction [34, 50]. Finally, recent data obtained in a small groups of MCI who did or did not convert to AD over time [31] showed that higher amounts of IL-33-producing CD14+ monocytes were seen in AD-non converters, in whom percentages of CD14+/IL-33+ cells positively correlated with the volumes of both left and right hippocampus.

Finally, recent data obtained in a small groups of MCI who did or did not convert to AD over time [31] showed that higher amounts of IL-33-producing CD14+ monocytes were seen in AD-non converters, in whom percentages of CD14+/IL-33+ cells positively correlated with the volumes of both left and right hippocampus. In the attempt to further analyze possible correlations between hippocampus volumes and IL-33, we are planning to verify the immunological effects of IL-33 on human microglia cell lines.

Conclusions

IL-33 was shown to have a neuroprotective role in AD secondary to the reduction of Aβ secretion and the activation of Aβ phagocytosis by the microglia [35]. Findings herein offer an immune explanation as well to the protective role of IL-33 in AD; these results warrant the investigation of this cytokine in treatment and rehabilitation programs for AD.

Abbreviations

AD: Alzheimer's disease; MCI: Mild cognitive impairment; HC: Healthy controls; IL: Interleukin; CSF: Cerebrospinal fluid; Aβ: Amyloid beta; TLR: Toll-like receptor; PBMC: Peripheral blood mononuclear cell; sST2: Soluble ST2

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Authors' contributions

MS, IM, and MC conceived and designed the research. MS, IM, FR, and FP performed the experiments. CF, DG, and ES are responsible for the clinical cohorts of patients. MS, IM, and MC analyzed the data and prepared the manuscript. The author(s) read and approved the final manuscript.

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Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article. The raw data of this study are available from the corresponding author [M.S.] on request.

Ethics approval and consent to participate

All patients and controls gave informed consent according to a protocol approved by the local ethics committee of the Don Gnocchi Foundation

Consent for publication

Not applicable

Competing interests

The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE

Evidence of retinal anterograde neurodegeneration in the very early stages of multiple sclerosis: a longitudinal OCT study

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Abstract

Background Neurodegenerative processes are present since the early stages of multiple sclerosis (MS), constituting the primary substrate of disability. As part of the CNS, retinal damage could be considered a reliable prognostic biomarker of neurodegeneration in MS.

Objectives To characterize longitudinal changes in the retinal layers' thickness and to investigate correlations between retinal atrophy and other prognostic biomarkers, i.e., cerebrospinal fluid (CSF) β-amyloid$_{1–42}$ (Aβ) levels.

Methods Forty-two eyes without a history of optic neuritis of 23 MS patients were recruited. All patients underwent spectral-domain-OCT scans (SD-OCT), brain magnetic resonance imaging (MRI), and lumbar puncture at baseline. SD-OCT and brain MRI were repeated after 12 months. Ten controls underwent the same OCT procedure.

Results At baseline, macular ganglion cell/inner plexiform layer (mGCIPL) thickness was reduced in patients compared to controls ($p = 0.008$), without retinal nerve fiber layer (RNFL) thinning, that was revealed only at follow-up ($p = 0.005$). Patients with lower CSF Aβ levels displayed reduced RNFL thickness values, both at baseline and follow-up.

Conclusions At very early clinical stages, mGCIPL thickness values were reduced without a concomitant peripapillary RNFL thinning. The longitudinal assessment demonstrated a RNFL loss in patients compared to HC, together with a plateau of mGCIPL thinning. Aβ$_{low}$ subgroup of patients showed a reduction of retinal nerve fiber layer thickness.

Keywords Multiple sclerosis · OCT · Neurodegeneration · β-Amyloid · Biomarkers

Introduction

Multiple sclerosis (MS) represents a potentially severe cause of disability throughout adult life [1]. Alongside with chronic inflammation of the central nervous system (CNS), neurodegenerative processes may be present since the early stages of MS, constituting the primary substrate of irreversible disability [2–8].

As part of the CNS, the optic nerve (ON) is a major target of MS [3]. Optical coherence tomography (OCT), and particularly macular segmentation using spectral-domain-OCT (SD-OCT), represents a sensitive easy-accessible tool to investigate retinal damage in MS, and is currently considered a reliable prognostic biomarker of neurodegeneration [9–13]. Peripapillary and macular retinal nerve fiber layer (pRNFL and mRNFL), and macular ganglion cell/inner plexiform layer (mGCIPL) thinning results from axonal loss and neuronal damage of the inner retina. They correlate with visual

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Faster Cortical Thinning and Surface Area Loss in Presymptomatic and Symptomatic *C9orf72* Repeat Expansion Adult Carriers

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Frontotemporal Dementia: Correlations Between Psychiatric Symptoms and Pathology

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Objective: The pathology of frontotemporal dementia, termed frontotemporal lobar degeneration (FTLD), is characterized by distinct molecular classes of aggregated proteins, the most common being TAR DNA-binding protein-43 (TDP-43), tau, and fused in sarcoma (FUS). With a few exceptions, it is currently not possible to predict the underlying pathology based on the clinical syndrome. In this study, we set out to investigate the relationship between pathological and clinical presentation at single symptom level, including neuropsychiatric features.

Methods: The presence or absence of symptoms from the current clinical guidelines, together with neuropsychiatric features, such as hallucinations and delusions, were scored and compared across pathological groups in a cohort of 150 brain donors.

Results: Our cohort consisted of 68.6% FTLD donors (35.3% TDP-43, 28% tau, and 5.3% FUS) and 31.3% non-FTLD donors with a clinical diagnosis of frontotemporal dementia and a different pathological substrate, such as Alzheimer's disease (23%). The presence of hyperorality points to FTLD rather than non-FTLD pathology ($p < 0.001$). Within the FTLD group, hallucinations in the initial years of the disease were related to TDP-43 pathology ($p = 0.02$), including but not limited to chromosome 9 open reading frame 72 (*C9orf72*) repeat expansion carriers. The presence of perseverative or compulsive behavior was more common in the TDP-B and TDP-C histotypes ($p = 0.002$).

Interpretation: Our findings indicate that neuropsychiatric features are common in FTLD and form an important indicator of underlying pathology. In order to allow better inclusion of patients in targeted molecular trials, the routine evaluation of patients with frontotemporal dementia should include the presence and nature of neuropsychiatric symptoms.

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The term frontotemporal dementia (FTD) defines a group of neurodegenerative syndromes with diverse clinical presentations, including the behavioral variant of frontotemporal dementia (bvFTD) and language dominant syndromes, such as primary progressive aphasia (PPA), including the nonfluent/agrammatic variant of PPA (nfPPA), and the semantic variant of PPA (svPPA).[1,2] Other syndromes that are part of this group are characterized by prominent movement symptoms, such as corticobasal syndrome, progressive supranuclear palsy, and FTD with motor neuron disease. Most patients present with mixed behavior, language, and motor symptoms, but

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ORIGINAL RESEARCH

Neuronal pentraxin 2: a synapse-derived CSF biomarker in genetic frontotemporal dementia

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ABSTRACT

Introduction Synapse dysfunction is emerging as an early pathological event in frontotemporal dementia (FTD), however biomarkers are lacking. We aimed to investigate the value of cerebrospinal fluid (CSF) neuronal pentraxins (NPTXs), a family of proteins involved in homeostatic synapse plasticity, as novel biomarkers in genetic FTD.

Methods We included 106 presymptomatic and 54 symptomatic carriers of a pathogenic mutation in *GRN*, *C9orf72* or *MAPT*, and 70 healthy non-carriers participating in the Genetic Frontotemporal dementia Initiative (GENFI), all of whom had at least one CSF sample. We measured CSF concentrations of NPTX2 using an in-house ELISA, and NPTX1 and NPTX receptor (NPTXR) by Western blot. We correlated NPTX2 with corresponding clinical and neuroimaging datasets as well as with CSF neurofilament light chain (NfL) using linear regression analyses.

Results Symptomatic mutation carriers had lower NPTX2 concentrations (median 643 pg/mL, IQR (301–872)) than presymptomatic carriers (1003 pg/mL (624–1358), p<0.001) and non-carriers (990 pg/mL (597–1373), p<0.001) (corrected for age). Similar results were found for NPTX1 and NPTXR. Among mutation carriers, NPTX2 concentration correlated with several clinical disease severity measures, NfL and grey matter volume of the frontal, temporal and parietal lobes, insula and whole brain. NPTX2 predicted subsequent decline in phonemic verbal fluency and Clinical Dementia Rating scale plus FTD modules. In longitudinal CSF samples, available in 13 subjects, NPTX2 decreased around symptom onset and in the symptomatic stage.

Discussion We conclude that NPTX2 is a promising synapse-derived disease progression biomarker in genetic FTD.

INTRODUCTION

Frontotemporal dementia (FTD), a common form of early-onset dementia, has an autosomal dominant inheritance in 20%–30% of patients, most often due to mutations in granulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*) or microtubule-associated protein tau (*MAPT*).[1] Developing sensitive biomarkers to detect disease onset at an early, even preclinical stage is of utmost importance for upcoming therapeutic interventions. Genetic forms of FTD provide a unique opportunity to study disease progression from presymptomatic to overt FTD and to identify novel biomarkers.

Our previous proteomics study identified neuronal pentraxin receptor (NPTXR) in cerebrospinal fluid (CSF) as the most promising candidate biomarker in genetic FTD, with markedly reduced levels in the symptomatic stage.[2] NPTXR forms complexes with NPTX1 and NPTX2 (also termed neuronal activity related protein, Narp) at excitatory synapses of pyramidal neurons onto parvalbumin interneurons and contributes to synaptic homeostatic plasticity.[3 4] Increasing evidence suggests that dysfunction and degeneration of synapses is an early pathological event in FTD,[5–7] especially in *GRN*-associated FTD,[8–10] a concept widely recognised in other neurodegenerative diseases.[5 11] Fluid biomarkers reflecting synaptic integrity in FTD might therefore contribute to an early diagnosis and monitoring of disease progression in clinical practice and clinical trials. Following studies in Alzheimer's disease (AD) that identified NPTXs as candidate biomarkers,[12–20] we hypothesised that NPTXs could be valuable synapse-derived biomarkers in genetic FTD.

In the present study, we measured CSF NPTXs in a large cohort of *GRN*, *C9orf72* and *MAPT*

a transient increase in NPTXs has been observed, with a subsequent decline as the disease progresses.[15 16 19 20] This discrepancy in NPTXs dynamics may result from differences in underlying pathophysiology.

The diagnostic accuracy of NPTX2 of 71% to distinguish symptomatic from presymptomatic mutation carriers is comparable to that of neurogranin, the most evaluated synapse-derived CSF biomarker for AD.[38] Its longitudinal evaluation, especially in the late-presymptomatic stage, might be more valuable than cross-sectional measurements. It is promising that Ma *et al*[39] observed a correlation between NPTX1 in plasma and brain tissue; further studies are warranted to investigate whether NPTX2 can also be reliably measured in the blood, which would offer opportunities for longitudinal studies with larger numbers of samples.

We found an inverse correlation between NPTX2 and NfL in mutation carriers. NfL is a sensitive marker of neuroaxonal degeneration which is elevated in CSF and blood in the symptomatic stage of genetic FTD[40] and in various other neurological disorders.[21] Although a trend was found for symptomatic carriers alone after exclusion of ALS patients (who are known to have very high NfL levels),[21] the lack of a stronger correlation probably reflects that NPTX2 and NfL are markers of different pathological processes which do not occur simultaneously.

Strengths of this study include the large sample size, despite the relative rarity of the disease, and the availability of corresponding clinical and brain imaging datasets. The inclusion of subjects with specific genetic defects allowed us to define pathologically homogeneous groups. Our NPTX2 findings are supported by similar results in NPTX1 and NPTXR, which correlated strongly with NPTX2, and indicate an overall reduction in NPTXs in genetic FTD.

The findings of this study must be viewed in light of some limitations. First, our longitudinal NPTX2 measurements were too limited in number to draw strong conclusions and require replication and more extensive statistical analyses in larger datasets. Second, using diagnostic criteria to label mutation carriers as presymptomatic or symptomatic may have failed to recognise subjects in a very early symptomatic stage. We calculated disease duration based on estimated time of symptom onset, rather than diagnosis, to ensure that any diagnostic delay did not affect correlative analyses. Third, three *C9orf72* mutation carriers had ALS without FTD, which, although increasingly recognised as part of the FTD spectrum,[1] represents a clinically distinct phenotype. We ensured that these subjects did not affect our main results by repeating group comparisons after exclusion of these subjects. Finally, although brief medical and neurological history and examination did not reveal any significant neurological comorbidities, asymptomatic diseases, including cerebrovascular disease, could have confounded NPTX measurements. Future research focusing on potential confounding factors will be an important next step.

In conclusion, we provide evidence for NPTX2 as a novel CSF biomarker in genetic FTD. Its synaptic localisation and correlation with disease progression indicates that NPTX2 decreases probably reflect synaptic dysfunction or loss, providing novel opportunities for in vivo monitoring of synaptic integrity in genetic FTD. Treatment strategies aimed at improving synaptic connectivity may benefit from the use of NPTX2 as a tool to select and monitor patients with neural circuit dysfunction. More longitudinal data on NPTXs in presymptomatic and symptomatic mutation carriers might verify their value as (pre-)clinical biomarkers.

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BRIEF COMMUNICATION OPEN

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Cerebrospinal fluid glutamate changes in functional movement disorders

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The aim of this study was to assess cerebrospinal fluid (CSF) concentrations of specific amino acids using a high-performance liquid chromatography system in a sample of patients with functional movement disorders (FMDs) and in a sample of controls. CSF levels of glutamate were significantly lower in patients with FMD than in controls. This finding argues in favor of glutamatergic dysfunction in the pathophysiology of FMD.

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Glutamate has an important role as the primary excitatory neurotransmitter in the central nervous system[1]. Growing evidence suggests that abnormalities in glutamatergic neurotransmission, via the *N*-methyl-D-aspartate receptor (NMDA-R), have a key role in the pathophysiology of several neuropsychiatric conditions, such as schizophrenia, mood disorders, and Alzheimer's disease[2–4]. Recently, a possible role of glutamate + glutamine (Glx) has been suggested in the pathophysiology of FMDs (also called conversion disorders), a neuropsychiatric condition characterized by the presence of motor symptoms that cannot be explained by typical neurological diseases or other medical conditions[5]. Although they are highly prevalent and have a considerable impact on national health systems, the pathophysiology of FMD remains unclear. In the past decade, new hypotheses based on the integration between psychology and neurobiology have been formulated, gradually shifting the attention of the scientific community from traditional psychoanalytic theories to models considering neurobiological factors[6]. Functional neuroimaging studies have underlined abnormalities in FMD at the level of brain network activity, connectivity, and specific anatomic areas of altered metabolic demand during tasks (mainly the limbic system)[7]. With respect to Glx, Demartini et al.[5] recently showed, through a brain magnetic resonance spectroscopy (MRS) technique, that patients with FMD presented significantly higher levels of Glx in the limbic system than healthy controls. The authors hypothesized that abnormal increase in Glx in the limbic system might have a central pathophysiological role in FMD onset and maintenance, possibly by altering limbic–motor interactions. However, no studies have been conducted assessing the levels of glutamate and glutamine in the cerebrospinal fluid (CSF) of patients with FMD.

Given the evidence of a potential role of Glx in the pathophysiology of FMD, we aimed to retrospectively assess CSF levels of glutamate and glutamine in a sample of patients with FMD and in a sample of controls. Moreover, we also assessed the CSF levels of two other amino acids, alanine and asparagine, serving as controls.

Eight patients with FMD and nine controls were recruited. The Kolmogorov–Smirnov test showed that all the continuous variables respected the assumption of a normal distribution (all $p > 0.05$).

Patients and controls did not differ with respect to gender, age, or time elapsed between the date of the lumbar puncture (LP) and date of CSF amino acid measurements (Table 1). No major psychiatric comorbidity was found in the whole sample. No significant correlations were found between amino acid levels and (i) severity of FMD or (ii) duration of disease (all $p > 0.05$). Multivariate analysis of variance (ANOVA) showed that CSF levels of glutamate were significantly lower in patients with FMD (mean 1.23 ± 0.66) than in controls (mean 2.17 ± 1.05) (F (1,15) $= 4.751$, $p = 0.046$). Neither CSF levels of glutamine (mean 341.25 ± 87.01 for patients with FMD, mean 340.99 ± 65.57 for controls), asparagine (mean 4.52 ± 2.60 for patients with FMD, mean 5.02 ± 0.97 for controls), nor alanine (mean 30.35 ± 10.03 for patients with FMD, mean 31.26 ± 8.47 for controls) differed between the two groups (F (1,15) $= 0.000$, $p = 0.994$ for glutamine, F (1,15) $= 0.293$, $p = 0.596$ for asparagine, F (1,15) $= 0.041$, $p = 0.841$ for alanine) (Fig. 1).

In this study, which was designed to provide information on CSF glutamate and glutamine and subsequently on pathophysiological mechanisms in FMD, the main finding is that patients with FMD have decreased CSF glutamate compared to a group of controls. To the best of our knowledge, this is the first study exploring the levels of glutamate and glutamine in the CSF of patients with FMD. Previous studies have assessed CSF glutamate and glutamine in different neuropsychiatric conditions, with contradictory results: some studies found glutamate or glutamine to be decreased in patients' CSF, while others found glutamate or glutamine to be increased[2–4]. The discrepancy in CSF glutamate and glutamine levels observed in previous studies might be due to different analytical methods used. Another reason for the inconsistency of results may be a time-dependent in vitro hydrolysis of glutamine to glutamate in the CSF[8]. In the present study, the mean storage time of patients with FMD and control

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Psychiatric Disorders in Alzheimer Disease With the Presenilin-1 L226F Mutation

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Abstract: The presenilin-1 (*PSEN1*) L226F mutation has been linked to very early onset of prominent behavioral and psychiatric disturbances followed by cognitive decline within a few years. We report a novel case of early-onset Alzheimer disease that was originally diagnosed as psychotic depression in a patient with this gene mutation. We also compare our patient's clinical data to those of other cases of this mutation that have been described in the literature. Because atypical behavioral and psychiatric disturbances in young (<40 years) individuals can herald Alzheimer disease, a tight collaboration between psychiatrists and neurologists is crucial for an early diagnosis.

Key Words: early-onset Alzheimer disease, *PSEN1*, genetic, behavioral symptoms

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AD = Alzheimer disease. **ADAD** = autosomal dominant Alzheimer disease. ***PSEN1*** = presenilin-1.

Genetic mutations in the presenilin-1 (*PSEN1*) gene are the most common cause of autosomal dominant Alzheimer disease (ADAD) (Lleò et al, 2004). The manifestation of ADAD may sometimes be difficult to recognize, particularly if the patient presents with early-onset psychiatric disturbances. A classical neurologic condition such as Alzheimer disease (AD) may present with initial psychiatric features such as depression or bipolar disorder; however, detection of a neurologic substrate underlying the early phases of ADAD, especially when psychiatric features are prominent in young (<40 years) individuals, remains unclear. In this report, we present a case of ADAD with the rare *PSEN1* L226F mutation. We also review the only other three cases of ADAD with the *PSEN1* L226F mutation that have been reported thus far.

PSEN1 L226F is a very rare gene mutation and has been reported in only three prior cases (Bagyinszky et al, 2016; Gómez-Tortosa et al, 2010; Zekanowski et al, 2006). First detected in 2006, *PSEN1* L226F is a missense point mutation on exon 7 of chromosome 14 (CTC→TTC) that results in the substitution of leucine with phenylalanine, which causes changes in the surface of a transmembrane domain of *PSEN1* by increasing hydrophobic interactions. The first case of an individual with this gene mutation was a man with prominent behavioral symptoms who had been clinically diagnosed with frontotemporal dementia at the age of 33 (Zekanowski et al, 2006). His autopsy revealed AD pathology. The second case was a 33-year-old woman who had developed depression followed by a speech and memory deficit; she was clinically diagnosed with AD, which was confirmed at autopsy (Gómez-Tortosa et al, 2010). The third case was a 37-year-old woman who had developed paranoid ideation and anxiety, which progressed to nonfluent aphasia and cognitive and memory deficits; she was clinically diagnosed with AD, but no autopsy was performed (Bagyinszky et al, 2016).

These three cases, plus the one presented here, had a prodromal phase that was dominated by isolated psychiatric disturbances that occurred while the patients were in their early 30s; this phase was followed by rapid cognitive decline and dementia.

CASE REPORT

A 36-year-old, left-handed woman of South American origin was referred to the neurology department of the Aldo Ravelli Center for Neurotechnology and Experimental Neurotherapeutics in Milan, Italy, in the spring of 2018 with a history of behavioral changes and cognitive impairment

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Portions of this work were presented at the annual meeting of the Italian Society of Neurology–Dementia, April 8, 2019, in Florence, Italy.
The authors declare no conflicts of interest.
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Behavioural Neurology

Social cognition impairment in genetic frontotemporal dementia within the GENFI cohort

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Parieto-occipital sulcus widening differentiates posterior cortical atrophy from typical Alzheimer disease

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ARTICLE INFO

Keywords:
Posterior Cortical Atrophy
Alzheimer Disease
Visual rating scale
Voxel based morphometry
Differential Diagnosis

ABSTRACT

Objectives: Posterior Cortical Atrophy (PCA) is an atypical presentation of Alzheimer disease (AD) characterized by atrophy of posterior brain regions. This pattern of atrophy is usually evaluated with Koedam visual rating scale, a score developed to enable visual assessment of parietal atrophy on magnetic resonance imaging (MRI). However, Koedam scale is complex to assess and its utility in the differential diagnosis between PCA and typical AD has not been demonstrated yet. The aim of this study is therefore to spot a simple and reliable MRI element able to differentiate between PCA and typical AD using visual rating scales.

Methods: 15 patients who presented with progressive complex visual disorders and predominant occipitoparietal hypometabolism on PET-FDG were selected from our centre and compared with 30 typical AD patients and 15 healthy subjects. We used previously validated visual rating scales including Koedam scale, which we divided into three major components: posterior cingulate, precuneus and parieto-occipital. Subsequently we validated the results using the automated software Brainvisa Morphologist and Voxel Based Morphometry (VBM).

Results: Patients with PCA, compared to typical AD, showed higher widening of the parieto-occipital sulcus, assessed both with visual rating scales and Brainvisa. In the corresponding areas, the VBM analysis showed an inverse correlation between the results obtained from the visual evaluation scales with the volume of the grey matter and a direct correlation between the same results with the cerebrospinal fluid volume.

Conclusions: A visually based rating scale for parieto-occipital sulcus can distinguish Posterior Cortical Atrophy from typical Alzheimer disease.

1. Introduction

Posterior Cortical Atrophy (PCA) is a clinico-radiological syndrome mainly characterized by the loss of tissue in the posterior regions of the brain and the current diagnostic criteria consider the presence of MRI atrophy in these regions as a supportive feature (Crutch et al., 2017). The most frequent neuropathologic cause of PCA is Alzheimer disease (AD), but other, much rarer, alternative aetiologies have been identified

Abbreviations: AD, Alzheimer disease; AT, Anterior temporal scale; AUC, Area under the ROC Curve; CON, Controls; CSF, Cerebrospinal Fluid; FDG, fluorodeoxyglucose; FEW, Family wise error; GM, grey matter; LBD, Lewy Body Dementia; MMSE, Mini Mental State Examination; MNI, Montreal Neurological Institute; MRI, magnetic resonance imaging; MTA, Medial temporal scale; OF, Orbito-Frontal scale; PA, Posterior scale; PCA, Posterior cortical atrophy; PCS, Posterior cingulate sulcus scale; PET, Positron emission tomography; POS, Parieto-occipital sulcus scale; PRE, Precuneus scale; VBM, Voxel Based Morphometry; VOSP, Visual object and space perception test; WM, white matter.

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New Algorithms Improving PML Risk Stratification in MS Patients Treated With Natalizumab

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Overview: We assessed the role of age and disease activity as new factors contributing to establish the risk of progressive multifocal leucoencephalopathy in multiple sclerosis

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Original Article

Low CSF β-amyloid levels predict early regional grey matter atrophy in multiple sclerosis

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ARTICLE INFO

Keywords:
Multiple sclerosis
β-amyloid
Brain M
Grey matter atrophy

ABSTRACT

Background and purpose: Grey matter (GM) atrophy is present from the earliest stages of multiple sclerosis (MS) and occurs largely in a nonrandom manner. However, the biological mechanisms underlying the progression of regional atrophy are still unclear. Aim of this study is to investigate whether amyloid pathology might be involved in determining the pattern of GM atrophy over time.

Methods: Forty-six subjects were recruited: 31 newly diagnosed relapsing-remitting (RR-) MS patients and 15 age- and sex-matched healthy controls (HC). Aβ levels were determined in CSF samples from all subjects. All participants underwent brain magnetic resonance imaging (MRI) at baseline, and 23 out of 31 patients at one year follow-up. T1-weighted scans were segmented using the Geodesic Information Flows software. Non-parametric statistical tests were used for between-group comparisons and multiple regression analyses.

Results: CSF Aβ concentration was the best predictor of global GM loss over time after age (β = 0.403; $p = 0.024$), in particular in the left precuneus ($p = 0.045$), in the left middle cingulate gyrus ($p = 0.009$), in the left precentral gyrus ($p = 0.021$) and in the right angular gyrus ($p = 0.029$).

Conclusions: CSF Aβ levels seem to be crucial in MS early brain volume loss as GM atrophy manifests in regions particularly vulnerable to early Aβ deposition.

1. Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS) (Reich et al., 2018). Although traditionally considered a white matter (WM) demyelinating disease, it is now recognized that axonal loss is critically involved in MS pathophysiology since early clinical stages (Frischer et al., 2009; Bjartmar et al., 2000). At present, however, the biological mechanisms underlying degeneration and grey matter (GM) atrophy in MS are still largely unknown: they could involve acute axonal transection in inflammatory lesions, network-mediated trans-synaptic degeneration, energy and metabolic deficits, cortical demyelination, diffuse neuroinflammation or meningeal inflammation (Stankoff and Louapre, 2018). As a consequence of these unanswered questions, the evaluation of neurodegeneration using new magnetic resonance imaging (MRI) techniques and its possible link with predictive biomarkers has become a crucial area of research in MS (Rocca et al., 2017).

MRI is an invaluable tool for the diagnostic work-up in MS patients (Polman et al., 2011; Filippi et al., 2016). However, it is known that no strong correlation exists between conventional MRI measures. Brain atrophy quantification is the most reliable parameter to assess axonal loss and neurodegeneration (Filippi et al., 2016; De Stefano et al., 2016). In longitudinal MRI studies MS patients exhibited brain tissue loss at a considerably faster rate than healthy age-matched controls (De Stefano et al., 2016). More specifically, the mean annualized brain atrophy rate in healthy adults is approximately −0.1 to −0.3%, compared with −0.4 to −1.0% in MS patients (De Stefano et al., 2014) Moreover, advances in brain atrophy analysis revealed that some regions are more susceptible to tissue loss than others (Steenwijk et al., 2016; Preziosa et al., 2017), suggesting that brain atrophy occurs

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nutrients

MDPI

Article

A Special Amino-Acid Formula Tailored to Boosting Cell Respiration Prevents Mitochondrial Dysfunction and Oxidative Stress Caused by Doxorubicin in Mouse Cardiomyocytes

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Abstract: Anthracycline anticancer drugs, such as doxorubicin (DOX), can induce cardiotoxicity supposed to be related to mitochondrial damage. We have recently demonstrated that a branched-chain amino acid (BCAA)-enriched mixture (BCAAem), supplemented with drinking water to middle-aged mice, was able to promote mitochondrial biogenesis in cardiac and skeletal muscle. To maximally favor and increase oxidative metabolism and mitochondrial function, here we tested a new original formula, composed of essential amino acids, tricarboxylic acid cycle precursors and co-factors (named α5), in HL-1 cardiomyocytes and mice treated with DOX. We measured mitochondrial biogenesis, oxidative stress, and BCAA catabolic pathway. Moreover, the molecular relevance of endothelial nitric oxide synthase (eNOS) and mechanistic/mammalian target of rapamycin complex 1 (mTORC1) was studied in both cardiac tissue and HL-1 cardiomyocytes. Finally, the role of Krüppel-like factor 15 (KLF15), a critical transcriptional regulator of BCAA oxidation and eNOS-mTORC1 signal, was investigated. Our results demonstrate that the α5 mixture prevents the DOX-dependent mitochondrial damage and oxidative stress better than the previous BCAAem, implying a KLF15/eNOS/mTORC1 signaling axis. These results could be relevant for the prevention of cardiotoxicity in the DOX-treated patients.

Keywords: branched-chain amino acids; cardiomyocytes; doxorubicin; endothelial nitric oxide synthase; Krüppel-like factor 15; mechanistic/mammalian target of rapamycin; mitochondria; oxidative stress; peroxisome proliferator-activated receptor γ coactivator 1 α; tricarboxylic acid cycle

1. Introduction

Anthracyclines, such as doxorubicin (DOX), are widely used and highly successful anticancer chemotherapeutics [1]. Unfortunately, DOX administration results in dose-dependent side effects to non-cancer tissues, including the development of cardiomyopathy, in addition to dyspnea, exercise intolerance, hepatotoxicity, and nephropathy [2]. The risk of cardiotoxicity is one of the greatest limiting factors to the clinical use of this drug, resulting in both acute and chronic cardiovascular events. Acute cardiac toxicity of DOX can develop within minutes to days after administration and normally is characterized by hypotension, arrhythmia, and most importantly left ventricular failure [1,3,4]. Although the molecular mechanisms of these side effects are not fully understood, also

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Lab resource: Stem Cell Line

Generation of the Becker muscular dystrophy patient derived induced pluripotent stem cell line carrying the *DMD* splicing mutation c.1705-8 $T>C$.

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A B S T R A C T

Becker Muscular dystrophy (BMD) is an X-linked syndrome characterized by progressive muscle weakness. BMD is generally less severe than Duchenne Muscular Dystrophy. BMD is caused by mutations in the dystrophin gene that normally give rise to the production of a truncated but partially functional dystrophin protein. We generated an induced pluripotent cell line from dermal fibroblasts of a BMD patient carrying a splice mutation in the dystrophin gene (c.1705-8 $T>C$). The iPSC cell-line displayed the characteristic pluripotent-like morphology, expressed pluripotency markers, differentiated into cells of the three germ layers and had a normal karyotype.

Resource Table:

Unique stem cell line identifier	CCMi004-A
Alternative name(s) of stem cell line	BMD3 c.13
Institution	*Centro Cardiologico Monzino-IRCCS*
Contact information of distributor	*Aoife Gowran; aoife.gowran@ccfm.it*
Type of cell line	*iPSC*
Origin	*Human*
Additional origin info	*Age: 5 (at skin biopsy)* *Sex: M* *Ethnicity if known: Caucasian*
Cell Source	*Dermal fibroblasts*
Clonality	*Clonal*
Method of reprogramming	*Episomal vectors containing the reprogramming factors: hL-MYC, hLIN28, hSOX2, hKLF4, hOCT4.*
Genetic Modification	*YES*
Type of Modification	*Spontaneous mutation*
Associated disease	*Becker Muscular dystrophy*
Gene/locus	*DMD gene, Xp21.2-p21.1*
Method of modification	*No modification*
Name of transgene or resistance	*N/A*
Inducible/constitutive system	*N/A*
Date archived/stock date	*July 2019*
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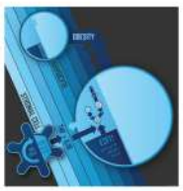
Autophagy



Autophagy

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Metformin rescues muscle function in BAG3 myofibrillar myopathy models

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RESEARCH PAPER

Check for updates

Metformin rescues muscle function in BAG3 myofibrillar myopathy models

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ABSTRACT

Dominant *de novo* mutations in the co-chaperone BAG3 cause a severe form of myofibrillar myopathy, exhibiting progressive muscle weakness, muscle structural failure, and protein aggregation. To elucidate the mechanism of disease in, and identify therapies for, BAG3 myofibrillar myopathy, we generated two zebrafish models, one conditionally expressing $BAG3^{P209L}$ and one with a nonsense mutation in *bag3*. While transgenic $BAG3^{P209L}$-expressing fish display protein aggregation, modeling the early phase of the disease, $bag3^{-/-}$ fish exhibit exercise dependent fiber disintegration, and reduced swimming activity, consistent with later stages of the disease. Detailed characterization of the $bag3^{-/-}$ fish, revealed an impairment in macroautophagic/autophagic activity, a defect we confirmed in *BAG3* patient samples. Taken together, our data highlights that while $BAG3^{P209L}$ expression is sufficient to promote protein aggregation, it is the loss of BAG3 due to its sequestration within aggregates, which results in impaired autophagic activity, and subsequent muscle weakness. We therefore screened autophagy-promoting compounds for their effectiveness at removing protein aggregates, identifying nine including metformin. Further evaluation demonstrated metformin is not only able to bring about the removal of protein aggregates in zebrafish and human myoblasts but is also able to rescue the fiber disintegration and swimming deficit observed in the $bag3^{-/-}$ fish. Therefore, repurposing metformin provides a promising therapy for BAG3 myopathy.

Abbreviations:ACTN: actinin, alpha; BAG3: BAG cochaperone 3; CRYAB: crystallin alpha B; DES: desmin; DMSO: dimethyl sulfoxide; DNAJB6: DnaJ heat shock protein family (Hsp40) member B6; dpf: days post fertilization; eGFP: enhanced green fluorescent protein; FDA: Food and Drug Administration; FHL1: four and a half LIM domains 1; FLNC: filamin C; hpf: hours post-fertilization; HSPB8: heat shock protein family B [small] member 8; LDB3/ZASP: LIM domain binding 3; MYOT: myotilin; TTN: titin; WT: wild-type.

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Autophagy; BAG3; metformin; muscle; myofibrillar myopathy; zebrafish

Introduction

Myofibrillar myopathies are a group of chronic muscle diseases characterized at the cellular level by accumulation of protein aggregates and structural failure of the muscle fiber. There is significant variability in the presentation of these diseases, with onset ranging from infantile to late seventies and muscle weakness ranging from mild reductions to severe impairment of skeletal, cardiac, and respiratory muscles resulting in early death. Causative mutations for myofibrillar myopathies have been identified in 10 genes: *DES* (desmin) [1], *CRYAB/αB-crystallin* [2], *MYOT* (myotilin) [3], *LDB3* (LIM domain binding 3)*/ZASP (*Z-band alternatively spliced PDZ motif-containing protein) [4], *FLNC* (filamin C) [5], *BAG3* (BAG cochaperone 3) [6], *FHL1* (four and a half LIM domains 1) [7], *TTN* (titin) [8], *DNAJB6* (DnaJ heat shock protein family [Hsp40] member B6) [9], and *HSPB8* (heat shock protein family B [small] member 8) [10]. All of these genes encode proteins found at the Z-disk, a key structure involved in the transmission of tension and contractile forces along the muscle fiber.

While structural failure of the muscle fiber is a feature of myofibrillar myopathies, not all of the proteins associated with the disease have a direct structural role. One such protein is BAG3, a multi domain co-chaperone that is predominantly expressed in skeletal and cardiac muscle, where it co-localizes

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Cell Death & Disease

ARTICLE Open Access

MICAL2 is essential for myogenic lineage commitment

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Abstract

Contractile myofiber units are mainly composed of thick myosin and thin actin (F-actin) filaments. F-Actin interacts with Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 2 (MICAL2). Indeed, MICAL2 modifies actin subunits and promotes actin filament turnover by severing them and preventing repolymerization. In this study, we found that MICAL2 increases during myogenic differentiation of adult and pluripotent stem cells (PSCs) towards skeletal, smooth and cardiac muscle cells and localizes in the nucleus of acute and chronic regenerating muscle fibers. In vivo delivery of Cas9–Mical2 guide RNA complexes results in muscle actin defects and demonstrates that MICAL2 is essential for skeletal muscle homeostasis and functionality. Conversely, MICAL2 upregulation shows a positive impact on skeletal and cardiac muscle commitments. Taken together these data demonstrate that modulations of MICAL2 have an impact on muscle filament dynamics and its fine-tuned balance is essential for the regeneration of muscle tissues.

Introduction

Muscle tissue represents 40% of human body mass and provides locomotion, posture support and it is required for breathing. Myogenesis is the process that ensures the generation of myoblasts which differentiate into skeletal muscle tissue[1]. Once the muscle is mature, a sub-population of *Pax7*$^{+}$ cells becomes quiescent as satellite cells (SCs) and muscle renewal and regeneration rely on the activated SCs that have the potential to fuse and form new fibers, as well as maintaining the stem cell niche[2–4]. This process occurs in adult tissue when muscles are damaged[5] to restore the damaged contractile myofiber units[6]. Thin actin filaments (F-actin) are crucial components of contractile myofiber units. F-Actin interacts with Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing proteins (MICALs), capable to make oxidation-reduction reactions (redox) by its FAD domain[7,8]. MICALs make redox reactions on filamentous-actin (F-actin), by binding FAD and using NADPH and O_2 to depolymerize F-actin[9–12]. MICALs were seen involved in many functions within different cell types, all depending on dynamic actin cytoskeleton remodeling[8,11–16]. So far, MICAL was identified only in Drosophila skeletal muscles[14,17] and its role in mammalian muscles is totally unexplored. However, it seems that MICALs are indirectly involved in cardiovascular integrity by regulating semaphorin 3a expression. Indeed, semaphorin 3a overexpression led to a reduction of post-myocardial infarction arrhythmia[18,19]. Nevertheless, evidences of a direct role of MICALs on cardiac and smooth muscle are still lacking. Among the MICALs, MICAL2 emerged as the one involved in angiogenesis, cell viability, gene transcription[19]. Indeed, MICAL2 modifies actin subunits and promotes actin turnover by ensuring disaggregation and preventing repolymerization[11]. In addition, in a

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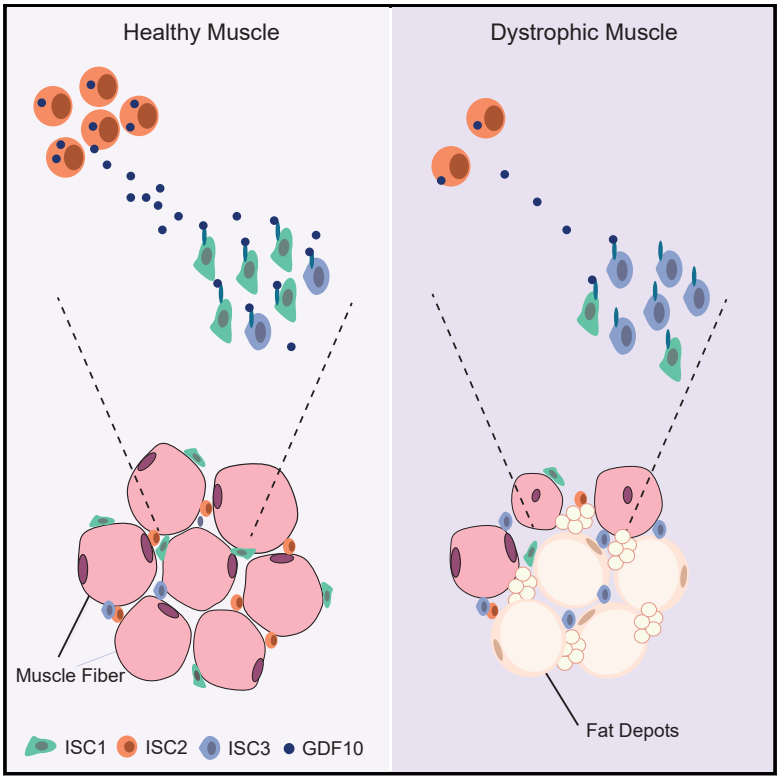
Article

Interstitial Cell Remodeling Promotes Aberrant Adipogenesis in Dystrophic Muscles

Graphical Abstract



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In Brief

Camps et al. describe a cell population that blocks fat turnover in the skeletal muscle by single-cell analysis. Furthermore, they show that this population acts through GDF10 secretion and is reduced in dystrophic muscle. This could be an essential turning point in the progressive deterioration of muscular dystrophy patients.

Highlights

- Single-cell RNA-seq reveals an altered cell landscape in dystrophic skeletal muscle
- Classification of interstitial stem cell states from healthy and dystrophic muscle
- Adipo-regulatory cells (Aregs) block adipogenesis through GDF10 secretion
- The amount of Aregs decreases in dystrophic muscle, thereby increasing fat depots

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Article

Interstitial Cell Remodeling Promotes Aberrant Adipogenesis in Dystrophic Muscles

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SUMMARY

Fibrosis and fat replacement in skeletal muscle are major complications that lead to a loss of mobility in chronic muscle disorders, such as muscular dystrophy. However, the *in vivo* properties of adipogenic stem and precursor cells remain unclear, mainly due to the high cell heterogeneity in skeletal muscles. Here, we use single-cell RNA sequencing to decomplexify interstitial cell populations in healthy and dystrophic skeletal muscles. We identify an interstitial CD142-positive cell population in mice and humans that is responsible for the inhibition of adipogenesis through GDF10 secretion. Furthermore, we show that the interstitial cell composition is completely altered in muscular dystrophy, with a near absence of CD142-positive cells. The identification of these adipo-regulatory cells in the skeletal muscle aids our understanding of the aberrant fat deposition in muscular dystrophy, paving the way for treatments that could counteract degeneration in patients with muscular dystrophy.

INTRODUCTION

The skeletal muscle exists out of muscle fibers, multinucleated contractile units that are structured in bundles or fascicles, surrounded by connective tissue, also called the interstitium (Frontera and Ochala, 2015). Upon acute injury, progenitors swiftly activate their repair mechanisms and completely regenerate the skeletal muscle in a time span of weeks (Wosczyna and Rando, 2018). An abundant assembly of cells has been reported to be involved in this repair process, of which muscle satellite cells (MuSCs) are the predominant cell type (Sacco et al., 2008). MuSCs are skeletal muscle stem cells located beneath the basal lamina of muscle fibers (Mauro, 1961). In homeostatic conditions, they remain quiescent, although upon injury they proliferate, differentiate, and fuse into new or existing myofibers (Feige et al., 2018). Besides MuSCs, fibro/adipogenic progenitors (FAPs) (Joe et al., 2010), mesenchymal progenitors (Uezumi et al., 2010), mesoangioblasts (MABs) (Dellavalle et al., 2007), $Pw1^{+}/Pax7^{+}$ interstitial cells (PICs) (Mitchell et al., 2010), and $TWIST2^{+}$ progenitors (Liu et al., 2017a) have been reported as stem cell populations that directly or indirectly support myogenic regeneration. However, the current characterization of these cells does not suffice, as many of these cell types share overlapping markers and functions.

In chronic injury, regeneration deteriorates, leading to increased adipogenesis and fibrosis at the expense of the muscle fibers (Hamrick et al., 2016; Mann et al., 2011). This deposition of fat and extracellular matrix (ECM) leads to progressive muscle weakening and dysfunction, leading to the loss of ambulation and respiratory complications (Mercuri and Muntoni, 2013). Although the mechanisms of fibrosis in muscular dystrophy are generally understood (Joe et al., 2010; Lemos et al., 2015; Mueller et al., 2016; Uezumi et al., 2011), it is still unclear how adipogenesis arises in chronic muscle regeneration. FAPs

frontiers in Physiology

ORIGINAL RESEARCH
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PTX3 Predicts Myocardial Damage and Fibrosis in Duchenne Muscular Dystrophy

*Andrea Farini[1], Chiara Villa[1], Dario Di Silvestre[2], Pamela Bella[1], Luana Tripodi[1], Rossana Rossi[2], Clementina Sitzia[3], Stefano Gatti[4], Pierluigi Mauri[2] and Yvan Torrente[1]**

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Pentraxin 3 (PTX3) is a main component of the innate immune system by inducing complement pathway activation, acting as an inflammatory mediator, coordinating the functions of macrophages/dendritic cells and promoting apoptosis/necrosis. Additionally, it has been found in fibrotic regions co-localizing with collagen. In this work, we wanted to investigate the predictive role of PTX3 in myocardial damage and fibrosis of Duchenne muscular dystrophy (DMD). DMD is an X-linked recessive disease caused by mutations of the dystrophin gene that affects muscular functions and strength and accompanying dilated cardiomyopathy. Here, we expound the correlation of PTX3 cardiac expression with age and Toll-like receptors (TLRs)/interleukin-1 receptor (IL-1R)-MyD88 inflammatory markers and its modulation by the so-called alarmins IL-33, high-mobility group box 1 (HMGB1), and S100β. These findings suggest that cardiac levels of PTX3 might have prognostic value and potential in guiding therapy for DMD cardiomyopathy.

Keywords: Duchenne muscular dystrophy (DMD), muscular dystrophy, cardiomyopathy, pentraxin 3 (PTX3), alarmins

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INTRODUCTION

Pentraxins (PTXs) are a superfamily of proteins containing the highly conserved C-terminal PTX domain. According to the primary structure of the promoter, they are divided into two distinct groups: short and long (Deban et al., 2011). Among the longer subfamily, the Pentraxin 3 (PTX3) is an inflammatory mediator, mainly produced during the first phase of the inflammatory processes by phagocytes, neutrophils, fibroblasts, endothelial cells, following the secretion of inflammatory cytokines (Doni et al., 2008). PTX3 is a fundamental component of humoral innate immunity and – in synergy with other proteins as the PTX C-reactive protein (CRP) and serum amyloid P-component (SAP) – mediates the innate resistance to pathogens, allows the activation of complement pathway, coordinates the functions of macrophages/dendritic cells (DCs), and

cells

MDPI

Review

Role of Insulin-Like Growth Factor Receptor 2 across Muscle Homeostasis: Implications for Treating Muscular Dystrophy

Yvan Torrente *, Pamela Bella, Luana Tripodi, Chiara Villa and Andrea Farini *

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Abstract: The insulin-like growth factor 2 receptor (IGF2R) plays a major role in binding and regulating the circulating and tissue levels of the mitogenic peptide insulin-like growth factor 2 (IGF2). IGF2/IGF2R interaction influences cell growth, survival, and migration in normal tissue development, and the deregulation of IGF2R expression has been associated with growth-related disease and cancer. IGF2R overexpression has been implicated in heart and muscle disease progression. Recent research findings suggest novel approaches to target IGF2R action. This review highlights recent advances in the understanding of the IGF2R structure and pathways related to muscle homeostasis.

Keywords: IGF2R; muscle homeostasis; inflammation; muscular dystrophy; pericytes

1. Introduction

The cation-independent mannose 6-phosphate/insulin-like growth factor 2 receptor (CI-M6P/IGF2R, hereafter IGF2R) is a type-1 transmembrane glycoprotein consisting of a large N-terminal extracytoplasmic domain, which allows it to bind to a wide variety of ligands [1,2]. The IGF2 and M6P ligands [3–5] of IGF2R have distinct but important roles in normal development and mesoderm differentiation [6]. Many studies have demonstrated the suppression action of IGF2R on insulin-like growth factor 1 receptor (IGF1R) signaling by scavenging extracellular IGF2 [7]. Furthermore, several lines of evidence demonstrate that IGF2 is highly expressed in rodent embryos, where it functions as an embryonic growth factor, while its amount is diminished at birth [8]. Smith et al. recently showed that a transgene-induced overexpression of IGF2 blocked programmed cell death, one of the main pathological features of cancer [9]. Furthermore, in some cancers such as mammary tumors, IGF2R behaves as a tumor suppressor gene [10], whereas in other cancers such as cervical tumors or glioblastomas, IGF2R acts as an oncogene [11,12]. Thus, these two traits of IGF2R might depend on cell type. Interestingly, cervical tumors and glioblastomas have common mesenchymal founders, namely myofibroblasts [13], which are also involved in muscle disease. Muscle repair is a complex and tightly regulated event that recruits different cell types, starting from macrophage and lymphocyte consecutive involvement and terminating with satellite cell (SC) activation and differentiation [14]. Among the common hallmarks of muscular dystrophy are the infiltration of immune cells into skeletal muscle fibers, and fibrotic cell proliferation [15–18].

Impaired muscle regeneration with SC pool exhaustion is considered an additional pathological feature of Duchenne muscular dystrophy (DMD) [19]. The main biological function of IGF2R is the suppression of IGF1R signaling via the deprivation of extracellular IGF2 ligands. Some studies have explained the tumor suppressive functions of IGF2R by its negative regulation of the

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REVIEW

Topical treatment of radiation-induced dermatitis: current issues and potential solutions

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Abstract

Approximately 95% of patients receiving radiotherapy (RT) will ultimately develop radiation-induced dermatitis (RID) during or after the course of treatment, with major consequences on quality of life and treatment outcomes. This paper reviews the pathophysiology of RID and currently used topical products for the prevention and treatment of RID. Although there is no consensus on the appropriate management, recent evidence suggests that the use of topical products supports to protect and promote tissue repair in patients with RID. Basic recommendations include advice to wear loose clothing, using electric razors if necessary, and avoiding cosmetic products, sun exposure or extreme temperatures. Based on mechanisms involved and on the clinical characteristics of oncological patients, the profile of the ideal topical product for addressing RID can be designed; it should have limited risk of adverse events, systemic adsorption and drug–drug interactions, should be characterized by multiple clinical activities, with a special focus on localized pain, and should have a careful formulation as some vehicles can block the RT beam.

Keywords: pain, quality of life, radiation-induced dermatitis, radiotherapy, skin toxicity, topical treatment.

Citation

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Introduction

Radiation-induced dermatitis (RID) is a very common side effect that is almost universally experienced by patients undergoing radiotherapy (RT) for cancer treatment. RID results from cutaneous or subcutaneous lesions due to external beam radiation. Indeed, it has been estimated that approximately 95% of patients receiving RT will ultimately develop RID during or after the course of treatment, with major consequences on quality of life and adherence to RT treatments, thereby affecting clinical outcomes.[1–3] However, at present, there is no consensus on the appropriate management of this condition. Therefore, there is urgent need for increased knowledge to guarantee a range of therapeutic options available for the treatment of RID.[1]

Recent evidence suggests that topical products may be used to protect and promote tissue repair in patients with RID, including within the prophylactic setting.[4,5]

The aim of this paper is to discuss current knowledge on RID and propose targets for the prevention/treatment of this condition. On these bases, the characteristics of the 'ideal' compound to address this side effect will be described.

Manuscripts for consideration in the present paper were retrieved via a PubMed search, using pertinent keywords (e.g. radiation-induced dermatitis). Papers were then selected for inclusion according to their relevance to the topic, as judged by the authors. The reference lists of the papers were also browsed to identify other suitable publications. Papers from personal collections of literature of the authors were also considered.

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ORIGINAL ARTICLE

WILEY

Reprogramming fibroblasts and peripheral blood cells from a *C9ORF72* patient: A proof-of-principle study

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Abstract

As for the majority of neurodegenerative diseases, pathological mechanisms of amyotrophic lateral sclerosis (ALS) have been challenging to study due to the difficult access to alive patients' cells. Induced pluripotent stem cells (iPSCs) offer a useful in vitro system for modelling human diseases. iPSCs can be theoretically obtained by reprogramming any somatic tissue although fibroblasts (FB) remain the most used cells. However, reprogramming peripheral blood cells (PB) may offer significant advantages. In order to investigate whether the choice of starting cells may affect reprogramming and motor neuron (MNs) differentiation potential, we used both FB and PB from a same *C9ORF72*-mutated ALS patient to obtain iPSCs and compared several hallmarks of the pathology. We found that both iPSCs and MNs derived from the two tissues showed identical properties and features and can therefore be used interchangeably, giving the opportunity to easily obtain iPSCs from a more manageable source of cells, such as PB.

KEYWORDS

amyotrophic lateral sclerosis, *C9ORF72*, fibroblasts, iPSCs, motor neuron, peripheral blood cells, repeat expansion, reprogramming, RNA foci, TDP-43

1 | INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease. This disorder is characterized by a progressive motor neuron (MNs) loss in brain and spinal cord, causing paralysis, respiratory failure and death within 5 years from the diagnosis.[1] At molecular level, the DNA/RNA-binding protein TAR (trans-activation response element) DNA-binding protein (TDP-43) was described to be the major component of the pathological aggregates found in brains of ALS patients.[2] The presence of the GGGGCC (G_4C_2)-repeat expansion in the noncoding region of the open reading frame 72 (*C9ORF72*) gene on the chromosome 9 is the most frequent genetic

Vincenzo Silani and Patrizia Bossolasco joint senior authors.

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Focus on the heterogeneity of amyotrophic lateral sclerosis

Caterina Bendotti, Valentina Bonetto, Elisabetta Pupillo, Giancarlo Logroscino, Ammar Al-Chalabi, Christian Lunetta, Nilo Riva, Gabriela Mora, Giuseppe Lauria, Jochen H. Weishaupt, Federica Agosta, Andrea Malaspina, Manuela Basso, Linda Greensmith, Ludo Van Den Bosch, Antonia Ratti, Massimo Corbo, Orla Hardiman, Adriano Chiò, Vincenzo Silani & Ettore Beghi

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REVIEW ARTICLE

Focus on the heterogeneity of amyotrophic lateral sclerosis

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Abstract

The clinical manifestations of amyotrophic lateral sclerosis (ALS) are variable in terms of age at disease onset, site of onset, progression of symptoms, motor neuron involvement, and the occurrence of cognitive and behavioral changes. Genetic background is a key determinant of the ALS phenotype. The mortality of the disease also varies with the ancestral origin of the affected population and environmental factors are likely to be associated with ALS at least within some cohorts. Disease heterogeneity is likely underpinned by the presence of different pathogenic mechanisms. A variety of ALS animal models can be informative about the heterogeneity of the neuropathological or genetic aspects of the disease and can support the development of new therapeutic intervention. Evolving biomarkers can contribute to the identification of differing genotypes and phenotypes, and can be used to explore whether genotypic and phenotypic differences in animal models might help to provide a better definition of the heterogeneity of ALS in humans. These include neurofilaments, peripheral blood mononuclear cells, extracellular vesicles, microRNA and imaging findings. These biomarkers might predict not only the development of the disease, but also the variability in progression, although robust validation is required. A promising area of progress in modeling the heterogeneity of human ALS is represented by the use of

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COVID-19

Neurology and the COVID-19 emergency

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Following the COVID-19 outbreak, significant changes have been implemented on a national level in the organization of neurology units and associated stroke units. Regionally-designated COVID-19 hospitals have implemented an aggressive policy to relocate as many beds as possible to COVID-19 patients. In order to do so, the preferred strategy has been to reduce the number of beds in neurology units, and in some cases several units have been consolidated into one. In other cases, particularly in the northern regions, entire neurology units have been closed and converted into COVID-19 departments entrusted to other medical specialties. Some stroke units have followed the same path and become "hubs" and "spokes" for acute neurological pathology. In other situations, especially when it was necessary to meet the need for paramedical staff, the number of beds in neurology/stroke wards were significantly reduced and the nurses transferred to other units as needed to manage the COVID-19 emergency.

During the COVID-19 emergency phase in March and April 2020, only patients with acute neurological diseases, including cerebrovascular disease, epilepsy, and other acute pathologies, were admitted to neurology units of COVID-19 hospitals. For all other patients with non-acute central and peripheral nervous system diseases, hospitalization was limited. Overall, the number of hospitalized neurological patients has undergone a drastic reduction. Some neurological patients have been hospitalized in non-COVID-19 hospitals or in other non-neurological wards, while a significant proportion of patients were forced to postpone their request for hospitalization. In addition to the reduced availability of beds, the COVID-19 emergency has also resulted in the suspension of neurological day hospitals, ambulatory care, complex outpatient activity, and outpatient clinics, as well as clinics dedicated to the diagnosis and treatment of specific categories of neurological patients (centers for epilepsy, neu-

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COVID-19

Rising evidence for neurological involvement in COVID-19 pandemic

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Dear Editor,

A novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged at the end of 2019 and resulted in a rapidly spreading acute respiratory illness epidemic in Wuhan, China. The World Health Organization (WHO) termed this severe respiratory illness Coronavirus Disease 2019 (COVID-19). Since then, COVID-19 has become a pandemic and has affected over 2 million individuals in more than 200 countries and resulted in more than 135,000 deaths worldwide

Clinically, COVID-19 is primarily a respiratory disease marked by fever, cough, and shortness of breath, and in a small percentage of cases, it can lead to severe respiratory complications and death. However, as the number of cases of COVID-19 grows globally and understanding of the disease is unfolding, there are hints that it might have a neurologic component as well.

Coronavirus infections and CNS

The novel SARS-CoV-2 belongs to the β-coronavirus cluster [1] and COVID-19 is the third known zoonotic coronavirus disease after SARS and Middle East Respiratory Syndrome (MERS), which are also caused by β-coronaviruses SARS-CoV and MERS-CoV, respectively. Evidence from former studies of SARS and MERS indicates that human coronaviruses (CoV) do not remain confined to the respiratory system. Instead, they can disseminate damaging several other regions, including the central nervous system (CNS). Indeed, many human coronaviruses are neurotropic and potentially neurovirulent. Possible mechanisms of entry into the CNS include the neuronal retrograde route and hematogenous dissemination. Within the neuronal route, trans-synaptic transfer via peripheral nerve terminals has been well documented for other coronaviruses. An alternative pathway for neuroinvasion implicates connections from mechano- and chemoreceptors in the lung and lower respiratory airways to specific nuclei in the brainstem.

Neurological manifestations were previously observed in patients with SARS and MERS. A group described four patients who developed axonal polyneuropathy, myopathy, or both approximately 3 weeks after the onset of SARS [2], while others have reported large artery ischemic strokes [3]. These findings raise the critical issue of possibly needing to adjust therapeutic strategies in certain patients. Similarly, patients with MERS-CoV infections have a high probability of experiencing neurological complications. A retrospective study of patients with MERS found that symptoms such as generalized fatigue, confusion, and myalgia are relatively common [4]. Another study reported that almost 1/5 of patients show specific neurological complications, including impaired consciousness, paralysis, ischemic stroke, Guillain-Barré syndrome, and other neuropathies which appeared 2–3 weeks after the respiratory symptoms [5].

Given the neurotropic properties of other CoV and the similarity between SARS-CoV and SARS CoV-2, it is likely that neuroinvasion plays an important role in COVID-19. Notably, earlier this year, a group found that up to one-third of COVID-19 patients presented at least one neurological symptom [6]. Most reported symptoms are non-specific like dizziness, headache, fatigue, and myalgia. However, specific symptoms such as acute cerebrovascular disease, seizure, and coma are also described and are more common in severe cases of COVID-19. The incidence of neurological features may vary significantly among different study populations. Further studies are

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Human salivary Raman fingerprint as biomarker for the diagnosis of Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease leading to progressive and irreversible muscle atrophy. The diagnosis of ALS is time-consuming and complex, with the clinical and neurophysiological evaluation accompanied by monitoring of progression and a long procedure for the discrimination of similar neurodegenerative diseases. The delayed diagnosis strongly slows the potential development of adequate therapies and the time frame for a prompt intervention. The discovery of new biomarkers could improve the disease diagnosis, as well as the therapeutic and rehabilitative effectiveness and monitoring of the pathological progression. In this work saliva collected from 19 patients with ALS, 10 affected by Parkinson's disease, 10 affected by Alzheimer's disease and 10 healthy subjects, was analysed using Raman spectroscopy, optimizing the parameters for detailed and reproducible spectra. The statistical multivariate analysis of the data revealed a significant difference between the groups, allowing the discrimination of the disease onset. Correlation of Raman data revealed a direct relationship with paraclinical scores, identifying multifactorial biochemical modifications related to the pathology. The proposed approach showed a promising accuracy in ALS onset discrimination, using a fast and sensitive procedure that can make more efficient the diagnostic procedure and the monitoring of therapeutic and rehabilitative processes in ALS.

Amyotrophic Lateral Sclerosis (ALS) is a complex and lethal neurodegenerative disease that progressively leads to irreversible muscle atrophy due to the death of motoneurons replaced by gliosis, with a life expectation from the onset of first symptoms between 2 and 5 years, depending on the cases[1]. This disorder affects both lower and upper motoneurons with symptoms including generalized muscle weakness, possible cognitive dysfunction, cramps, fasciculations, spasticity, serious functional limitations with parallel and progressive paralysis leading to death, typically resulting from ventilatory failure[2]. An American study showed that there are 223,000 people affected by ALS worldwide with an incidence of 1.75/100,000 and a predicted increase of 69% in 2040 due to the population aging[3]. The causes for ALS disease are still unclear with different mechanisms proposed including genetic, environmental, viral, immunological and epidemiological factors[4]. Compared to other neurodegenerative diseases, the identification of potential biomarkers in ALS has been hampered by the long lag-time between symptoms onset and diagnosis (approximately 12 months) and to the low annual incidence that makes general screening strategies not feasible[5]. Nowadays, no diagnostic test can specifically detect ALS at onset and discriminate ALS from other motoneuron and similar neurodegenerative diseases, thus hindering the diagnosis, prognosis, patients' stratification, treatment monitoring or the objective evaluation of the effects of new possible therapies. Currently, the diagnosis of ALS is achieved by the combination of clinical data and neurophysiological evidence together with the monitoring of the symptoms progression in a time-consuming process that limits the time frame for a prompt intervention and the choice of a personalized therapy[6]. The discovery of a new biomarker easily accessible and quickly detectable represents a priority for ALS early diagnosis, stratification and evaluation of the therapeutic and rehabilitative effectiveness.

In recent years, several potential biomarkers were isolated from different tissues and highly specific techniques have been proposed. The road taken by researchers regards the analysis of biofluids, whose molecular

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Progression of brain functional connectivity and frontal cognitive dysfunction in ALS

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ABSTRACT

Objective: To investigate the progression of resting-state functional connectivity (rs-FC) changes in patients with amyotrophic lateral sclerosis (ALS) and their relationship with frontal cognitive alterations.
Methods: This is a multicentre, observational and longitudinal study. At baseline and after six months, 25 ALS patients underwent 3D T1-weighted MRI, resting-state functional MRI (rs-fMRI), and the computerized Test of Attentional Performance (TAP). Using independent component analysis, rs-FC changes of brain networks involving connections to frontal lobes and their relationship with baseline cognitive scores and cognitive changes over time were assessed. With a seed-based approach, rs-FC longitudinal changes of the middle frontal gyrus (MFG) were also explored.
Results: After six months, ALS patients showed an increased rs-FC of the left anterior cingulate, left middle frontal gyrus (MFG) and left superior frontal gyrus within the frontostriatal network, and of the left MFG, left supramarginal gyrus and right angular gyrus within the left frontoparietal network. Within the frontostriatal network, a worse baseline performance at TAP divided attention task was associated with an increased rs-FC over time in the left MFG and a worse baseline performance at the category fluency index was related with increased rs-FC over time in the left frontal superior gyrus. After six months, the seed-based rs-FC analysis of the MFG with the whole brain showed decreased rs-FC of the right MFG with frontoparietal regions in patients compared to controls.
Conclusions: Rs-FC changes in ALS patients progressed over time within the frontostriatal and the frontoparietal networks and are related to frontal-executive dysfunction. The MFG seems a potential core region in the framework of a frontoparietal functional breakdown, which is typical of frontotemporal lobar degeneration. These findings offer new potential markers for monitoring extra-motor progression in ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is the most common type of motor neuron disease, a fatal and heterogeneous neurodegenerative disorder characterized by progressive damage to upper and lower motor neurons. (Swinnen and Robberecht, 2014) At present, its multisystem nature and clinical, pathological, and genetic overlap with frontotemporal lobar degeneration (FTLD) are firmly established. (Proudfoot et al., 2018) Cognitive and behavioural disturbances in ALS have been observed in about 50% of patients, (van Es et al., 2017) with executive

Abbreviations: rs-FC, resting-state functional connectivity; rs-fMRI, rs functional MRI; TAP, Test of Attentional Performance; MFG, middle frontal gyrus; IC, independent component.

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MEDICINE

Cervical transverse MRI in ALS diagnosis and possible link to VEGF and MMP9 single nucleotide polymorphisms. Case Report

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Accepted: 7 April 2020

Abstract

We report the case of a patient diagnosed with ALS and presenting with a "snake eyes" sign on axial cervical MRI. Given the peculiar radiological presentation and our patient's young age, we decided to perform a genetic analysis to better investigate the molecular mechanisms underlying the pathogenesis of his clinical phenotype. In our study, we identified different polymorphisms of interest at the level of VEGF and MMP9 promoters that we suggest could contribute to neuronal sufferance by promoting vascular abnormalities and tissue hypoxia, in accordance with previous published papers. When combined with repetitive mechanical stress at cervical level, as evident in our patient's case, the resultant radiological image appears to be the "snake eyes" sign in the anterior horns of the cervical spinal cord, while the clinical phenotype is that of an atypical ALS case, possibly an ALS-mimic. To correctly identify and diagnose such patients, in particular when disease course and neuroradiological data do not correspond to those of a classical ALS case, we underline the importance of axial cervical MRI studies and suggest the possibility of their implementation into routine clinical practice.

Keywords MRI · ALS · Motor neuron disease · Spinal cord · Snake-eyes sign

Case Report

A 29-year-old man, ex professional kart pilot, with past medical history of familial vein varicosity, presented clinically with lower (LMN) and upper (UMN) motor neuron signs (particularly of C4-C8 myomeres), confirmed by electrophysiological studies and stable over 2 years. On exam, diffuse fasciculations were present together with atrophy mainly of proximal upper limb and trapezius muscles. In addition, the patient showed marked hyperreflexia involving both upper and lower extremities. Cranial nerves were intact. There were no sensory deficits. Accurate imaging studies revealed a "snake eyes" sign at the level of the cervical spinal cord (C3-C7) in axial (Fig. 1a) and sagittal sections (Fig. 1b). No spinal atrophy or cerebellar tonsil ectopia was noted. A thorough examination of the differential diagnosis was performed, including Hirayama disease (suggested by the history of repetitive cervical flexion sustained by the patient during his professional training as well as by the neuroimaging finding of the "snake eyes sign") and cervical spondylotic amyotrophy (consistent with both MRI findings and proximal upper limb muscular atrophy). However, because of the

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COVID-19

An Italian multicenter retrospective-prospective observational study on neurological manifestations of COVID-19 (NEUROCOVID)

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Abstract

Neurological manifestations of COVID-19 have been described in both single case reports and retrospective scanty case series. They may be linked to the potential neurotropism of the SARS-COV-2 virus, as previously demonstrated for other coronaviruses. We report here the description of a multicenter retrospective-prospective observational study promoted by the Italian Society of Neurology (SIN), involving the Italian Neurological Departments, who will consecutively recruit patients with neurological symptoms and/or signs, occurred at the onset or as a complication of COVID-19. Hospitalized patients will be recruited either in neurological wards or in COVID wards; in the latter cases, they will be referred from other specialists to participant neurologists. Outpatients with clinical signs of COVID and neurological manifestations will be also referred to participating neurologists from primary care physicians. A comprehensive data collection, in the form of electronic case report form (eCRF), will register all possible neurological manifestations involving central nervous systems, peripheral nerves, and muscles, together with clinical, laboratory (including cerebrospinal fluid, if available), imaging, neurological, neurophysiological, and neuropsychological data. A follow-up at hospital discharge (in hospitalized patients), and for all patients after 3 and 6 months, is also planned. We believe that this study may help to intercept the full spectrum of neurological manifestations of COVID-19 and, given the large diffusion at national level, can provide a large cohort of patients available for future more focused investigations. Similar observational studies might also be proposed at international level to better define the neurological involvement of COVID-19.

Keywords COVID-19 · Neurological manifestations · NEUROCOVID · Observational study

Introduction

In the recent outbreak of COVID-19 pandemic, emerging in China and then exported first to Iran and Italy, particularly in the northern Lombardy region, and thereafter to a growing number of European countries and America, single case reports of co-occurrence of neurological disorders are being reported [1, 2]. A recent self-reported questionnaire on 59

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Review Article

New technologies and Amyotrophic Lateral Sclerosis – Which step forward rushed by the COVID-19 pandemic?

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Artificial intelligence
Robotics
COVID-19

ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fast-progressive neurodegenerative disease leading to progressive physical immobility with usually normal or mild cognitive and/or behavioural involvement. Many patients are relatively young, instructed, sensitive to new technologies, and professionally active when developing the first symptoms. Older patients usually require more time, encouragement, reinforcement and a closer support but, nevertheless, selecting user-friendly devices, provided earlier in the course of the disease, and engaging motivated carers may overcome many technological barriers. ALS may be considered a model for neurodegenerative diseases to further develop and test new technologies. From multidisciplinary teleconsults to telemonitoring of the respiratory function, telemedicine has the potentiality to embrace other fields, including nutrition, physical mobility, and the interaction with the environment. Brain-computer interfaces and eye tracking expanded the field of augmentative and alternative communication in ALS but their potentialities go beyond communication, to cognition and robotics. Virtual reality and different forms of artificial intelligence present further interesting possibilities that deserve to be investigated. COVID-19 pandemic is an unprecedented opportunity to speed up the development and implementation of new technologies in clinical practice, improving the daily living of both ALS patients and carers.

The present work reviews the current technologies for ALS patients already in place or being under evaluation with published publications, prompted by the COVID-19 pandemic.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease in which death occurs mainly due to respiratory insufficiency and respiratory infections. ALS patients are followed every 1.5–3 months by multidisciplinary teams, interval usually extended for slow progressors. Extra visits may be needed for respiratory, nutritional and psychological support [1].

Contrary to patients suffering a specific event in a specific moment, neurological or not, as a cerebrovascular injury or a fracture, in whom the natural course of the disease, assisted by co-adjuvant therapies including rehabilitation, progresses frequently from a considerable initial functional impact to higher levels of independence, ALS patients face the opposite track. From fully independent, patients with initial lower limb (LL) weakness need progressive assistance with gait and balance until being unable to climb stars, walk or maintain posture. Ankle-foot orthosis assist patients with drop foot but progressive disto-proximal LL weakness will require a cane/ walker, until wheelchair and bed confinement ensue. Upper limb (UL) involvement initially limits fine finger-hand motor skills, as needed for handling a needle. Other activities of daily life (ADL) are progressively affected as holding a pen and write, using the cutlery and self-feeding, grabbing a glass and

Abbreviations: ADL, Activities of daily life; AI, Artificial intelligence; ALS, Amyotrophic Lateral Sclerosis; ALSFRS-R, Revised ALS functional rating scale; AAC, Augmentative and alternative communication; AR, Augmented Reality; BCI, Brain-computer interfaces; ECAS, Edinburgh Cognitive and Behavioural ALS Screen; EQoL-5D, European quality of life questionnaire; ENCALS, European Network for the Cure of ALS; ET, Eye tracking; EU, European Union; FVC, Forced vital capacity; GDPR, General Data Privacy Regulation; HAD scale, Hospital Anxiety and Depression scale; MIP, Maximal inspiratory pressure; ML, Machine Learning; QoL, Quality of life; LL, Lower limb; MIE, Mechanical insufflator-exsuflattor; NEALS, Northeast ALS Consortium; NIV, Non-invasive ventilation; PLS, Primary lateral sclerosis; pt, patient; UL, Upper limb; VR, Virtual Reality; WHO, World Health Organization

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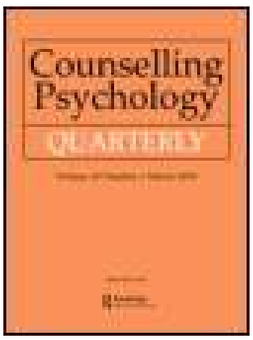

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Telepsychotherapy: a leaflet for psychotherapists in the age of COVID-19. A review of the evidence

Barbara Poletti , Sofia Tagini , Agostino Brugnera , Laura Parolin , Luca Pievani , Roberta Ferrucci , Angelo Compare & Vincenzo Silani

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ARTICLE

Telepsychotherapy: a leaflet for psychotherapists in the age of COVID-19. A review of the evidence

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ABSTRACT

COVID-19 outbreak imposes the adoption of extraordinary containment measures, including the strict necessity to limit interpersonal contact. Face-to-face psychotherapy collides with this requirement and, above all, it might endanger both therapists and patients' safety. Telepsychotherapy might come to the aid, ensuring therapeutic continuity and the possibility to reach people who might benefit of extra psychological support. Infectious outbreaks have been indeed associated with major psychopathological outcomes. The aim of the present work is to review the most recent experimental evidence about telepsychotherapy, focusing on its effectiveness, possible determinants of efficacy and therapists/patients' attitudes, to rapidly inform psychotherapists. Out of the 857 records found, 18 studies have been included in the review. Our results show that, despite therapists and public's skepticism, telepsychotherapy is a trustworthy alternative to be adopted, which can be used efficaciously to treat common mental-health disorders such as anxiety, depression and post-traumatic distress. As well as in the traditional setting, a higher number of sessions and the proper management of patients' expectations seem to be associated with better outcomes. On the contrary, low familiarity with web-based means of communication and technical issues might reduce specifically the effectiveness of telepsychotherapy.

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Introduction

On December 2019, a pneumonia of unknown cause was first reported to the World Health Organization (WHO) in China. Lately, this disease was found to be caused by a new coronavirus (SARS-Cov-2) and was named COVID-19 (World Health Organization, 2020). Since then, the new coronavirus rapidly spread worldwide and on 11 March 2020 the WHO declared COVID-19 outbreak a pandemic. Accordingly, extraordinary containment

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LETTER TO THE EDITOR

Advance care planning and mental capacity in ALS: a current challenge for an unsolved matter

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Dear Editor in Chief,

Recently, a law was approved in Italy, establishing the right for patients to refuse exams and treatments, including the suspension of previously accepted treatments, although assisted suicide and euthanasia remain illegal. Such legal establishment, in line with patients' right to autonomy and self-determination, should be carefully handled in Amyotrophic Lateral Sclerosis (ALS). The progressive and fatal course of the disease and the presence of cognitive/behavioral alterations and frontotemporal dementia (FTD), together with the disrupt of communication abilities, have indeed relevant implications for advanced health directives (AHD). In particular, patients' perspectives regarding medical decision-making and end-of-life interventions may be strongly influenced by cognitive-behavioral aspects [1], with frontal integrity and executive functions playing a crucial role on patients' decisional capacity [2]. Moreover, the paucity of longitudinal studies in ALS lead to a poorly defined scenario about cognitive-behavioral impairment progression due to the absence of screening tools compensating for verbal-motor impairment, leaving this issue still unsolved. Even if a cognitive-behavioral tool has been recently developed and applied to overcome such limitations [3], it does not fully compensate for motor and speech impairment and thus cannot be performed in moderate-severe stages of the disease. Recently, new technologies such as Eye Tracking and Brain Computer Interface have been used to administer neuropsychological tests [4]; however, to date, a full verbal-motor–independent battery specifically designed to assess cognitive components of mental capacity in ALS patients along the course of the disease is not available.

Patients' attitudes towards end-of-life interventions are also influenced by psychological symptoms. An association between refusing an intervention and depression has not been definitely observed in ALS patients [5]; on the contrary, the presence of fear of suffocation and life-sustaining strategies dependency are frequently described in such patients and could play a more relevant role. Recent studies have highlighted the importance of apathy in being compliant with initiative and planning the future [6] and prognosis [7]. Apathy is a prominent symptom of ALS, and it is the most common behavioral change [8]. In recent years, apathy has been mainly studied in a multidimensional way [9], also highlighting the importance of ecological tasks (i.e., planning and goal management), when measuring it. Furthermore, severe apathy revealed to be an independent, negative prognostic factor in ALS patients [7] and associated with lower overall quality of life (QOL) [10]. Additionally, the issue of pseudobulbar effect, important for evaluation of mood, behavior and prognosis needs to be carefully addressed [11].

Both cognitive and behavioral consequences of the disease can have an impact on adherence to treatment, and patients' psychological state could influence ADH decision making. The psychological impact of the disease on patients necessarily leads to the need for psychological support [12]. Indeed, psychological or psychosocial interventions could be supportive for patients and should be taken into account when considering ADH, even if there is still a lack of research on the efficacy of psychological interventions in ALS [13].

These findings highlight the need for adequate and timely patient's information regarding disease's related symptoms along disease, including psychological symptoms other than depression within evaluation of patients' competency in AHD. Finally, loss of insight is an under-considered issue that could play a role on patients' decision-making process and should be measured within ALS patients' current behavioral assessment.

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Chronic stress induces formation of stress granules and pathological TDP-43 aggregates in human ALS fibroblasts and iPSC-motoneurons

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative diseases characterized by the presence of neuropathological aggregates of phosphorylated TDP-43 (P-TDP-43) protein. The RNA-binding protein TDP-43 participates also to cell stress response by forming stress granules (SG) in the cytoplasm to temporarily arrest translation. The hypothesis that TDP-43 pathology directly arises from SG has been proposed but is still under debate because only sub-lethal stress conditions have been tested experimentally so far. In this study we reproduced a mild and chronic oxidative stress by sodium arsenite to better mimic the persistent and subtle alterations occurring during the neurodegenerative process in primary fibroblasts and induced pluripotent stem cell-derived motoneurons (iPSC-MN) from ALS patients carrying mutations in *TARDBP* and *C9ORF72* genes. We found that not only the acute sub-lethal stress usually used in literature, but also the chronic oxidative insult was able to induce SG formation in both primary fibroblasts and iPSC-MN. We also observed the recruitment of TDP-43 into SG only upon chronic stress in association to the formation of distinct cytoplasmic P-TDP-43 aggregates and a significant increase of the autophagy marker p62. A quantitative analysis revealed differences in both the number of cells forming SG in mutant ALS and healthy control fibroblasts, suggesting a specific genetic contribution to cell stress response, and in SG size, suggesting a different composition of these cytoplasmic foci in the two stress conditions. Upon removal of arsenite, the recovery from chronic stress was complete for SG and P-TDP-43 aggregates at 72 h with the exception of p62, which was reduced but still persistent, supporting the hypothesis that autophagy impairment may drive pathological TDP-43 aggregates formation. The gene-specific differences observed in fibroblasts in response to oxidative stress were not present in iPSC-MN, which showed a similar formation of SG and P-TDP-43 aggregates regardless their genotype. Our results show that SG and P-TDP-43 aggregates may be recapitulated in patient-derived neuronal and non-neuronal cells exposed to prolonged oxidative stress, which may be therefore exploited to study TDP-43 pathology and to develop individualized therapeutic strategies for ALS/FTD.

Abbreviations: ALS, amyotrophic lateral sclerosis; ARS, sodium arsenite; FTD, frontotemporal dementia; iPSC-MN, iPSC-derived motoneurons; LCD, low-complexity domain; LLPS, liquid-liquid phase separation; mC9ORF72, mutant C9ORF72; mTDP-43, mutant TARDBP p.A382T; P-TDP-43, phosphorylated TDP-43; RBP, RNA-binding protein; SG, stress granules; TEM, Transmission electron microscopy

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Fiberoptic endoscopic evaluation of swallowing in early-to-advanced stage Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder characterized by motor disturbances, cognitive decline, and behaviour changes. A well-recognized feature of advanced HD is dysphagia, which leads to malnutrition and aspiration pneumonia, the latter being the primary cause of death in HD. Previous studies have underscored the importance of dysphagia in HD patients with moderate-to-advanced stage disease, but it is unclear whether dysphagia affects patients already at an early stage of disease and whether genetic or clinical factors can predict its severity. We performed fiberoptic endoscopic evaluation of swallowing (FEES) in 61 patients with various stages of HD. Dysphagia was found in 35% of early-stage, 94% of moderate-stage, and 100% of advanced-stage HD. Silent aspiration was found in 7.7% of early-stage, 11.8% of moderate-stage, and 27.8% of advanced-stage HD. A strong correlation was observed between disease progression and dysphagia severity: worse dysphagia was associated with worsening of motor symptoms. Dysphagia severity as assessed by FEES correlated with Huntington's Disease Dysphagia Scale scores (a self-report questionnaire specific for evaluating swallowing in HD). The present findings add to our understanding of dysphagia onset and progression in HD. A better understanding of dysphagia onset and progression in HD may inform guidelines for standard clinical care in dysphagia, its recognition, and management.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG expansion in the *IT-15* gene; its prevalence in the Caucasian population is 7–11 per 100,000 (OMIM#143100). HD is characterized by motor, cognitive, and behavioural symptoms that have their onset usually between age 30 and 50 years, after which they slowly progress for 15–20 years until death. Most HD patients with moderate-to-advanced stages complain of swallowing difficulties. Severe dysphagia often leads to aspiration pneumonia, the main cause of death in HD[1]. The natural history of dysphagia in HD remains unclear.

Neuropathological changes in HD include prominent loss of striatal GABAergic neurons and progressive involvement of the cerebral cortex, pallidum, thalamus, brainstem, and cerebellum[2]. Such widespread neurodegeneration results in movement disorders. Besides chorea, the hallmark motor symptom in HD, other typical motor disorders include dystonia, incoordination, Parkinsonism, and ideomotor apraxia. When these heterogeneous movement disorders involve the oropharyngeal musculature, swallowing difficulties ensue[3].

Dysphagia in HD has been investigated by subjective and objective swallowing evaluation tests and described in case reports and case series[1,3–6]. The Huntington's Disease Dysphagia Scale (HDDS), a self-report questionnaire specifically designed to assess swallowing in HD, has demonstrated good construct. In patients with cognitive

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LETTER

Clinical features and outcomes of the flail arm and flail leg and pure lower motor neuron MND variants: a multicentre Italian study

INTRODUCTION

Motor neuron disease (MND) is a heterogeneous group of neurodegenerative disorders defined by a progressive upper motor neuron (UMN) and lower motor neuron (LMN) loss in a varying combination, encompassing a heterogeneous clinical spectrum depending on a different body region involvement at onset, extent and rate of motor neuron (MN) loss and disease spread. Amyotrophic lateral sclerosis (ALS) is the most common and severe form of MND, leading to death in approximately 4 years from symptoms onset. To date, the mainstay neuroprotective therapy is riluzole, despite its limited efficacy, while the role of edaravon is still debated. Phenotypic heterogeneity is increasingly recognised within the MND spectrum, ranging from selective UMN or LMN involvement, to classic ALS, when widespread combination of UMN and LMN dysfunction occurs.[1] The clinical spectrum of MND has been further detailed with the recognition of flail arm (FA), flail leg (FL) and pure lower motor neuron (PLMN) phenotypes, considered to be restricted MND phenotypes characterised by a predominant or selective LMN disease (LMND), when UMN dysfunction is absent or marginal.[1 2] Furthermore, patients with MND can show an extra-motor involvement such as cognitive impairment with the development, in approximately 10%–15% of cases, of frontotemporal dementia.

Few studies have previously focused on these LMN-restricted phenotypes, therefore, the aim of the present study is to retrospectively investigate the differentiating features of FA, FL and PLMN phenotypes in a large Italian MND cohort.

MATERIALS AND METHODS

2648 patients with MND were recruited in 13 Italian ALS referral centres from January 2009 to December 2013 and data collected in a common database, which was cleaned before data analysis. To highlight the distinguishing features of patients with FA, FL and PLMN, the classic and bulbar phenotypes were used as controls. The final dataset consisted of 1944 patients. ALS diagnosis was established in accordance with revised El Escorial criteria (r-EEC) at time of diagnosis; however, a subgroup of patients with FA, FL and PLMN did not fulfil r-EEC and were grouped into the additional category 'unclassified'.[2] Treating neurologists collected a detailed clinical profile of each patient.[3] In a subset of patients, the results of genetic screening for mutations in common ALS-related genes were available. Information concerning non-invasive mechanical ventilation (NIMV), percutaneous endoscopic gastrostomy (PEG), tracheostomy and time of death was collected. Comparisons between groups were assessed using a binary logistic regression for categorical variables and one-way analysis of variance, followed by Bonferroni post-hoc test, for continuous variables. The Kaplan-Meier univariate analysis was carried out to determine the effect of phenotypes on survival and time to King's stage 4 (defined as time from symptoms onset to significant feeding or respiratory failure). Subsequently, we perform a Cox multivariate proportional hazards model corrected for well-known prognostic variables to estimate the proportional HRs of phenotype on survival and time to King's stage 4.[3] P value was set at $p<0.05$.[3] Full description of materials and methods is available in online supplementary file.

RESULTS

Demographic and clinical characteristics of each MND phenotype are summarised in table 1 and online supplementary table 2. We observed a significantly higher male prevalence in LMND compared with bulbar patients (men: FA: 70.9%, FL: 59.5%, PLMN: 64.2% vs bulbar: 41.5%; $p<0.001$); male prevalence was significantly higher in FA also compared with the classic phenotype (56.8%; $p=0.004$) (online supplementary table 3). The mean age at symptom onset was different between groups (FA: 62.3, FL: 63.7, PLMN: 60.6, bulbar: 68.3, classic: 63.1; $p<0.001$). Patients with FA, FL and PLMN exhibited a significantly longer mean diagnostic delay compared with both bulbar and classic patients (FA: 20.3, FL: 18.1, PLMN: 22.3 vs bulbar: 10.9 and classic: 12.5 months; $p<0.001$). LMND phenotypes also exhibited a reduced proportion of patients undergoing PEG compared with both classic and bulbar patients (FA: 20.5%, FL: 14.9%, PLMN: 11.9% vs classic: 30.5% and bulbar: 45.7%; $p<0.001$). No difference emerged in the proportion of NIMV users. Moreover, patients with LMND showed lower rates of comorbid dementia (FA: 5.1%, FL: 2.0%, PLMN: 0% vs bulbar: 10.9% and classic: 7.7%; $p=0.001$). The results of genetic analysis were available for 585 patients (30.1 %) and in 23.6% of cases a variant in one of the major ALS genes analysed was detected (online supplementary table 4). Overall mutation carrier rate was higher among patients with classic-ALS (FA: 10.3%, FL: 10.8%, PLMN: 26.7% vs classic: 30.1% and bulbar: 16.5%; $p<0.001$). Furthermore, the rate of patients harbouring the *C9orf72* expansion was lower across LMND phenotypes (FA: 2.6%, FL: 1.6%, PLMN: 0% vs bulbar: 12.6% and classic: 17.1%; $p=0.001$).

Kaplan-Meier analysis showed a significant survival difference across MND phenotypes ($p<0.001$) (online supplementary figure 1A). Similarly, median time to reach King's stage 4 (online supplementary figure 2A) was significantly different among MND phenotypes ($p<0.001$). Finally, multivariate Cox regression analysis (online supplementary table 1) demonstrated an independent effect of MND phenotypes on both survival and time to reach King's stage 4 (online supplementary figures 1B and 2B).

DISCUSSION

Our study, performed in a large Italian cohort of patients with MND, demonstrates distinguishing clinical and prognostic features of patients with FA, FL and PLMN. These phenotypes differed in terms of gender distribution, age at onset, diagnostic delay, rate of performing PEG and risk of developing dementia. In addition, this group of patients with MND presented a slower disease progression, calculated in terms of survival and time to reach King's stage 4. More specifically, FA, FL and PLMN phenotypes showed a significant gender effect, occurring more frequently in men. Gender distribution was significant in the FA group, in line with previous reports.[1 2 4] Importantly, FA, FL and PLMN phenotypes also showed a longer diagnostic delay and lower rate of definite diagnosis according to r-EEC, reflecting the slower disease progression and mild or absent UMN involvement. The milder central nervous system involvement in these phenotypes is also confirmed by the lower rates of comorbid dementia detected in these groups.

Kaplan-Meier analysis showed longer survival of LMND, consistent with previous reports.[1 2] Interestingly, in

of mutation carriers for the major ALS genes among classic patients, with no differences in the ALS familiarity rate, suggesting that heritability for the patients with FA, FL and PLMN could be related to different genetic factors, even if almost any clinical phenotype has been described as 'inherited-ALS'. Additionally, we noticed a higher rate of bulbar and classic patients harbouring the *C9orf72* repeat expansion.[4]

In summary, we showed that the patients with FA, FL and PLMN present distinguishing features and clinical course compared with bulbar and classic patients. Our results suggest that a detailed phenotypic classification could be important to predict prognosis, for a more individualised approach to patient management and to correctly stratify patients in clinical trials. Furthermore, phenotypic heterogeneity and the disease progression across MND phenotypes could be related to a distinct underlying pathological mechanism or different genetic factors. Further investigations are needed to clarify the pathogenesis underlying each MND spectrum phenotype.

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ORIGINAL RESEARCH

Primary lateral sclerosis: consensus diagnostic criteria

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ABSTRACT

Primary lateral sclerosis (PLS) is a neurodegenerative disorder of the adult motor system. Characterised by a slowly progressive upper motor neuron syndrome, the diagnosis is clinical, after exclusion of structural, neurodegenerative and metabolic mimics. Differentiation of PLS from upper motor neuron-predominant forms of amyotrophic lateral sclerosis remains a significant challenge in the early symptomatic phase of both disorders, with ongoing debate as to whether they form a clinical and histopathological continuum. Current diagnostic criteria for PLS may be a barrier to therapeutic development, requiring long delays between symptom onset and formal diagnosis. While new technologies sensitive to both upper and lower motor neuron involvement may ultimately resolve controversies in the diagnosis of PLS, we present updated consensus diagnostic criteria with the aim of reducing diagnostic delay, optimising therapeutic trial design and catalysing the development of disease-modifying therapy.

INTRODUCTION

Primary lateral sclerosis (PLS) is a characteristically slowly progressive and selective neurodegenerative disorder primarily affecting the adult central motor system. Progressive muscle stiffness leads to an insidious loss of mobility typically with the development of corticobulbar dysfunction, which may be the initial symptom for a minority. Diagnostic criteria for PLS proposed 75 years ago recognised the potential for clinical overlap in the early symptomatic phase with the more common disorder amyotrophic lateral sclerosis (ALS).[1] Like PLS, upper motor neuron (UMN)-predominant ALS has a significantly slower rate of progression compared with classical forms of ALS, with survival frequently extending into a second decade from onset of symptoms.[2] The development of clinically obvious and functionally significant, progressive lower motor neuron (LMN) involvement is inevitable in ALS, in contrast to PLS, but may not emerge for several years from the initial clinical UMN syndrome.[3] As a result, criteria for the definite diagnosis of PLS have enshrined a minimum duration of symptoms, varying from 3 to 5 years.[1 4 5]

Among the earliest reported cases, many of those that were said to have a hereditary component[1] would now be recognised within the spectrum of hereditary spastic paraplegia (HSP). The development of non-invasive neuroimaging has brought further structural, inflammatory and metabolic mimic disorders into consideration (see later). A 'gold standard' *postmortem* histopathological signature for PLS has proved elusive. While neuronal and glial cytoplasmic inclusions of the 43 kDa transactive response DNA-binding protein, TDP-43, are common to 97% of cases of ALS (across disparate monogenetic and apparently sporadic cases), there have been very few *postmortem* studies of PLS in the modern era of immunohistochemistry.[6 7] Debate as to whether PLS represents an extreme end of a continuum with ALS, or a distinct disorder is ongoing.

The clinical imprecision in the diagnosis, along with some uncertainty about overlap with UMN-predominant ALS has become an obstacle to therapeutic development for PLS. As the result of a meeting of international PLS experts (3 May 2019, Philadelphia, Pennsylvania, USA), a working group set forth to create more permissive diagnostic criteria, in an effort to spur therapeutic development and to accelerate research into the basic histopathology of PLS.

The core clinical syndrome

There have been consistent clinical observations reported across multiple case series in PLS.[8] Mean age at symptom onset is around 50 years which is at least a decade earlier than non-familial ALS, and a decade later than HSP. While there have been cases reported with symptoms beginning in childhood, many of those might now be linked to developmental or monogenetically mediated disorders. A male predominance has been consistently noted in PLS (range 2–4:1).

An insidious onset is the rule in PLS, so that individuals are unlikely to reach specialised neurological services soon after the very earliest symptoms. For the majority of patients, symptoms emerge in the lower limbs first, but for a significant minority in the corticobulbar pathways with dysarthria and often prominent emotionality (pseudobulbar affect). Although dysphagia may become marked, the value of gastrostomy is far less clear than in ALS, and the need for non-invasive ventilation in PLS more exceptional. Lower limb involvement in the early symptomatic phase may be articulated as a sense of dysequilibrium or loss of fluidity in gait. Prominent sensory involvement should not be evident. Spasticity with pathological hyperreflexia are invariable examination findings. Although PLS

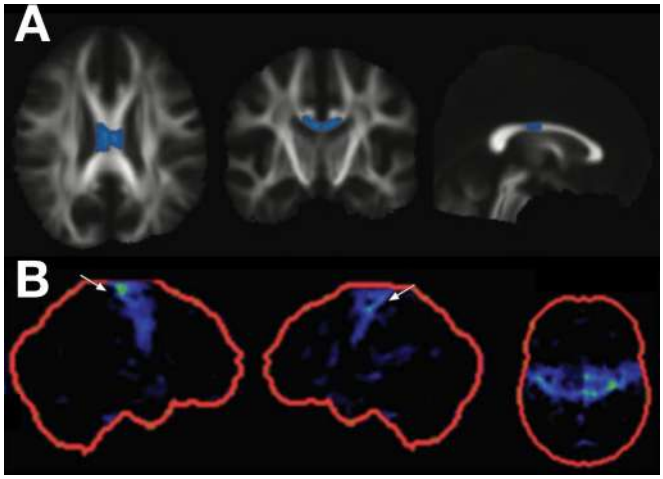

Figure 1 Diffusion tensor imaging and fluorodeoxyglucose (FDG) positron emission tomography (PET) findings in cases of primary lateral sclerosis (PLS). Mean diffusivity in the mid-portion of the corpus callosum is increased in cases of PLS compared with amyotrophic lateral sclerosis (row A, PLS vs ALS group findings highlighted on axial, coronal and sagittal views of the white matter tract skeleton; with kind permission of the author, see Iwata *et al*[37]). Focal hypometabolism may be seen in the primary motor cortices in PLS (row B, FDG PET z-score images in the right sagittal, left sagittal and axial planes; with kind permission of the author, see Claassen *et al*[35]). Neither of these techniques yet has sufficient sensitivity or specificity to be applied in isolation for the diagnosis of PLS.

delay. The development of an international registry that includes all those with 'probable PLS' will allow a more precise delineation of the pathogenesis from ALS, and accelerate therapeutic developments.

Beyond primary disease-modifying therapy, the most pressing unmet need for patients with PLS may well be the treatment of core symptoms rather than extension of survival. The development of more effective relief of spasticity that does not sacrifice muscle strength would have a great impact for those living with PLS, notwithstanding the long-term desire for neuroprotective or regenerative therapy. Recognising that advances in molecular phenotyping may well supersede purely clinical diagnostics, it is hoped that these pragmatic criteria will provide greater confidence to reduce diagnostic delay, thus allowing access to potentially disease-modifying therapies at lower levels of disability.

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Cerebrospinal fluid phosphorylated neurofilament heavy chain and chitotriosidase in primary lateral sclerosis

INTRODUCTION

Primary lateral sclerosis (PLS) is a rare degenerative disease of upper motor neurons (UMNs) manifesting with progressive spasticity. Disease progression is definitely slower than in amyotrophic lateral sclerosis (ALS). Clinical distinction between PLS and ALS can be challenging, as the UMN-dominant form of ALS may sometimes not manifest lower motor neuron (LMN) signs for a long time after symptom onset.[1] Therefore, traditional PLS diagnostic criteria allowed diagnosis only after 3 or 4 years of documented absence of LMN signs,[2] which poses a psychological burden on patients, hinders correct clinical management and prevents enrolment in clinical trials. In order to overcome these issues, new diagnostic criteria have been recently formulated, shortening the time required for a diagnosis of probable PLS to 2 years.[1]

Diagnostic uncertainties in PLS are complicated by the lack of specific neurochemical biomarkers. Whereas cerebrospinal fluid (CSF) neurofilament levels are clearly increased in ALS, making them a well-established ALS biomarker reflecting axonal degeneration, few studies reported lower increases in PLS[3]; a similar pattern was observed for the putative ALS microglial biomarker chitotriosidase (Chit1).[4] Here we measured phosphorylated neurofilament heavy chain (pNFH) and Chit1 in the CSF of patients with PLS, ALS and non-neurodegenerative neurological conditions, focusing on the ability of each biomarker to distinguish PLS from controls and from ALS.

PATIENTS AND METHODS

Patients

In this retrospective study we included those 10 patients (5 men, 5 women) from our consecutive PLS series (n=52) whose CSF was stored in our biobank. They all fulfilled—at the time of sampling or on later evaluations—the recently published diagnostic criteria for definite (n=9) or probable (n=1) PLS.[1] Patients with ALS and neurological controls (NCs) were selected randomly from our biobank to form two cohorts with sex and age distributions similar to PLS. Patients with ALS (n=28; 16 men, 12 women) fulfilled the revised El Escorial diagnostic criteria of definite or probable ALS.[5] NCs (n=30; 13 men, 17 women) had neurological complaints but no evidence of neurodegenerative diseases (online supplemental table 1).

Laboratory markers

Centrifuged CSF samples were stored at −80°C within 2 hours after lumbar puncture. pNFH was measured in all patients; Chit1 was measured in 10 of 10 patients with PLS, 20 of 28 patients with ALS, and 21 of 30 NCs. Commercial ELISAs from Biovendor and MBL, respectively, were used.

Statistical analysis

Comparisons of continuous variables among >2 groups were performed with Kruskal-Wallis test followed by Dunn's multiple comparisons test, whereas Mann-Whitney U test was used for comparisons between two groups. Diagnostic performances of each single biomarker and of combined biomarkers (sum of z-scores of log-transformed values of both biomarkers) were evaluated with receiver operating characteristic (ROC) curves, choosing cut-offs maximising the Youden index. Correlations between continuous variables were analysed with non-parametric Spearman correlation. The distributions of non-continuous variables in different categories were analysed with the χ^2 test. Analysis of survival was performed with Kaplan-Meier curves and log-rank test; the endpoint was death or beginning of invasive ventilation for patients who underwent it. The level of statistical significance for all tests was set at $p<0.05$.

RESULTS

pNFH and Chit1 in the different diagnostic categories

Patient characteristics are summarised in online supplemental table 2. Median levels of both pNFH and Chit1 differed in the three diagnostic categories, with lowest values in NCs and with patients with ALS showing higher values than patients with PLS (figure 1A,B, online supplemental table 3; individual data in online supplemental table 4). For both biomarkers, the Dunn's pairwise comparisons showed significant differences both between ALS and NCs and between PLS and NCs, but not between PLS and ALS. pNFH and Chit1 correlated with each other in patients with ALS and motor neuron disease (MND) (ie, ALS+PLS) (r=0.454 and r=0.581, respectively). Neither pNFH nor Chit1 differed between patients with PLS, ALS and MND with spinal versus bulbar onset. pNFH correlated positively with age at sampling only in NCs (r=0.418) and with progression rate at sampling in patients with ALS (r=0.529) and in patients with MND (r=0.620), whereas it correlated negatively with disease duration at sampling only in patients with MND (r=−0.538). Chit1 showed a positive correlation with age at sampling only in NCs (r=0.452) and with progression rate at sampling only in patients with MND (r=0.504). Neither pNFH nor Chit1 correlated with Penn UMN Score in patients with PLS, ALS or MND. Finally, in patients with ALS, pNFH levels above the median were negatively associated with survival (HR=4.42), which was not observed for Chit1 (online supplementary figure, online supplemental table 5).

Diagnostic performance of pNFH and Chit1

Both pNFH and Chit1 demonstrated a good diagnostic performance in distinguishing patients with ALS, PLS and MND from NCs (area under the curve (AUC) for pNFH: 0.996, 0.933 and 0.980, respectively; AUC for Chit1: 0.981, 0.848 and 0.937, respectively) (figure 1C,D). For the differentiation between PLS and ALS, pNFH had an AUC of 0.771, with the best cut-off providing 67.9% sensitivity and 90.0% specificity. The corresponding AUCs for Chit1 and for the combined biomarkers pNFH+Chit1 were 0.740 and 0.790, respectively (figure 1E, online supplemental table 6). Comparison of the ROC curves of pNFH and Chit1 did not show superiority of either biomarker in any diagnostic discrimination, nor did the combined biomarkers outperform either biomarker alone for the differentiation between ALS and PLS (online supplemental table 7).

DISCUSSION

The main finding of the study is that both pNFH and Chit1 differ in the three diagnostic categories, with PLS showing somewhat intermediate levels between ALS and NCs. Pertaining to pNFH, this could reflect slower degeneration of corticospinal axons in PLS compared with ALS, which is supported by correlation of pNFH with disease progression rate in MND. Similarly, the lower elevation of Chit1 in PLS could reflect a lesser extent of microglial neuroinflammation, which

in turn may be linked to axonal loss, as suggested by the correlation between the two biomarkers. From a clinical standpoint, in addition to confirming the already known clearly raised CSF levels of pNFH and Chit1 in ALS and the prognostic value of pNFH in this disease,[3] our investigation suggests the potential usefulness of both pNFH and Chit1 for the diagnosis of PLS, although given the limited cohort size it should be regarded as exploratory. Even though pNFH showed higher AUCs compared with Chit1, the discriminatory performances of the two biomarkers were not significantly different: this warrants verification in larger cohorts, which could also enable alternative methods of combination of the two biomarkers. Most importantly, effectiveness of pNFH (or Chit1) in discriminating between PLS and ALS would represent a considerable advance towards early diagnosis of PLS: the latter cannot still be fully achieved even after the introduction of the newer diagnostic criteria, which also await clinical and neuropathological validation.[1] Early diagnosis would expedite enrolment of patients in therapeutic trials designed for PLS, whose paucity is one of the main reasons for the current lack of a specific disease-modifying therapy. Notably, while our ALS cohort was a composite population representing different ALS phenotypes, future studies should focus on comparison between PLS and slowly progressive UMN-dominant forms of ALS. Furthermore, the potential role of pNFH and Chit1 in the diagnosis of PLS suggested by our study deserves further investigation in larger cohorts undergoing prospective (including early) and multimodal (clinical, neurochemical, neuroimaging and neurophysiological) evaluations.

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