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 NAZIONALI E INTERNAZIONALI

E

FRONTESPIZI

LAVORI SCIENTIFICI

2021

"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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CLINICAL/SCIENTIFIC NOTES OPEN ACCESS

Homozygous *SOD1* Variation L144S Produces a Severe Form of Amyotrophic Lateral Sclerosis in an Iranian Family

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Abstract

Objectives

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder characterized by degeneration of motor neurons determining progressive muscular atrophy, weakness, and death from respiratory failure.

Methods

Here, we report clinical and molecular findings of a novel Iranian family affected with a severe form of early-onset familial ALS.

Results

Three siblings born to consanguineous parents developed a form of ALS characterized by early-onset lower limb involvement and a fast progression, proving fatal at age 16 years for 1 of them. Molecular analysis of the *SOD1* gene revealed the homozygous substitution c.434T>C in exon 5 resulting in the amino acid change p.Leu144Ser (L144S), previously reported as a dominant variant. Both parents were heterozygous carriers. The probands' mother recently developed a late-onset ALS with predominant upper motor neuron involvement.

Discussion

This report adds p.L144S to the short list of homozygous *SOD1* variants and suggests that the development of an earlier-onset and/or faster disease progression can occur when 2 mutated alleles are present.

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CLINICAL RESEARCH ARTICLE

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Characterization of patients with Becker muscular dystrophy by histology, magnetic resonance imaging, function, and strength assessments

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Abstract

Introduction/Aims: Becker muscular dystrophy (BMD) is characterized by variable disease severity and progression, prompting the identification of biomarkers for clinical trials. We used data from an ongoing phase II study to provide a comprehensive characterization of a cohort of patients with BMD, and to assess correlations between histological and magnetic resonance imaging (MRI) markers with muscle function and strength.

Methods: Eligible patients were ambulatory males with BMD, aged 18 to 65 years (200 to 450 meters on 6-minute walk test). The following data were obtained: function test results, strength, fat-fraction quantification using chemical shift-encoded MRI (whole thigh and quadriceps), and fibrosis and muscle fiber area (MFA) of the brachial biceps.

Results: Of 70 patients screened, 51 entered the study. There was substantial heterogeneity between patients in muscle morphology (histology and MRI), with high fat replacement. Total fibrosis correlated significantly and mostly moderately with all functional endpoints, including both upper arm strength assessments (left and right elbow flexion rho −.574 and −.588, respectively [both $P < .0001$]), as did MRI fat fraction (whole thigh and quadriceps), for example, with four-stair-climb velocity −.554 and −.550, respectively (both $P < .0001$). Total fibrosis correlated significantly and moderately with both MRI fat fraction assessments (.500 [$P = .0003$] and .423 [.0024], respectively).

Discussion: In this BMD cohort, micro- and macroscopic morphological muscle parameters correlated moderately with each other and with functional parameters,

Abbreviations: 6MWT, 6-minute walk test; BMD, Becker muscular dystrophy; CINRG, Cooperative International Neuromuscular Research Group; DMD, Duchenne muscular dystrophy; LOESS, locally estimated scatterplot smoothing; MFA, muscle fiber area; MFM, Motor Function Measure; MRI, magnetic resonance imaging; SSRI, selective serotonin reuptake inhibitor.

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Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with a lifetime risk of one in 350 people and an unmet need for disease-modifying therapies. We conducted a cross-ancestry genome-wide association study (GWAS) including 29,612 patients with ALS and 122,656 controls, which identified 15 risk loci. When combined with 8,953 individuals with whole-genome sequencing (6,538 patients, 2,415 controls) and a large cortex-derived expression quantitative trait locus (eQTL) dataset (MetaBrain), analyses revealed locus-specific genetic architectures in which we prioritized genes either through rare variants, short tandem repeats or regulatory effects. ALS-associated risk loci were shared with multiple traits within the neurodegenerative spectrum but with distinct enrichment patterns across brain regions and cell types. Of the environmental and lifestyle risk factors obtained from the literature, Mendelian randomization analyses indicated a causal role for high cholesterol levels. The combination of all ALS-associated signals reveals a role for perturbations in vesicle-mediated transport and autophagy and provides evidence for cell-autonomous disease initiation in glutamatergic neurons.

ALS is a fatal neurodegenerative disease affecting one in 350 individuals. Due to degeneration of both upper and lower motor neurons, patients suffer from progressive paralysis, ultimately leading to respiratory failure within 3–5 years after disease onset[1]. In ~10% of patients with ALS, there is a clear family history for ALS, suggesting a strong genetic predisposition, and currently a pathogenic mutation can be found in more than half of these cases[2]. On the other hand, apparently sporadic ALS is considered a complex trait for which heritability is estimated at 40–50% (refs. [3,4]). There is no widely accepted definition of familial or sporadic ALS[5], and they are likely to represent the ends of a spectrum with overlapping genetic architectures for which the same genes have been implicated in both familial and sporadic disease[6–11]. To date, partially overlapping GWASs have identified up to six genome-wide significant loci, explaining a small proportion of the genetic susceptibility to ALS[11–16]. Indeed, some of these loci found in GWASs harbor rare variants with large effects also present in familial cases (for example, *C9orf72* and *TBK1*)[6,17,18]. For other loci, the role of rare variants remains unknown.

While ALS is referred to as a motor neuron disease, cognitive and behavioral changes are observed in up to 50% of patients, sometimes leading to frontotemporal dementia (FTD). The overlap with FTD is clearly illustrated by the pathogenic hexanucleotide repeat expansion in *C9orf72*, which causes familial ALS and/or FTD[17,18] and the genome-wide genetic correlation between ALS and FTD[19]. Further expanding the ALS–FTD spectrum, a genetic correlation with progressive supranuclear palsy (PSP) has been described[20]. Shared pathogenic mechanisms between ALS and other neurodegenerative diseases, including common diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), can further reveal ALS pathophysiology and inform new therapeutic strategies.

Here, we combine new and existing individual-level genotype data in the largest GWAS of ALS to date. We present a comprehensive screen for pathogenic rare variants and short tandem repeat (STR) expansions as well as regulatory effects observed in brain cortex-derived RNA sequencing (RNA-seq) and methylation datasets to prioritize causal genes within ALS-risk loci. Furthermore, we reveal similarities and differences between ALS and other neurodegenerative diseases as well as the biological processes in disease-relevant tissues and cell types that affect ALS risk.

Results

Cross-ancestry meta-analysis reveals 15 risk loci for ALS. To generate the largest GWAS of ALS to date, we merged individual-level genotype data from 117 cohorts into six strata matched by genotyping platform. A total of 27,205 patients with ALS and 110,881 control participants of European ancestries passed quality control (including 6,374 newly genotyped cases and 22,526 control participants; Methods and Supplementary Tables 1 and 2). Patients were not selected for a family history of ALS. Through meta-analysis of these six strata, we obtained association statistics for 10,461,755 variants down to a minor allele frequency (MAF) of 0.1% in the Haplotype Reference Consortium resource[21]. We observed moderate inflation of the test statistics ($\lambda_{GC}=1.12$, $\lambda_{1000}=1.003$), and linkage disequilibrium (LD) score regression yielded an intercept of 1.029 (s.e.=0.0073), indicating that the majority of inflation was due to the polygenic signal in ALS (LD score regression (LDSC): $h^2_l=0.028$, s.e.=0.003, $K=350^{-1}$, $P=5.5\times10^{-21}$). The European ancestry analysis identified 12 loci reaching genome-wide significance ($P<5.0\times10^{-8}$; Extended Data Fig. 1). For nine loci, the top SNP or a strong LD proxy ($r^2=0.996$) was present in GWAS of ALS in Asian ancestries (2,407 patients with ALS and 11,775 control participants)[15,16], and all showed a consistent direction of effects ($P_{binom}=2.0\times10^{-3}$). The three SNPs that were not present in the Asian ancestry GWAS were low-frequency variants (MAF of 0.6–1.6% in European ancestries, Table 1). The genetic overlap between ALS risk in European and Asian ancestries resulted in a trans-ancestry genetic correlation of 0.57 (s.e.=0.28) for genetic effect and 0.58 (s.e.=0.30) for genetic impact, which were not statistically significantly different from unity ($P=0.13$ and $P=0.16$, respectively).

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Letter to the Editor

CACNA1S mutation associated with a case of juvenile-onset congenital myopathy

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Dear Editor,

We present the case of an Italian family, whose affected members showed different degrees of muscle involvement and histopathological features compatible with congenital myopathies (CM), in the presence of a rare *CACNA1S* heterozygous mutation. CMs are a heterogeneous group of genetic muscle disorders ranging from severe and life-threatening neonatal manifestations to adult-onset milder forms [1] and classified according to specific histological and ultrastructural muscle biopsy features [2]. Main clinical characteristics include hypotonia and weakness of proximal and facial muscles, often associated with respiratory failure and cardiac involvement [1]. Disease course is slowly progressive and serum creatine-kinase (CK) levels are normal to mildly elevated [1]. Over the last decades, many disease-causing genes have been associated with CMs [2]. Genes belonging to the *CACNA1* family encode for tissue-specific alpha subunits of the voltage-gated calcium channel and have been associated with ataxias, hemiplegic migraine, blindness and deafness [3,4]. *CACNA1S* expressed in skeletal muscle encodes for $Ca_v1.1$ protein, the pore-forming subunit of the dihydropyridine receptor (DHPR) which is coupled to the Ryanodine receptor Ca^{2+}-release channel-1 (RYR1) in muscle excitation-contraction [4,5]. *CACNA1S* mutations have been formerly associated with malignant hyperthermia, hypokalemic and normokaliemic periodic paralysis [4], and, more recently CMs [6–10].

The proband we present, is a single-born 61-year-old woman who developed bilateral palpebral ptosis, progressive symmetric proximal muscular weakness, cramps, hypophonia and obstructive sleep apnea requiring nighttime non-invasive ventilation by the age of 50 years. No clear signs of post-activation exhaustion were demonstrated. No abnormalities were noticed during the neonatal period and childhood. A long-limb constitution, scoliosis and pectus excavatum were firstly noticed during puberty. The disease slowly progressed causing distal muscle weakness without exhaustion after repeated exercise, eyelid ptosis worsened by Simpson's test and dysphagia for liquid foods. CK levels were normal. Antibodies against Acetylcholine Receptor (AChR), Muscle-Specific Kinase (MuSK), titin and ryanodine were absent and the chest computed tomography excluded the presence of thymoma. Pyridostigmine and tizanidine therapies produced partial benefit on cramps. At neurophysiological study needle examination revealed myogenic potentials, whereas motor and sensitive nerves conduction studies, single-fiber electromyography (EMG) and repetitive nerve stimulation testing with low stimulation frequencies (3–5 Hz) of facial, accessory and ulnar nerves were normal.

Skeletal muscle biopsy performed at left biceps brachialis showed mild neurogenic alterations, namely rare nuclear clumps, fiber size variability and few type II angulated and grouped hypotrophic fibers, with normal connective tissue representation and absence of inflammatory cells (Fig. 1A-B). Oxidative enzyme and acid phosphatase activities were normal (Fig. 1C-D). Electron microscopy analysis revealed focal zones of myofibrillar disorganization without alterations of nuclei; the outstanding feature was represented by the streaming or absence of *Z*-disc, with both M-band and other contractile components regularly arranged. Taken together, these features pointed towards a diagnosis of CM [1] (Fig. 1E-F). Muscle magnetic resonance imaging (MRI) showed diffuse hypotrophy, more prominent in the right deltoid muscle, while no muscular inflammation or specific fatty infiltration patterns were observed (Fig. 1G-J), consistent with a previous report of *CACNA1S*-associated CM [6]. Echocardiogram and electrocardiogram exams were normal. No consanguinity was reported through her relatives. The 85-year-old mother of the proband underwent blepharoplasty surgery for palpebral ptosis in her seventies. Since the age of 80, she developed progressive head drop. She never complained of respiratory problems. The only-son of the proband, aged 35 years, is long-limbed and presents a thin constitution, However, he has not developed muscular disturbances and EMG has not revealed myopathic signs so far. No other family members presented neuromuscular signs or symptoms. A wide Next Generation Sequencing (NGS) panel analysis for congenital neuromuscular diseases revealed the heterozygous potentially pathogenic c.3364 T > C, p.Tyr1122His (NM_000069) *CACNA1S* mutation in the proband, confirmed by Sanger sequencing. The identified variant is rare in public databases (Genome Aggregation Database (gnomAD): MAF 1.6×10^{-5}) replaces a highly conserved tyrosine and is predicted as pathogenic by *in silico* tools. The mutation was confirmed by Sanger sequencing in the mother and in the son of the proband. Although not novel, it is present in the gnomAD database only in the Asiatic population, thus suggesting that it might be extremely rare in Caucasians. *CACNA1S* mRNA analysis excluded macrodeletions in the proband. This

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given the increasing involvement of *CACNA1S* in CMs, we propose including this gene in NGS panels aimed to screen these disorders, especially in case of suggestive histopathological and ultrastructural features at muscle biopsy.

Ethic statement

The case report has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Informed written consent was obtained from the participant prior to the inclusion.

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Declaration of Competing Interest

None.

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JAMA Neurology | Original Investigation

Association of Variants in the *SPTLC1* Gene With Juvenile Amyotrophic Lateral Sclerosis

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IMPORTANCE Juvenile amyotrophic lateral sclerosis (ALS) is a rare form of ALS characterized by age of symptom onset less than 25 years and a variable presentation.

OBJECTIVE To identify the genetic variants associated with juvenile ALS.

DESIGN, SETTING, AND PARTICIPANTS In this multicenter family-based genetic study, trio whole-exome sequencing was performed to identify the disease-associated gene in a case series of unrelated patients diagnosed with juvenile ALS and severe growth retardation. The patients and their family members were enrolled at academic hospitals and a government research facility between March 1, 2016, and March 13, 2020, and were observed until October 1, 2020. Whole-exome sequencing was also performed in a series of patients with juvenile ALS. A total of 66 patients with juvenile ALS and 6258 adult patients with ALS participated in the study. Patients were selected for the study based on their diagnosis, and all eligible participants were enrolled in the study. None of the participants had a family history of neurological disorders, suggesting de novo variants as the underlying genetic mechanism.

MAIN OUTCOMES AND MEASURES De novo variants present only in the index case and not in unaffected family members.

RESULTS Trio whole-exome sequencing was performed in 3 patients diagnosed with juvenile ALS and their parents. An additional 63 patients with juvenile ALS and 6258 adult patients with ALS were subsequently screened for variants in the *SPTLC1* gene. De novo variants in *SPTLC1* (p.Ala20Ser in 2 patients and p.Ser331Tyr in 1 patient) were identified in 3 unrelated patients diagnosed with juvenile ALS and failure to thrive. A fourth variant (p.Leu39del) was identified in a patient with juvenile ALS where parental DNA was unavailable. Variants in this gene have been previously shown to be associated with autosomal-dominant hereditary sensory autonomic neuropathy, type 1A, by disrupting an essential enzyme complex in the sphingolipid synthesis pathway.

CONCLUSIONS AND RELEVANCE These data broaden the phenotype associated with *SPTLC1* and suggest that patients presenting with juvenile ALS should be screened for variants in this gene.

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Supplemental content

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fects of each variant on SPTLC1 enzyme-substrate preference,[20] and we observed similar differences in substrate utilization across the variants that we had studied at the enzymatic level (Figure 3A). Alternatively, the phenotypes associated with variants in HSAN1 may represent a continuum between sensory neuropathy and ALS. Future postmortem studies that determine the central nervous system pathology (eg, TAR DNA-binding protein 43, tau, β-amyloid deposition) underlying the motor neuron deficits and the cognitive impairment may resolve the nature of this overlap with other neurodegenerative diseases.

Perturbed sphingolipid metabolism underlies many neurological disorders, such as Niemann-Pick disease and Gaucher disease,[24] and may play a role in the pathogenesis of Alzheimer disease.[25] Sphingolipid metabolism has also been implicated in motor neuron degeneration. For example, patients with partial deficiency of hexosaminidase A enzyme activity (also known as GM2 gangliosidosis, a form of sphingolipidosis) may have clinical manifestations mimicking ALS.[26] The accumulation of ceramides and cholesterol esters also occurs within the spinal cords of patients with ALS and an *SOD1* transgenic mouse model of ALS.[27]

Owing to the poor prognosis observed among patients with juvenile ALS and work published by other groups,[20,28] patient 2 was commenced on high-dose (10 g per day) oral serine supplementation on a compassionate basis. Her body weight increased during this off-label treatment, which was the first time she had gained weight in several years. The patient's ceramide levels were within normal range and trending downwards, indicating that ceramide toxic effects, a theoretical possibility with serine treatment, were not present (eFigure 5 and eTable 5 in the Supplement). We did not observe evidence of neurological improvement, although prolonged therapy would be required to detect such an effect.[29]

Serine is a nonessential amino acid that is available as a low-cost nutritional supplement. A 10% serine-enriched diet was associated with a reduction in neurotoxic deoxysphingolipid plasma levels both in transgenic mice expressing the p.Cys133Trp *SPTLC1* variant and in human patients diagnosed with HSAN1.[28] Furthermore, a safety trial involving 20 patients with adult-onset ALS demonstrated that high doses of oral serine are well tolerated and that this polar amino acid is actively transported across the blood-brain barrier.[30] Nutritional supplementation has proven to be remarkably effective in other forms of ALS.[31] Despite these supportive data, future clinical trials are needed to determine the effectiveness and safety profile of serine supplementation in patients with juvenile ALS owing to *SPTLC1* variants.

Limitations

Our study had limitations. DNA was not available from the parents of patient 4, so it was not possible to determine whether or not the variation arose spontaneously. Nevertheless, the lack of a family history supports the possibility that this variant was de novo in origin; there is only a 3.1% chance that none of her 5 siblings would have inherited an autosomal-dominant variant from a transmitting parent. Our evidence also demonstrates that variants in *SPTLC1* are not a common cause of adult-onset ALS. Overall, our data imply that the genetic causes of juvenile ALS and adult-onset ALS are distinct.

Conclusions

In conclusion, our data broaden the phenotype associated with variants in *SPTLC1* to include juvenile ALS and implicate sphingolipid metabolism as a pathway in motor neuron disease. Our findings are relevant in light of the fact that nutritional supplementation with serine has been postulated to ameliorate the toxic effect of abnormal sphingolipid metabolites if instituted at an early stage in the disease.[28] In such cases, abnormal plasma metabolites could be used as a marker of target engagement.[32] This provides an early opportunity for future clinical trials to test the precision medicine approach in an otherwise fatal neurodegenerative disease.

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BRIEF COMMUNICATION

Co-occurrence of *DMPK* expansion and *CLCN1* mutation in a patient with myotonia

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Abstract

Introduction Myotonic disorders are a group of diseases affecting the muscle, in different ways. Myotonic dystrophy type 1 (DM1) is related to (CTG)n expansion in the 3-untranslated region of the dystrophia myotonica protein kinase (DMPK) gene and is the most frequent and disabling form, causing muscular, visibility, respiratory, and cardiac impairment. Non-dystrophic myotonias (NDMs) affect the skeletal muscle alone. In particular, mutations in the chloride channel (CLCN1) gene cause myotonia congenita (MC), which can have autosomal dominant or recessive inheritance.

Case report We describe a patient with a family history of asymptomatic or paucisymptomatic myotonia, who presented handgrip myotonia which sharply reduced after mexiletine administration. Molecular analysis showed both a paternally inherited DMPK expansion and a maternally inherited CLCN1 mutation.

Conclusions Only one other similar case was reported so far; however, the segregation of the two mutations and the characteristics of the muscle were not studied. Since our patient lacked the classical phenotypical and muscle histopathological characteristics of DM1 and showed mild splicing alterations despite a pathogenic DMPK expansion and the nuclear accumulation of toxic RNA, we may speculate that the co-occurrence of a CLCN1 mutation could have attenuated the severity of DM1 phenotype.

Keywords Myotonia · Myotonic dystrophy type 1 · Myotonia congenita · *DMPK* · *CLCN1*

Myotonic disorders have as common feature the presence of delayed muscle relaxation due to increased muscle excitability. Both dystrophic and non-dystrophic myotonias are known. Myotonic dystrophies are multisystem disorders in which myotonia is very often accompanied by muscle atrophy, cataract, respiratory and cardiac involvement, and endocrine disturbances. The non-dystrophic myotonias (NDMs) exclusively affect the skeletal muscle. Herein, we report a double-troubled patient clinically presenting with handgrip myotonia and carrying both a paternally inherited *DMPK* expansion and a maternally inherited *CLCN1* mutation.

Highlights
- We describe a myotonic patient harboring both a *DMPK* expansion and a *CLCN1* mutation.
- Both clinical phenotype and bioptic findings were mild.
- *CLCN1* mutation could have mitigated DM1 phenotype.

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A 21-year-old woman (proband) presented with a 2-year history of myotonia involving hand muscles and occasionally masticatory muscles. Neurological examination did not show alterations apart from handgrip myotonia with warm-up phenomenon. Ophthalmological evaluation did not reveal lens opacities. Routine blood tests, electrocardiogram, and echocardiogram were normal. Electromyography (EMG) demonstrated abundant myotonic discharges in proximal and distal muscles, in absence of myopathic changes. Family history revealed cataract in the 52-year old father, while the 51-year old mother was asymptomatic; however, a 53-year old maternal aunt had been clinically diagnosed with myotonia during pregnancy because of impaired muscle relaxation, which disappeared after delivery. Thus, we evaluated all three relatives: while clinical examination was completely normal, EMG uncovered myotonic discharges in all of them. Therefore, we performed molecular analysis of the genes responsible for myotonia in all subjects. Results obtained on blood samples confirmed our suspicion (Fig. 1A): the proband carried both a *DMPK* expansion

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BRIEF COMMUNICATION

p.Asn1180Ile mutation of SCN4A gene in an Italian family with myopathy and myotonic syndrome

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Abstract

Introduction Mutations of the skeletal muscle sodium channel gene *SCN4A* are associated with several neuromuscular disorders including hyper/hypokaliemic periodic paralysis, paramyotonia congenita and sodium channel myotonia. These disorders are distinguished from dystrophic myotonias by the absence of progressive weakness and extramuscular systemic involvement.

Methods We present an Italian family with 2 subjects carrying a p.Asn1180Ile mutation in *SCN4A* gene showing a peculiar clinical picture characterized by the association of myopathic features and myotonia.

Results The clinical, electromyographic and histological findings of these patients are reported. The possible pathogenicity of the mutation was tested by three different software, all giving positive results.

Discussion This is the first report of a dominant, heterozygous mutation in *SCN4A* causing a complex phenotype of non-congenital myopathy and myotonic syndrome. We suggest that, in patients with myotonia and myopathy not related to dystrophic myotonias, the sequence analysis of *SCN4A* gene should be performed.

Keywords Myotonia · Myopathy · Channellopathies · SCN4A · Mutation

Introduction

Mutations of the skeletal muscle sodium channel gene (*SCN4A*, sodium voltage-gated channel alpha subunit 4, $Na_v1.4$; OMIM: 603,967;17q23.3) are associated with several neuromuscular disorders including hyper/hypokaliemic periodic paralysis, paramyotonia congenita and sodium channel myotonia. These disorders are distinguished from dystrophic myotonias mainly by the absence of progressive muscle wasting and extramuscular systemic involvement [1, 2]. We present an Italian family with 2 subjects carrying a mutation in SCN4A gene showing a peculiar phenotype characterized by the association of myopathy and myotonia.

Case report

The case index (I-1) is a 76-year-old man hospitalized for a syncope due to torsade des pointes. During his stay in cardiology, hypokinetic cardiopathy (ejection fraction 30%), severe atrio-ventricular block and elongated QT were documented. General examination disclosed a peculiar aspect with elongated face, bilateral ptosis and horizontal smile. Neurological examination showed bifacial, neck flexor (3 Medical Research Council (MRC) grading system), proximal (3 MRC) and distal (4 MRC) limb weakness. Distal sensory deficit, predominantly involving touch and vibration, was also contemporary present. Deep tendon reflexes

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CLINICAL RESEARCH ARTICLE

MUSCLE&NERVE WILEY

Impact of COVID-19 on the quality of life of patients with neuromuscular disorders in the Lombardy area, Italy

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Abstract

Introduction:/Aims: Patients with neuromuscular disorders (NMDs), including many elderly, immunosuppressed, and disabled individuals, may have been particularly affected during the coronavirus disease 2019 (COVID-19) pandemic in Lombardy, a COVID-19 high-incidence area between February and May 2020. We aimed to evaluate the effects of the COVID-19 pandemic on the quality of life (QoL) and perceived disease burden of this group of patients.

Methods: We conducted a cross-sectional phone-based survey study between June 1 and June 14, 2020, on a sample of 240 NMD patients followed at our clinic in Milan, Italy. We asked about perceived NMD burden and QoL before and during the COVID-19 pandemic. We collected responses on access to outpatient care and ancillary services. We investigated the presence of symptoms suggestive of COVID-19 infection and confirmed cases.

Results: We collected 205 responses: 53 patients (25.9%) reported a subjective worsening of the underlying NMD. QoL measures showed a significant worsening between pre and pandemic time frames (odds ratio, 2.14 95%; confidence interval, 1.82–2.51). Outpatient visits were postponed in more than half of cases (57.1%), with 104 patients (50.7%) experiencing a cancellation of scheduled diagnostic tests. 79 patients (38.5%) reported at least one symptom attributable to COVID-19 infection. Among the 10 patients tested with nasopharyngeal swabs, 6 tested positive and 3 died from respiratory failure, including 2 patients on corticosteroid/ immunosuppressive therapy.

Abbreviations: ADLs, activities of daily living; ALS, amyotrophic lateral sclerosis; CI, confidence interval; CIDP, chronic inflammatory demyelinating polyneuropathy; COVID-19, coronavirus disease 2019; IQR, interquartile range; MG, myasthenia gravis; MMN, multifocal motor neuropathy; MNDs, motor neuron diseases; MRC, Medical Research Council; NIV, non-invasive ventilation; NM, necrotizing myopathy; NMDs, neuromuscular disorders; OPMD, oculopharyngeal muscular dystrophy; OR, odds ratio; PEG, percutaneous endoscopic gastrostomy; QoL, quality of life; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Delia Gagliardi and Gianluca Costamagna contributed equally to the work.
Stefania Corti and Giacomo Pietro Comi contributed equally to the work.

frontiers in Neurology

BRIEF RESEARCH REPORT
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Early Findings in Neonatal Cases of *RYR1*–Related Congenital Myopathies

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Ryanodine receptor type 1-related congenital myopathies are the most represented subgroup among congenital myopathies (CMs), typically presenting a central core or multiminicore muscle histopathology and high clinical heterogeneity. We evaluated a cohort of patients affected with Ryanodine receptor type 1-related congenital myopathy (*RYR1*-RCM), focusing on four patients who showed a severe congenital phenotype and underwent a comprehensive characterization at few months of life. To date there are few reports on precocious instrumental assessment. In two out of the four patients, a muscle biopsy was performed in the first days of life (day 5 and 37, respectively) and electron microscopy was carried out in two patients detecting typical features of congenital myopathy. Two patients underwent brain MRI in the first months of life (15 days and 2 months, respectively), one also a fetal brain MRI. In three children electromyography was performed in the first week of life and neurogenic signs were excluded. Muscle MRI obtained within the first years of life showed a typical pattern of *RYR1*-CM. The diagnosis was confirmed through genetic analysis in three out of four cases using Next Generation Sequencing (NGS) panels. The development of a correct and rapid diagnosis is a priority and may lead to prompt medical management and helps optimize inclusion in future clinical trials.

Keywords: congenital myopathy, *RyR1*, fetal brain MRI, neonatal, muscle MRI, muscle biopsy

INTRODUCTION

Ryanodine receptor type 1-related congenital myopathies (*RYR1*-RCM) are the most represented subgroup among congenital myopathies (CM) and are associated with mutations in *RYR1* (1–3). *RYR1* is a large gene encoding calcium-(Ca2+) channel (RyR1), which is a homotetrameric protein embedded in the sarcoplasmic reticulum membrane of skeletal muscle (4, 5). Mutations in *RYR*1 can be extremely heterogeneous even in patients showing the same genotype (6–8). *RYR1*-RCM manifests with symptoms ranging from perinatal onset with floppy baby syndrome to late-onset

PLOS ONE

RESEARCH ARTICLE

North Star Ambulatory Assessment changes in ambulant Duchenne boys amenable to skip exons 44, 45, 51, and 53: A 3 year follow up

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Abstract

Introduction

The aim of this study was to report 36-month longitudinal changes using the North Star Ambulatory Assessment (NSAA) in ambulant patients affected by Duchenne muscular dystrophy amenable to skip exons 44, 45, 51 or 53.

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ORIGINAL PAPER

Missense mutations in small muscle protein X-linked (*SMPX*) cause distal myopathy with protein inclusions

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Abstract
Using deep phenotyping and high-throughput sequencing, we have identified a novel type of distal myopathy caused by mutations in the Small muscle protein X-linked (*SMPX*) gene. Four different missense mutations were identified in ten patients from nine families in five different countries, suggesting that this disease could be prevalent in other populations as well. Haplotype analysis of patients with similar ancestry revealed two different founder mutations in Southern Europe and France, indicating that the prevalence in these populations may be higher. In our study all patients presented with highly similar clinical features: adult-onset, usually distal more than proximal limb muscle weakness, slowly progressing over decades with preserved walking. Lower limb muscle imaging showed a characteristic pattern of muscle involvement and fatty degeneration. Histopathological and electron microscopic analysis of patient muscle biopsies revealed myopathic findings with rimmed vacuoles and the presence of sarcoplasmic inclusions, some with amyloid-like characteristics. In silico predictions and subsequent cell culture studies showed that the missense mutations increase aggregation propensity of the SMPX protein. In cell culture studies, overexpressed SMPX localized to stress granules and slowed down their clearance.

Keywords X-linked · Distal myopathy · Proteinopathy · Amyloidogenesis · Stress granules

Introduction

Distal myopathies are a clinically, histopathologically and genetically heterogeneous group of inherited skeletal muscle diseases. In some entities the muscle weakness remains restricted to the distal muscles, and in others involvement of the proximal muscles may occur as the disease progresses [26]. Over the years, deep phenotyping, including muscle imaging and histopathological evidence, and molecular genetics have resulted in the identification of more than 25 genes related to distal myopathies [33]. Advancements in high-throughput sequencing (HTS) have increased the rate of molecular diagnosis for families with inherited rare neuromuscular disorders. However, over 40% of patients, in particular singletons, still remain without conclusive molecular diagnosis often due to lack of sufficient family data and material [12, 32]. To consider pathogenicity of previously unknown genes as causative for a neuromuscular disease, either more than one family with similar phenotype or a very large family is needed.

Using deep phenotyping, HTS and subsequent functional studies, we describe here a novel adult-onset distal myopathy observed in ten patients sharing common clinical features, characteristic muscle imaging (MRI) features, histopathological findings and missense variants in the novel myopathy gene *SMPX*.

SMPX (also known as Chisel, CSL) is a proline-rich protein of 88 amino acids (9 kDa). It is predominantly expressed in skeletal muscles and heart, with a costameric and intermyofibrillar localization and highest expression in slow muscle fibers [29]. When overexpressed in mouse myoblasts, it associates with focal adhesion proteins, promotes myoblast fusion, and modulates actin turnover and cell shape upstream of Rac1 and p38 [29, 35]. While mutations causing total

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ANNALS of Clinical and Translational Neurology
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CASE STUDY

Charcot–Marie–Tooth disease type 2F associated with biallelic *HSPB1* mutations

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Elena Abati, Stefania Magri, and Megi Meneri equally contributed to the work; Davide Pareyson, Franco Taroni, and Stefania Corti equally contributed to the work.

Abstract

Objective: This work aims to expand knowledge regarding the genetic spectrum of *HSPB1*-related diseases. *HSPB1* is a gene encoding heat shock protein 27, and mutations in *HSPB1* have been identified as the cause of axonal Charcot–Marie–Tooth (CMT) disease type 2F and distal hereditary motor neuropathy (dHMN). **Methods**: Two patients with axonal sensorimotor neuropathy underwent detailed clinical examinations, neurophysiological studies, and next-generation sequencing with subsequent bioinformatic prioritization of genetic variants and in silico analysis of the likely causal mutation. **Results**: The *HSPB1* p.S135F and p.R136L mutations were identified in homozygosis in the two affected individuals. Both mutations affect the highly conserved alpha-crystallin domain and have been previously described as the cause of severe CMT2F/dHMN, showing a strictly dominant inheritance pattern. **Interpretation**: Thus, we report for the first time two cases of biallelic *HSPB1* p.S135F and p.R136L mutations in two families.

Introduction

Charcot–Marie–Tooth (CMT) disease is a spectrum of primary hereditary sensorimotor neuropathies with an overall prevalence of 1/1,200–2,500, making it the most common genetic neuromuscular disorder.[1] CMTs are classified according to their neurophysiological properties and inheritance pattern.[1] Motor nerve conduction velocity (MNCV) allows to distinguish demyelinating CMT type 1 (slow MNCV) from axonal CMT type 2 (preserved MNCV).[1] Both these forms mainly display autosomal dominant transmission, although recessive inheritance

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Review

Advancing Drug Discovery for Neurological Disorders Using iPSC-Derived Neural Organoids

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Abstract: In the last decade, different research groups in the academic setting have developed induced pluripotent stem cell-based protocols to generate three-dimensional, multicellular, neural organoids. Their use to model brain biology, early neural development, and human diseases has provided new insights into the pathophysiology of neuropsychiatric and neurological disorders, including microcephaly, autism, Parkinson's disease, and Alzheimer's disease. However, the adoption of organoid technology for large-scale drug screening in the industry has been hampered by challenges with reproducibility, scalability, and translatability to human disease. Potential technical solutions to expand their use in drug discovery pipelines include Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) to create isogenic models, single-cell RNA sequencing to characterize the model at a cellular level, and machine learning to analyze complex data sets. In addition, high-content imaging, automated liquid handling, and standardized assays represent other valuable tools toward this goal. Though several open issues still hamper the full implementation of the organoid technology outside academia, rapid progress in this field will help to prompt its translation toward large-scale drug screening for neurological disorders.

Keywords: induced pluripotent stem cells (iPSCs); brain organoids; CRISPR-Cas9; drug discovery; disease modeling; neurological diseases; machine learning; single-cell sequencing; bioengineering; organoid imaging

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1. Introduction

Organoids are stem cell-derived, three-dimensional (3D) cultures that are artificially generated. Organoids contain different cell types that self-organize through cell-sorting and spatially restricted lineage commitment, similarly to in vivo organs [1]. Different cell types have been used to generate organoids in vitro, including primary cultured cells from human tissues, embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) [2]. iPSC cultures have provided invaluable information for modeling neurological and neuromuscular disorders [3,4]. However, organoids present some advantages over traditional two-dimensional cultures. They exhibit near-physiologic cellular composition, and they can grow extensively in culture while maintaining genomic stability [5,6], making them potentially valuable for high-throughput screenings [7]. Compared with animal models, organoids can reduce experimental complexity and allow the study of human development features that may be difficult to investigate in animal models. Examples of 3D cultures recapitulating human organs in vitro include the peripheral nerve, [8] the spinal cord [9], and the brain [1]. These neural organoids proved to be useful platforms to model neurodevelopmental, neuropsychiatric [10], and neurodegenerative disorders [11], such as microcephaly [12], Miller-Dieker Syndrome [13], and Alzheimer's disease [14].

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The nonsense mutation stop+4 model correlates with motor changes in Duchenne muscular dystrophy

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Abstract

The aim was to assess 3-year longitudinal data using 6MWT in 26 ambulant boys affected by DMD carrying nonsense mutations and to compare their results to other small mutations. We also wished to establish, within the nonsense mutations group, patterns of change according to several variables. Patients with nonsense mutations were categorized according to the stop codon type newly created by the mutation and also including the adjacent 5′ (upstream) and 3′ (downstream) nucleotides. No significant difference was found between nonsense mutations and other small mutations ($p > 0.05$) on the 6MWT. Within the nonsense mutations group, there was no difference in 6MWT when the patients were subdivided according to: Type of stop codon, frame status of exons involved, protein domain affected. In contrast, there was a difference when the stop codon together with the 3′ adjacent nucleotide ("stop+4 model") was considered ($p < 0.05$) with patients with stop codon TGA and 3′ adjacent nucleotide G (TGA*G*) having a more rapid decline. Our finding suggest that the stop+4 model may help in predicting functional changes. This data will be useful at the time of interpreting the long term follow up of patients treated with Ataluren that are becoming increasingly available.

Keywords: Duchenne; Nonsense mutation; Stop+4 model.

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IM - ORIGINAL

Clinical features and disease course of patients with acute ischaemic stroke just before the Italian index case: Was COVID-19 already there?

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Abstract

Since the end of February 2020, Italy has suffered one of the most severe outbreaks of coronavirus disease 2019 (COVID-19). However, what happened just before the Italian index case has not yet been investigated. To answer this question, we evaluated the potential impact of COVID-19 on the clinical features of a cohort of neurological inpatients admitted right before the Italian index case, as compared to the same period of the previous year. Demographic, clinical, treatment and laboratory data were extracted from medical records. The data collected included all inpatients who had been admitted to the Neurology and Stroke Units of the Ospedale Maggiore Policlinico, Milan, Italy, from December 15, 2018 to February 20, 2019 and from December 15, 2019 to February 20, 2020. Of the 248 patients, 97 subjects (39.1%) were admitted for an acute cerebrovascular event: 46 in the 2018/2019 period (mean [SD] age, 72.3 [15.6] years; 22 men [47.8%]), and 51 in the 2019/2020 interval (mean [SD] age, 72.8 [12.4] years; 24 men [47.1%]). The number of cryptogenic strokes has increased during the 2019–2020 year, as compared to the previous year (30 [58.8%] vs. 18 [39.1%], $p=0.05$). These patients had a longer hospitalization (mean [SD] day, 15.7 [10.5] days vs. mean [SD] day, 11.7 [7.2] days, $p=0.03$) and more frequent cerebrovascular complications (9 [30.0%] vs. 2 [11.1%]), but presented a lower incidence of cardiocerebral risk factors (18 [60.0%] vs. 14 [77.8%]). Right before the Italian index case, an increase in cryptogenic strokes has occurred, possibly due to the concomitant COVID-19.

Keywords COVID-19 · Acute ischaemic stroke · Cryptogenic stroke · Cerebrovascular events

Introduction

Since February 20, 2020, Italy, and in particular its most populous region, Lombardy, has suffered one of the most severe outbreaks of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of December 10, 2020, in this region, inhabited by about 10,000,000 people, the cases registered by the authorities were more than 430,000 and the total deaths were about 23,000 [1].

Early molecular epidemiological tracing suggests that COVID-19 spread in Lombardy weeks before the first reported cases of infection, confirming that SARS-CoV-2 virus entered northern Italy as early as January, 2020 [2]. A very recent paper confirmed this finding, describing the earliest evidence of SARS-CoV-2 RNA in a patient in Lombardy in early December, 2019, 3 months before Italy's first reported COVID-19 case [3].

The clinical manifestations of COVID-19 mostly affect the respiratory system, configuring varied clinical pictures ranging from mild upper respiratory tract illness to severe pneumonia with respiratory failure and death [4, 5]. Asymptomatic infections are reported [6]. More recently,

The members of the Neurology-COVID-19 Group was processed under acknowledgements section.

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A Novel Homozygous *VPS11* Variant May Cause Generalized Dystonia

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In this work, we describe the association of a novel homozygous *VPS11* variant with adult-onset generalized dystonia, providing a detailed clinical report and biological evidence of disease mechanism. Vps11 is a subunit of the homotypic fusion and protein sorting (HOPS) complex, which promotes the fusion of late endosomes and autophagosomes with the lysosome. Functional studies on mutated fibroblasts showed marked lysosomal and autophagic abnormalities, which improved after overexpression of the wild type Vps11 protein. In conclusion, a deleterious *VPS11* variant, damaging the autophagic and lysosomal pathways, is the probable genetic cause of a novel form of generalized dystonia.

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Dystonia is a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions causing abnormal movements and/or postures. If the trunk and at least 2 other sites are involved, dystonia is defined as generalized.[1] Inherited dystonias can be classified as isolated (dystonia is the only motor feature, except for tremor), combined (dystonia is associated with other movement disorders), or complex (dystonia co-occurs with other neurologic or systemic manifestations).[2]

Typically, isolated and combined dystonia have not characteristic features at brain magnetic resonance imaging (MRI). On the other hand, complex dystonia often shows pathognomonic MRI changes. Indeed, complex dystonia is one of the most frequent clinical presentations of neurodegeneration with brain iron accumulation (NBIA), which is a group of genetic disorders displaying progressive iron accumulation in basal ganglia.[2]

Vps11 aggregates with other Vps proteins (ie, Vps16, Vps18, Vps33, Vps41, and Vps39) to form the "homotypic fusion and protein sorting complex (HOPS)." The HOPS complex promotes the fusion of late endosomes and autophagosomes with lysosomes.[3,4]

Homozygous *VPS11* mutations were associated with hypomyelinating leukodystrophy 12 (HLD12), characterized by appendicular spasticity, truncal hypotonia, opisthotonic posturing, and seizures. Brain MRIs of affected subjects present a thin corpus callosum and diffused hypomyelination. Two homozygous mutations were described so far (c.2536T>G p.C846G and c.1158_1184del p.L387-G395del; Fig 1A).[5–7]

Here, we describe a novel homozygous *VPS11* variant probably causative of adult-onset generalized dystonia. We provide strong evidence of variant deleteriousness and demonstrate its highly damaging impact on the autophagy-lysosomal pathway.

Materials and Methods

Clinical Data

The subject underwent several neurological examinations, brain MRI, and neurophysiological studies. Blood samples and a skin biopsy were collected. The Ethics Committee of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) approved the study. Written informed consent for

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Neuron

Article

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

Graphical Abstract

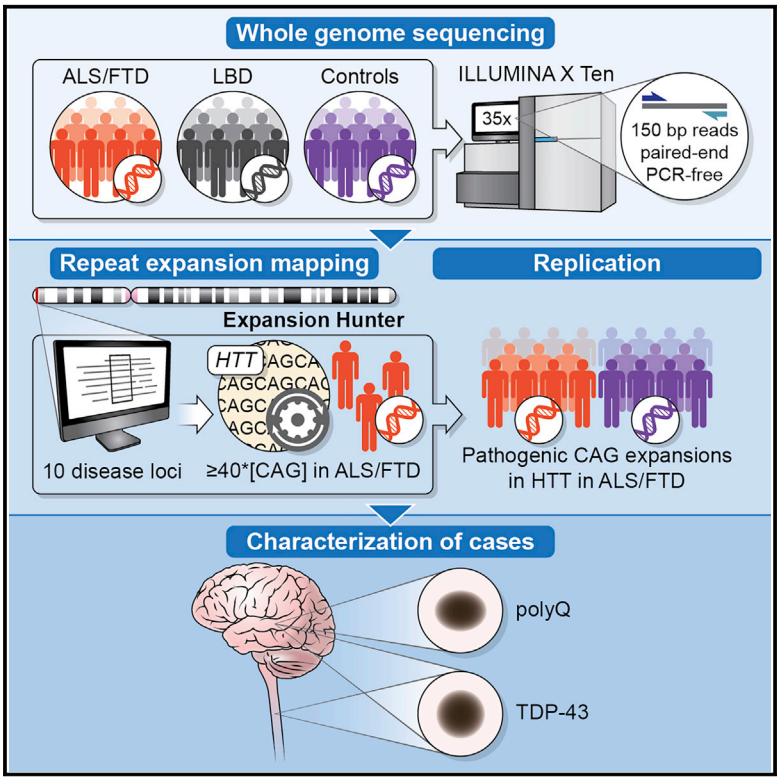

Figure360▷ For a Figure360 author presentation of this figure, see https://doi.org/10.1016/j.neuron.2020.11.005.

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

Authors

Ramita Dewan, Ruth Chia, Jinhui Ding, ..., Clifton L. Dalgard, Sonja W. Scholz, Bryan J. Traynor

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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

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

Gianluca Costamagna[1] · Elena Abati[1] · Nereo Bresolin[1,2] · Giacomo Pietro Comi[1,3] · Stefania Corti[1,2]

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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

Gianluca Costamagna and Elena Abati have equally contributed to this work.

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SHORT REPORT

CLINICAL GENETICS WILEY

Improving clinical interpretation of five *KRIT1* and *PDCD10* intronic variants

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Abstract

Cerebral cavernous malformation (CCM) is a vascular malformation of the central nervous system which may occur sporadically or segregate within families due to heterozygous variants in *KRIT1/CCM1*, MGC4607/*CCM2* or *PDCD10/CCM3*. Intronic variants are not uncommon in familial CCM, but their clinical interpretation is often hampered by insufficient data supporting in silico predictions. Here, the mRNA analysis for two intronic unpublished variants (*KRIT1* c.1147-7 T > G and *PDCD10* c.395 + 2 T > G) and three previously published variants in *KRIT1* but without data supporting their effects was carried out. This study demonstrated that all variants can induce a frameshift with the lack of residues located in the C-terminal regions and involved in protein–protein complex formation, which is essential for vascular homeostasis. These results support the introduction of mRNA analysis in the diagnostic pathway of familial CCM and expand the knowledge of abnormal splicing patterning in this disorder.

KEYWORDS
cerebral cavernous malformations, *KRIT1*, mRNA analysis, *PDCD10*, splicing

1 | INTRODUCTION

Cerebral cavernous malformation (CCM, OMIM #116860) is a disorder of the brain microvasculature characterized by severe endothelial dysfunction manifesting with intracerebral hemorrhage, seizures and headache. Familial CCM (FCCM) is inherited following an autosomal dominant pattern due to heterozygous deleterious variants in *KRIT1/CCM1*, *MGC4607/CCM2* or *PDCD10/CCM3*.[1] Loss-of-function is the prevalent molecular mechanism in FCCM.[2] More rarely, FCCM occurs in combination with missense and in-frame small

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ORIGINAL ARTICLE

WILEY

Diagnostic and prognostic value of CSF neurofilaments in a cohort of patients with motor neuron disease: A cross-sectional study

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Abstract

Motor neuron disease (MND) is a rare group of disorders characterized by degeneration of motor neurons (MNs). The most common form of MND, amyotrophic lateral sclerosis (ALS), is an incurable disease with a variable rate of progression. The search of robust biomarkers able to discriminate among different ALS forms is paramount to properly stratify patients, and to identify those who could most likely benefit from experimental therapies. Phosphorylated-neurofilament heavy chain (p-NfH) and neurofilament light chain (NfL) are neuron-specific components of the cytoskeleton and may represent reliable markers of neuronal injury in neurological disorders. In this study, we described our cohort of ALS patients in order to investigate whether and how cerebrospinal fluid (CSF) p-NfH and NfL levels may reflect progression rate, MN involvement and the extent of neurodegeneration. CSF p-NfH and NfL were significantly increased in ALS compared with healthy and disease controls, including patients with other forms of MND, and were higher in patients with more aggressive disease course, reflecting progression rate. We also evaluated neurofilament diagnostic accuracy in our centre, identifying with high sensitivity and 100% specificity cut-off values of 0.652 ng/mL for CSF p-NfH ($P < .0001$) and of 1261 pg/mL for NfL ($P < .0001$) in discriminating ALS from healthy controls. CSF neurofilaments were significantly correlated with ALS progression rate. Overall, CSF neurofilaments appear to reflect the burden of neurodegeneration in MND and represent reliable diagnostic and prognostic biomarkers in ALS.

KEYWORDS

amyotrophic lateral sclerosis, biomarkers, cerebrospinal fluid, motor neuron disease, neurofilaments, spinal muscular atrophy

Delia Gagliardi and Irene Faravelli These authors have equally contributed to this work.

frontiers in Neurology

BRIEF RESEARCH REPORT
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Sodium Levels Predict Disability at Discharge in Guillain-Barré Syndrome: A Retrospective Cohort Study

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Guillain-Barré syndrome (GBS) is an inflammatory polyradiculopathy with potentially severe complications. Clinical tools for risk stratification have been developed, but no definitive prognostic biomarker has been reported. Hyponatremia is frequent in GBS patients, but the impact of serum sodium levels on clinical outcomes is still ill-defined. In this retrospective cohort study, we included all adult patients diagnosed with GBS spectrum disorders at our center from January 2010 to July 2020. Disability at discharge was assessed with the GBS Disability Score (GDS), and all clinical and laboratory data was retrieved from medical charts. Thirty (58.8%) of the 51 subjects included in the study were discharged with severe residual disability (GDS $\geq$ 3). After accounting for relevant confounders, the odds of experiencing severe disability decreased by 27% ($p = 0.027$) for each unitary increase in serum sodium concentration. Thirteen (25.5%) patients were diagnosed with mild to moderate hyponatremia; the use of intravenous immune globulin (IVIG) independently increased the odds of developing hyponatremia. In conclusion, we found a significant, independent association between baseline serum sodium levels and severe disability at discharge in GBS patients. In our cohort, hyponatremia was more frequently observed after treatment with IVIG, suggesting dilutional pseudohyponatremia as a probable cause.

Keywords: hyponatremia, Guillain-Barré, polyradiculopathy, disability, intravenous immunoglobulin

INTRODUCTION

Guillain-Barré syndrome (GBS) is an inflammatory polyradiculopathy with a worldwide incidence of 100,000 cases per year, which can lead to permanent severe disability in a significant fraction of patients (1). Several clinical tools for risk stratification have been developed to tailor therapeutic strategies (2), but no reliable prognostic biomarker has been reported to date.

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Consumption of complement in a 26-year-old woman with severe thrombotic thrombocytopenia after ChAdOx1 nCov-19 vaccination

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ABSTRACT

Extremely rare reactions characterized by thrombosis and thrombocytopenia have been described in subjects that received ChAdOx1 nCoV-19 vaccination 5–16 days earlier. Although patients with vaccine-induced thrombotic thrombocytopenia (VITT) have high levels of antibodies to platelet factor 4 (PF4)–polyanion complexes, the exact mechanism of the development of thrombosis is still unknown. Here we reported serum studies as well as proteomics and genomics analyses demonstrating a massive complement activation potentially linked to the presence of anti-PF4 antibodies in a patient with severe VITT. At admission, complement activity of the classical and lectin pathways were absent (0% for both) with normal levels of the alternative pathway (73%) in association with elevated levels of the complement activation marker sC5b-9 (630 ng/mL [n.v. 139–462 ng/mL]) and anti-PF4 IgG (1.918 OD [n.v. 0.136–0.300 OD]). The immunoblotting analysis of C2 showed the complete disappearance of its normal band at 110 kDa. Intravenous immunoglobulin treatment allowed to recover complement activity of the classical pathway (91%) and lectin pathway (115%), to reduce levels of sC5b-9 (135 ng/mL) and anti-PF4 IgG (0.681 OD) and to normalize the C2 pattern at immunoblotting. Proteomics and genomics analyses in addition to serum studies showed that the absence of complement activity during VITT was not linked to alterations of the C2 gene but rather to a strong complement activation leading to C2 consumption. Our data in a single patient suggest monitoring complement parameters in other VITT patients considering also the possibility to target complement activation with specific drugs.

1. Introduction

Vaccine-induced thrombotic thrombocytopenia (VITT) is an extremely rare condition that may develop after vaccination with the recombinant adenoviral vector encoding the spike protein antigen of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (ChAdOx1 nCov-19, AstraZeneca) [1]. At the beginning, it was observed in 1 case per 100,000 exposures; however, at present some additional cases have been reported [2]. Thrombotic events, which begin 5–16 days after vaccination, mainly include cerebral venous thrombosis, splanchnic vein thrombosis and pulmonary embolism [1]. Low platelet counts, high levels of D-dimer and low fibrinogen have been described at

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Onasemnogene abeparvovec gene therapy for symptomatic infantile-onset spinal muscular atrophy type 1 (STR1VE-EU): an open-label, single-arm, multicentre, phase 3 trial

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See Comment page 777

*Study group members are listed in the appendix (p 2)

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Summary

Background Spinal muscular atrophy is a rare, autosomal recessive, neuromuscular disease caused by biallelic loss of the survival motor neuron 1 (*SMN1*) gene, resulting in motor neuron dysfunction. In this STR1VE-EU study, we aimed to evaluate the safety and efficacy of onasemnogene abeparvovec gene replacement therapy in infants with spinal muscular atrophy type 1, using broader eligibility criteria than those used in STR1VE-US.

Methods STR1VE-EU was a multicentre, single-arm, single-dose, open-label phase 3 trial done at nine sites (hospitals and universities) in Italy (n=4), the UK (n=2), Belgium (n=2), and France (n=1). We enrolled patients younger than 6 months (180 days) with spinal muscular atrophy type 1 and the common biallelic pathogenic *SMN1* exon 7–8 deletion or point mutations, and one or two copies of *SMN2*. Patients received a one-time intravenous infusion of onasemnogene abeparvovec ($1·1\times10^{14}$ vector genomes [vg]/kg). The outpatient follow-up consisted of assessments once per week starting at day 7 post-infusion for 4 weeks and then once per month until the end of the study (at age 18 months or early termination). The primary outcome was independent sitting for at least 10 s, as defined by the WHO Multicentre Growth Reference Study, at any visit up to the 18 months of age study visit, measured in the intention-to-treat population. Efficacy was compared with the Pediatric Neuromuscular Clinical Research (PNCR) natural history cohort. This trial is registered with ClinicalTrials.gov, NCT03461289 (completed).

Findings From Aug 16, 2018, to Sept 11, 2020, 41 patients with spinal muscular atrophy were assessed for eligibility. The median age at onasemnogene abeparvovec dosing was 4·1 months (IQR 3·0–5·2). 32 (97%) of 33 patients completed the study and were included in the ITT population (one patient was excluded despite completing the study because of dosing at 181 days). 14 (44%, 97·5% CI 26–100) of 32 patients achieved the primary endpoint of functional independent sitting for at least 10 s at any visit up to the 18 months of age study visit (*vs* 0 of 23 untreated patients in the PNCR cohort; p<0·0001). 31 (97%, 95% CI 91–100) of 32 patients in the ITT population survived free from permanent ventilatory support at 14 months compared with six (26%, 8–44) of 23 patients in the PNCR natural history cohort (p<0·0001). 32 (97%) of 33 patients had at least one adverse event and six (18%) had adverse events that were considered serious and related to onasemnogene abeparvovec. The most common adverse events were pyrexia (22 [67%] of 33), upper respiratory infection (11 [33%]), and increased alanine aminotransferase (nine [27%]). One death, unrelated to the study drug, occurred from hypoxic-ischaemic brain damage because of a respiratory tract infection during the study.

Interpretation STR1VE-EU showed efficacy of onasemnogene abeparvovec in infants with symptomatic spinal muscular atrophy type 1. No new safety signals were identified, but further studies are needed to show long-term safety. The benefit–risk profile of onasemnogene abeparvovec seems favourable for this patient population, including those with severe disease at baseline.

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Introduction

Spinal muscular atrophy is a rare, autosomal recessive, neurological disease caused by biallelic loss-of-function mutations in the survival motor neuron 1 (*SMN1*) gene, leading to motor neuron dysfunction and degeneration. Although the paralogous *SMN2* gene is retained in spinal muscular atrophy,[1] *SMN2* alone produces insufficient amounts of full length SMN protein.

Before the advent of *SMN*-targeting therapies, patients with spinal muscular atrophy type 1, the most severe postnatal phenotype, typically had disease onset by age 6 months followed by a rapid progressive decline in motor function, resulting in death or the need for permanent ventilation by age 2 years.[2–4] Patients given only supportive care were not expected to achieve any motor milestones.[5,6] Motor function in spinal muscular

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REVIEW ARTICLE

Spinal muscular atrophy: state of the art and new therapeutic strategies

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Abstract

Spinal muscular atrophy (SMA) is a severe disorder of motor neurons and the most frequent cause of genetic mortality, due to respiratory complications. We are facing an exciting era with three available therapeutic options in a disease considered incurable for more than a century. However, the availability of effective approaches has raised up ethical, medical, and financial issues that are routinely faced by the SMA community. Each therapeutic strategy has its weaknesses and strengths and clinicians need to know them to optimize clinical care. In this review, the state of the art and the results and challenges of the new SMA therapeutic strategies are highlighted.

Keywords Antisense oligonucleotides · Gene therapy · Spinal muscular atrophy · Therapy · Nusinersen

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of alpha motor neurons of spinal cord and destruction of motor neuron nuclei in the lower brain-stem [1]. SMA is caused by homozygous deletion or, less commonly, smaller mutations of *SMN1*, leading to deficiency of the ubiquitously expressed housekeeping protein "survival motor neuron" (SMN). This deficiency leads to muscle wasting and weakness, and feeding and respiratory difficulties [2, 3].

The estimated incidence of SMA is 1 in 6000 to 1 in 10,000 live births, with a carrier frequency of 1/40–1/60 [4, 5].

SMA is clinically classified into four phenotypes on the basis of age of onset and maximal motor function achieved. SMA type I (SMAI) accounts for ~50–60% of incident SMA and is the most severe form. The disease onset occurs within the first 6 months of lvhife. Affected babies exhibit generalized hypotonia, difficulty in swallowing, and paradoxical breathing and they never develop the ability to sit. Usually, they die of respiratory failure before the age of 2 years [6, 7].

SMA-II is characterized by onset of weakness before 18 months of age. Affected children achieve the ability to sit but they never walk unaided.

In children with SMA-III, the disease occurs after the age of 18 months. They typically achieve the independent walking

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Review

Perspectives on hiPSC-Derived Muscle Cells as Drug Discovery Models for Muscular Dystrophies

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Abstract: Muscular dystrophies are a heterogeneous group of inherited diseases characterized by the progressive degeneration and weakness of skeletal muscles, leading to disability and, often, premature death. To date, no effective therapies are available to halt or reverse the pathogenic process, and meaningful treatments are urgently needed. From this perspective, it is particularly important to establish reliable in vitro models of human muscle that allow the recapitulation of disease features as well as the screening of genetic and pharmacological therapies. We herein review and discuss advances in the development of in vitro muscle models obtained from human induced pluripotent stem cells, which appear to be capable of reproducing the lack of myofiber proteins as well as other specific pathological hallmarks, such as inflammation, fibrosis, and reduced muscle regenerative potential. In addition, these platforms have been used to assess genetic correction strategies such as gene silencing, gene transfer and genome editing with clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), as well as to evaluate novel small molecules aimed at ameliorating muscle degeneration. Furthermore, we discuss the challenges related to in vitro drug testing and provide a critical view of potential therapeutic developments to foster the future clinical translation of preclinical muscular dystrophy studies.

Keywords: stem cell model; cellular differentiation; muscular dystrophy; iPSC; dystrophin; skeletal muscle; drug screening platforms

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1. Introduction

Muscular dystrophies (MDs) are inherited disorders characterized by progressive skeletal muscle degeneration, clinically resulting in weakness in specific muscle groups, loss of ambulation, breathing and swallowing difficulties and, in most cases, reduced life span [1]. On a histopathological level, MDs share common "dystrophic" features, including muscle fiber degeneration, necrosis, replacement of muscle with connective and adipose tissues, inflammatory signs and reduced tissue regeneration capability impairing overall muscle structure and function [2]. These degenerative processes are induced by the lack or dysfunction of key myofiber proteins secondary to genetic mutations in their encoding genes [1].

MDs are characterized by the genetic and clinical heterogeneity of causative genes, pattern of inheritance, age of onset, rate of progression and type of muscle affected [1]. Cardiac and respiratory muscles are also frequently involved. To date, more than 50 genes have been implicated in up to 70 forms of MD [1]. The most common MDs are dystrophinopathies (Duchenne muscular dystrophy (DMD)) and Becker muscular dystrophy (BMD)) [3], myotonic dystrophies (DMs) [4,5], facioscapulohumeral muscular dystrophy (FSHD), and limb girdle muscular dystrophies (LGMDs) [6–8].

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Molecular Therapy
Original Article

Cell-penetrating peptide-conjugated Morpholino rescues SMA in a symptomatic preclinical model

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Spinal muscular atrophy (SMA) is a motor neuron disease and the leading genetic cause of infant mortality. Recently approved SMA therapies have transformed a deadly disease into a survivable one, but these compounds show a wide spectrum of clinical response and effective rescue only in the early stages of the disease. Therefore, safe, symptomatic-suitable, non-invasive treatments with high clinical impact across different phenotypes are urgently needed. We conjugated antisense oligonucleotides with Morpholino (MO) chemistry, which increase SMN protein levels, to cell-penetrating peptides (CPPs) for better cellular distribution. Systemically administered MOs linked to r6 and $(RXRRBR)_2XB$ peptides crossed the blood-brain barrier and increased SMN protein levels remarkably, causing striking improvement of survival, neuromuscular function, and neuropathology, even in symptomatic SMA animals. Our study demonstrates that MO-CPP conjugates can significantly expand the therapeutic window through minimally invasive systemic administration, opening the path for clinical applications of this strategy.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal-recessive, degenerative motor neuron disease, and is the main genetic cause of infant mortality.[1] SMA patients show progressive loss of motor neurons (MNs) in the ventral horns of the spinal cord, causing progressive muscle weakness, paralysis, and premature death. Homozygous mutations of the survival motor neuron 1 gene (*SMN*) account for reduced levels of SMN protein, which is critically important for MN maintenance and survival.[1,2] Humans have a nearly identical copy of the *SMN* gene, *SMN2*, which differs from *SMN* in five nucleotides. One of them determines the exclusion of exon 7 in *SMN2*, producing a truncated, non-functional SMN protein in 90% of cases.[3] *SMN2* copy number varies among individuals and is the most important influence on the clinical phenotype.[4]

Currently, three disease-modifying treatments are approved by the US Food and Drug Administration: nusinersen, onasemnogene abeparvovec, and risdiplam. Nusinersen is an antisense oligonucleotide (ASO) that modulates *SMN2* splicing by promoting the inclusion of exon 7 and the production of a functional SMN protein. It requires repeated intrathecal administration,[5,6] a relatively invasive procedure with side effects related to lumbar puncture, such as headache, local pain, etc. In addition, late-onset patients are often affected by scoliosis, have undergone previous spine fusion operations, and frequently have joint contractures and respiratory insufficiency, which complicate lumbar puncture.[7] Indeed, with currently available ASOs, limited distribution of the molecules to the rostral spinal and brain regions in some patients likely hamper the clinical response of their motor units in these regions.[8] Moreover, recent reviews have provided evidence that nusinersen can improve with heterogeneity motor functions in SMA type I and II but not always in SMA type III subjects.[9] Onasemnogene abeparvovec is a gene therapy that provides wild-type full-length SMN cDNA. It is systemically delivered, but its long-term persistence in peripheral organs is not yet determined and it has been linked to serious immunological side effects, particularly in the liver.[10] As yet, no clinical data are available regarding its use in SMA II–IV. Risdiplam is a small molecule that increases SMN production from *SMN2* mRNA. It has the great advantage of being orally administered and systemically distributed, but possible nonspecific effects of the molecule can lead to unexpected adverse side reactions. All SMN-based approved therapies show a very narrow therapeutic window: the compounds are strikingly efficient only in the pre- or early symptomatic phases, for reasons not completely understood,[11] and delayed intervention leads to a less efficient rescue of the pathological phenotype.[12] As SMA patients are a very heterogeneous group, the only identified factor that is predictive of SMN-augmenting treatment success is the age of the patient at treatment initiation, which is closely related to disease duration.[11] Nevertheless, universal newborn screening remains a very distant prospect. Thus, we sorely lack a drug

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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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Impairment of the neurotrophic signaling hub B-Raf contributes to motoneuron degeneration in spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a motoneuron disease caused by deletions of the *Survival of Motoneuron 1 gene* (*SMN1*) and low SMN protein levels. SMN restoration is the concept behind a number of recently approved drugs which result in impressive yet limited effects. Since SMN has already been enhanced in treated patients, complementary SMN-independent approaches are needed. Previously, a number of altered signaling pathways which regulate motoneuron degeneration have been identified as candidate targets. However, signaling pathways form networks, and their connectivity is still unknown in SMA. Here, we used presymptomatic SMA mice to elucidate the network of altered signaling in SMA. The SMA network is structured in two clusters with AKT and 14-3-3 ζ/δ in their centers. Both clusters are connected by B-Raf as a major signaling hub. The direct interaction of B-Raf with 14-3-3 ζ/δ is important for an efficient neurotrophic activation of the MEK/ERK pathway and crucial for motoneuron survival. Further analyses in SMA mice revealed that both proteins were down-regulated in motoneurons and the spinal cord with B-Raf being reduced at presymptomatic stages. Primary fibroblasts and iPSC-derived motoneurons from SMA patients both showed the same pattern of down-regulation. This mechanism is conserved across species since a *Caenorhabditis elegans* SMA model showed less expression of the B-Raf homolog *lin-45*. Accordingly, motoneuron survival was rescued by a cell autonomous *lin-45* expression in a *C. elegans* SMA model resulting in improved motor functions. This rescue was effective even after the onset of motoneuron degeneration and mediated by the MEK/ERK pathway.

spinal muscular atrophy | SMA | neurotrophic signaling | Raf | 14-3-3

Spinal muscular atrophy (SMA) is a neurodegenerative disease of newborns, infants, and young adults which preferentially affects lower motoneurons in the ventral horn of the spinal cord. As a consequence, patients suffer from muscle weakness and atrophy often resulting in respiratory insufficiency and early death. SMA is caused by homozygous deletions or mutations of the *Survival of Motoneuron 1 (SMN1)* gene (1). However, humans harbor the similar *SMN2* gene which codes for the same protein but differs in a critical cytosine to thymine exchange within exon seven in an exonic splice enhancer region (2). Consequently, the *SMN2* pre-mRNA is insufficiently spliced, resulting in low levels of functional full-length mRNA and protein (3). Thus, SMA is caused by low SMN protein levels. The number of *SMN2* gene copies critically modifies the disease phenotype with a low number associated with severe forms and higher numbers with milder forms (4).

SMA is characterized by a neuromuscular phenotype starting in proximal muscles with hypotonia, fatigue, and paralysis. The disease is categorized in five different subtypes based on the clinical presentation (5). A small number of patients suffer from congenital SMA-Type 0 with a prenatal onset and a rapid disease progression. The majority of the patients are classified as severe Type 1. The onset is postnatal between 2 wk and 6 mo of age. Untreated patients are never able to roll or sit independently, and about two-thirds decease within the first 2 y of life. Type 2 patients develop first symptoms between 6 and 18 mo of age and are unable to walk independently. Type 3 patients have mild progressive muscle weaknesses with a normal life-expectancy, and Type 4 patients have some difficulties with gross motor functions only (5).

However, recently approved treatments dramatically changed the clinical situation. Nusinersen, an antisense oligonucleotide, and Risdiplam, a small molecule compound, both correct *SMN2* pre-mRNA splicing (6–8). Onasemnogene Abeparvovec is a gene-therapy based on the delivery of a SMN cDNA by an adeno-associated virus (AAV) (9, 10). All treatments substantially enhance the survival and motor functions which will enhance the SMA prevalence. However, clinical and preclinical studies demonstrate that delayed interventions after disease onset led to limited clinical improvements. Moreover, there are a substantial number of nonresponders (7, 11, 12). Onasemnogene Abeparvovec, Nusinersen, and Risdiplam enhance the SMN-protein level thereby termed SMN-dependent treatments. Since SMN levels have already been restored by those drugs, other complementary therapies are needed which do not change

Significance

The mechanisms of neurodegeneration are important targets for future treatments of the devastating motoneuron disease spinal muscular atrophy (SMA). Here, we show an altered signaling network in SMA models and patient cells with a reduced B-Raf expression in the network center. B-Raf is crucial for motoneuron survival, and we present data that B-Raf restoration ameliorates motoneuron loss and symptoms in SMA models.

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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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Article

Dysregulation of Muscle-Specific MicroRNAs as Common Pathogenic Feature Associated with Muscle Atrophy in ALS, SMA and SBMA: Evidence from Animal Models and Human Patients

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‡ This paper is dedicated to the memory of Pia Bernasconi, enthusiastic mentor and eclectic scientist, passionate about life and knowledge (1965–2020).
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Abstract: Motor neuron diseases (MNDs) are neurodegenerative disorders characterized by upper and/or lower MN loss. MNDs include amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA), and spinal and bulbar muscular atrophy (SBMA). Despite variability in onset, progression, and genetics, they share a common skeletal muscle involvement, suggesting that it could be a primary site for MND pathogenesis. Due to the key role of muscle-specific microRNAs (myomiRs) in skeletal muscle development, by real-time PCR we investigated the expression of miR-206, miR-133a, miR-133b, and miR-1, and their target genes, in G93A-SOD1 ALS, Δ7SMA, and KI-SBMA mouse muscle

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Short communication

Screening of *LRP10* mutations in Parkinson's disease patients from Italy

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ABSTRACT

Introduction: Parkinson's disease (PD) belongs to a family of neurodegenerative diseases characterized by alpha-synuclein accumulation in neurons, whose etiopathogenesis remains largely uncovered. Recently, *LRP10* has been associated with PD, Parkinson's disease Dementia (PDD) and Dementia with Lewy Bodies (DLB) by linkage analysis and positional cloning in an Italian family with late-onset PD. After the first characterization of a *LRP10* pathogenic variant, other eight mutations have been detected in an international series of 660 probands with either a clinical or pathological diagnosis of PD, PDD or DLB. However, the results of following replication studies were inconclusive and the pathogenic role of *LRP10* is still debated. The aim of this study is to sequence the *LRP10* gene in an Italian cohort of clinically-diagnosed PD patients and to compare the frequency of the identified variants with the ones found in a large cohort of Italian exomes.

Methods: A cohort of 664 PD patients was analyzed by targeted Next Generation Sequencing approach. Identified *LRP10* variants were subsequently confirmed by Sanger sequencing and searched for in an in-house database including 3596 Italian exomes.

Results: We identified three PD patients carrying a rare heterozygous, potentially pathogenic variant (p.R296C, p.R549Q, p.R661C). None of them was detected in 3596 Italian exomes. Two of them (p.R296C and p.R661C) have been previously reported in one sporadic PD and one definite Progressive supranuclear palsy patients respectively. All three carriers had late-onset PD responsive to levodopa, characterized by both motor and non-motor features, but no cognitive impairment.

Conclusion: We report three rare possibly-pathogenic *LRP10* variants in PD patients from Italy. Further investigations are required to definitively establish their role in alpha-synucleinopathies.

1. Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease after Alzheimer's disease [1], belongs to the family of alpha-synucleinopathies. The progressive development of novel techniques in the field of genetics has brought, in the last two decades, to the discovery of several loci and disease-causing genes associated with alpha-synucleinopathies. Nonetheless, monogenic forms with classical Mendelian inheritance still represent only the 5–10% of cases [2].

Recently, the low-density lipoprotein receptor-related protein 10 (*LRP10*) has been associated with PD, Parkinson's disease Dementia (PDD) and Dementia with Lewy Bodies (DLB) [3]. After the first identification of a *LRP10* pathogenic variant by linkage analysis and positional cloning in a large Italian family with late onset PD, eight additional mutations have been detected in an international multicenter series of 660 probands with either a clinical or pathological diagnosis of

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LETTER TO THE EDITOR

Expanding the genotypic and phenotypic spectrum of Beta-propeller protein-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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ARTICLE

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Sumoylation regulates the assembly and activity of the SMN complex

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SMN is a ubiquitously expressed protein and is essential for life. SMN deficiency causes the neurodegenerative disease spinal muscular atrophy (SMA), the leading genetic cause of infant mortality. SMN interacts with itself and other proteins to form a complex that functions in the assembly of ribonucleoproteins. SMN is modified by SUMO (Small Ubiquitin-like Modifier), but whether sumoylation is required for the functions of SMN that are relevant to SMA pathogenesis is not known. Here, we show that inactivation of a SUMO-interacting motif (SIM) alters SMN sub-cellular distribution, the integrity of its complex, and its function in small nuclear ribonucleoproteins biogenesis. Expression of a SIM-inactivated mutant of SMN in a mouse model of SMA slightly extends survival rate with limited and transient correction of motor deficits. Remarkably, although SIM-inactivated SMN attenuates motor neuron loss and improves neuromuscular junction synapses, it fails to prevent the loss of sensory-motor synapses. These findings suggest that sumoylation is important for proper assembly and function of the SMN complex and that loss of this post-translational modification impairs the ability of SMN to correct selective deficits in the sensory-motor circuit of SMA mice.

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RESEARCH ARTICLE

Genetic defects are common in myopathies with tubular aggregates

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Abstract

Objective: A group of genes have been reported to be associated with myopathies with tubular aggregates (TAs). Many cases with TAs still lack of genetic clarification. This study aims to explore the genetic background of cases with TAs in order to improve our knowledge of the pathogenesis of these rare pathological structures. **Methods**: Thirty-three patients including two family members with biopsy confirmed TAs were collected. Whole-exome sequencing was performed on 31 unrelated index patients and a candidate gene search strategy was conducted. The identified variants were confirmed by Sanger sequencing. The wild-type and the mutant p.Ala11Thr of *ALG14* were transfected into human embryonic kidney 293 cells (HEK293), and western blot analysis was performed to quantify protein expression levels. **Results**: Eleven index cases (33%) were found to have pathogenic variant or likely pathogenic variants in *STIM1*, *ORAI1*, *PGAM2*, *SCN4A*, *CASQ1* and *ALG14*. Among them, the c.764A>T (p.Glu255Val) in *STIM1* and the c.1333G>C (p.Val445Leu) in *SCN4A* were novel. Western blot analysis showed that the expression of ALG14 protein was severely reduced in the mutant *ALG14* HEK293 cells (p.Ala11Thr) compared with wild type. The *ALG14* variants might be associated with TAs in patients with complex multisystem disorders. **Interpretation**: This study expands the phenotypic and genotypic spectrums of myopathies with TAs. Our findings further confirm previous hypothesis that genes related with calcium signalling pathway and N-linked glycosylation pathway are the main genetic causes of myopathies with TAs.

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Article

Movement Disorders in Children with a Mitochondrial Disease: A Cross-Sectional Survey from the Nationwide Italian Collaborative Network of Mitochondrial Diseases

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ORIGINAL COMMUNICATION

A 5-year clinical follow-up study from the Italian National Registry for FSHD

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Abstract

Background The natural history of facioscapulohumeral muscular dystrophy (FSHD) is undefined.

Methods An observational cohort study was conducted in 246 FSHD1 patients. We split the analysis between index cases and carrier relatives and we classified all patients using the Comprehensive Clinical Evaluation Form (CCEF). The disease progression was measured as a variation of the FSHD score performed at baseline and at the end of 5-year follow-up (ΔFSHD score).

Findings Disease worsened in 79.4% (112/141) of index cases versus 38.1% (40/105) of carrier relatives and advanced more rapidly in index cases (ΔFSHD score 2.3 versus 1.2). The 79.1% (38/48) of asymptomatic carriers remained asymptomatic. The highest ΔFSHD score (1.7) was found in subject with facial and scapular weakness at baseline (category A), whereas in subjects with incomplete phenotype (facial or scapular weakness, category B) had lower ΔFSHD score (0.6) $p < 0.0001$.

Conclusions The progression of disease is different between index cases and carrier relatives and the assessment of the CCEF categories has strong prognostic effect in FSHD1 patients.

Keywords FSHD · D4Z4 reduced allele · Clinical categories · Follow-up

Abbreviations

CCEF	Comprehensive Clinical Evaluation Form
DRA	D4Z4 reduced alleles
FSHD	Facioscapulohumeral muscular dystrophy
INRF	Italian National Registry for FSHD
INCF	Italian National Consortium for FSHD
MRC	Medical Research Council
NIV	Non-invasive ventilation

Liliana Vercelli, Fabiano Mele and Lucia Ruggiero contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00415-020-10144-7) contains supplementary material, which is available to authorized users.

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Introduction

Facioscapulohumeral muscular dystrophy (FSHD, OMIM # 158900) is a hereditary myopathy with prevalence of 1 in 8500–20,000 individuals [1, 2]. The classical FSHD phenotype is characterized by a distinctive distribution of muscular weakness [3].

Two genetically distinct disease subtypes, FSHD1 and FSHD2 have been described. The vast majority of FSHD subjects, named FSHD1, carry contractions of a polymorphic tandemly arrayed 3.3 kb D4Z4 repeat element on the telomeric region of chromosome 4, at 4q35 [4]. Detection of one D4Z4 alleles with 10 or fewer repeats associated with the 4qA polymorphism is considered a molecular hallmark for FSHD diagnosis [5]. FSHD2, which represents 5–10% of cases, is contraction-independent, with affected individuals carrying two D4Z4 arrays in the healthy range (> 10 RUs) [6].

Since the discovery of the D4Z4 locus for FSHD diagnosis it was clear that many different phenotypes and reduced

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FRIEDREICH'S ATAXIA

Treatment with ROS detoxifying gold quantum clusters alleviates the functional decline in a mouse model of Friedreich ataxia

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Friedreich ataxia (FRDA) is caused by the reduced expression of the mitochondrial protein frataxin (FXN) due to an intronic GAA trinucleotide repeat expansion in the *FXN* gene. Although FRDA has no cure and few treatment options, there is research dedicated to finding an agent that can curb disease progression and address symptoms as neurobehavioral deficits, muscle endurance, and heart contractile dysfunctions. Because oxidative stress and mitochondrial dysfunctions are implicated in FRDA, we demonstrated the systemic delivery of catalysts activity of gold cluster superstructures (Au_8-pXs) to improve cell response to mitochondrial reactive oxygen species and thereby alleviate FRDA-related pathology in mesenchymal stem cells from patients with FRDA. We also found that systemic injection of Au_8-pXs ameliorated motor function and cardiac contractility of YG8sR mouse model that recapitulates the FRDA phenotype. These effects were associated to long-term improvement of mitochondrial functions and antioxidant cell responses. We related these events to an increased expression of frataxin, which was sustained by reduced autophagy. Overall, these results encourage further optimization of Au_8-pXs in experimental clinical strategies for the treatment of FRDA.

INTRODUCTION

Friedreich ataxia (FRDA) is a multisystemic autosomal recessive disorder caused by a GAA repeat expansion mutation within intron 1 of the FXN gene (*1*) that determines reduced expression of the mitochondrial protein FXN (*2*). Frataxin dysregulation is linked to dysfunctions of the mitochondrial energy conversion and oxidative phosphorylation (OXPHOS) through iron accumulation and increased production of reactive oxygen species (ROS), leading to oxidative stress especially in mitochondria (*3*). Cellular antioxidant response is regulated by nuclear factor E2–related factor 2 (Nrf2) transcription factor that induces the expression of ROS-response antioxidant genes, by binding to the antioxidant response element (ARE) on nuclear DNA, including an ARE site within the FXN gene (*4*). In patients with FRDA and mouse models, Nrf2 is compromised in response to oxidative insults, thus leading to reduced expression of antioxidant genes such as superoxide dismutase (SOD) SOD1 and SOD2 (*5*). In addition, frataxin deficiency is associated with the down-regulation of PGC-1α [peroxisome proliferator–activated receptor γ (PPARγ) coactivator 1α], a regulator of mitochondrial biogenesis (*6*), suggesting an early impairment of mitochondrial pathways (*7*). Another hallmark of FRDA is the formation of giant or larger disorganized mitochondria in rodent muscles (*8*) or in cultured cells (*9*) triggered by increased expression of the mitochondrial fusion protein mitofusin 1 (Mfn1). Because metabolic stress triggers salvaging processes, by which macromolecules and organelles are targeted by autophagic vesicles to lysosomes for degradation and recycling of their constituents, autophagy may be considered a key node for the regulation of ROS amount and ROS-dependent pathway (*10*).

It is likely that alterations in ROS and imbalance of mitochondrial dynamics and autophagy are critically implicated in FRDA pathology (*9*), and they represent a common pathogenic element to several neurodegenerative diseases such as Parkinson's disease, Charcot-Marie-Tooth disease, Alzheimer's disease, Huntington's disease, or amyotrophic lateral sclerosis (*11*). Pathologically, frataxin insufficiency leads to spinocerebellar neurodegeneration, ataxia, muscle weakness, cardiomyopathy, diabetes mellitus, and skeletal deformities (*12*). No cure or effective treatment has been reported for FRDA. Emerging therapies are directed at the augmentation of mitochondrial function (such as antioxidants, Nrf2 up-regulators, and deuterated fatty acids) and frataxin restoration [such as gene and cell therapy, erythropoietin, interferon-γ, nicotinamide, and histone deacetylase inhibitor (HDACi)] (*13*–*15*). Buffering the abundance of ROS and the oxidative stress–induced damages in FRDA was the initial challenge of exploiting gold quantum clusters [Au_8 gold cluster superstructures (Au_8-pXs)] as potential therapeutic strategy for FRDA. Au_8-pX is a recently developed highly biocompatible material based on gold quantum clusters, subnanometer structures made from few units to hundred gold atoms, which have excellent ROS scavenger potential (*16*, *17*), probably related to their catalytic activity (*18*). In our study, we examined the effects of Au_8-pX treatment on bone marrow–derived

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Short Communication

Detection of the *SQSTM1* Mutation in a Patient with Early-Onset Hippocampal Amnestic Syndrome

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Abstract. Genetics has a major role in early-onset dementia, but the correspondence between genotype and phenotype is largely tentative. We describe a 54-year-old with familial early-onset slowly-progressive episodic memory impairment with the P392L-variant in *SQSTM1*. The patient showed cortical atrophy and hypometabolism in the temporal lobes, but no amyloidosis biomarkers. As symptoms/neuroimaging were suggestive for Alzheimer's disease—but biomarkers were not—and considering the family-history, genetic analysis was performed, revealing the P392L-variant in *SQSTM1*, which encodes for sequestosome-1/p62. Increasing evidence suggests a p62 involvement in neurodegeneration and *SQSTM1* mutations have been found to cause amyotrophic lateral sclerosis/frontotemporal dementia. Our report suggests that the clinical spectrum of *SQSTM1* variants is wider.

Keywords: Alzheimer's disease, early-onset dementia, next-generation sequencing, p62, SQSTM1

INTRODUCTION

Early-onset dementia (EOD) affects patients younger than 65 years of age and represents approximately 5% of all dementia cases. As compared to late-onset dementia, heritability has a major role in EOD, and many different genetic mutations have been identified in familial EOD, leading to a variety of clinical syndromes. However, the correspondence between genotype and phenotype remain largely tentative. We here describe a 54-year-old patient with early-onset slowly-progressive episodic memory impairment associated to the P392L variant in *SQSTM1*.

CASE REPORT

A 54-year-old man was referred to our Department due to a two-year history of slowly progressive

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FEATURED ARTICLE

Apathy in presymptomatic genetic frontotemporal dementia predicts cognitive decline and is driven by structural brain changes

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White Matter Hyperintensities Are No Major Confounder for Alzheimer's Disease Cerebrospinal Fluid Biomarkers

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FEATURED ARTICLE

Brain functional network integrity sustains cognitive function despite atrophy in presymptomatic genetic frontotemporal dementia

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ORIGINAL ARTICLE

Biomarkers and phenotypic expression in Alzheimer's disease: exploring the contribution of frailty in the Alzheimer's Disease Neuroimaging Initiative

Marco Canevelli · Ivan Arisi · Ilaria Bacigalupo · Andrea Arighi · Daniela Galimberti · Nicola Vanacore · Mara D'Onofrio · Matteo Cesari · Giuseppe Bruno · for the Alzheimer's Disease Neuroimaging Initiative

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Abstract The present study aimed at investigating if the main biomarkers of Alzheimer's disease (AD) neuropathology and their association with cognitive disturbances and dementia are modified by the individual's frailty status. We performed a cross-sectional analysis of data from participants with normal cognition, mild cognitive impairment (MCI), and AD dementia enrolled in the Alzheimer's Disease Neuroimaging Initiative 2 (ADNI2) study. Frailty was operationalized by computing a 40-item Frailty Index (FI). The following AD biomarkers were considered and analyzed according to the participants' frailty status: CSF $A\beta_{1\text{-}42}$, ^{181}P-tau, and

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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Review

Fluid biomarkers in frontotemporal dementia: past, present and future

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ABSTRACT

The frontotemporal dementia (FTD) spectrum of neurodegenerative disorders includes a heterogeneous group of conditions. However, following on from a series of important molecular studies in the early 2000s, major advances have now been made in the understanding of the pathological and genetic underpinnings of the disease. In turn, alongside the development of novel methodologies for measuring proteins and other molecules in biological fluids, the last 10 years have seen a huge increase in biomarker studies within FTD. This recent past has focused on attempting to develop markers that will help differentiate FTD from other dementias (particularly Alzheimer's disease (AD)), as well as from non-neurodegenerative conditions such as primary psychiatric disorders. While cerebrospinal fluid, and more recently blood, markers of AD have been successfully developed, specific markers identifying primary tauopathies or TDP-43 proteinopathies are still lacking. More focus at the moment has been on non-specific markers of neurodegeneration, and in particular, multiple studies of neurofilament light chain have highlighted its importance as a diagnostic, prognostic and staging marker of FTD. As clinical trials get under way in specific genetic forms of FTD, measures of progranulin and dipeptide repeat proteins in biofluids have become important potential measures of therapeutic response. However, understanding of whether drugs restore cellular function will also be important, and studies of key pathophysiological processes, including neuroinflammation, lysosomal function and synaptic health, are also now becoming more common. There is much still to learn in the fluid biomarker field in FTD, but the creation of large multinational cohorts is facilitating better powered studies and will pave the way for larger omics studies, including proteomics, metabolomics and lipidomics, as well as investigations of multimodal biomarker combinations across fluids, brain imaging and other domains. Here we provide an overview of the past, present and future of fluid biomarkers within the FTD field.

INTRODUCTION

The frontotemporal dementia (FTD) spectrum encompasses a group of conditions that overlap in their clinical, neuroanatomical, genetic and pathological features.[1] Clinically, FTD can be divided into a behavioural form (behavioural variant frontotemporal dementia (bvFTD)), a language variant (primary progressive aphasia (PPA)) and a motor presentation (either FTD with amyotrophic lateral sclerosis (FTD-ALS) or an atypical parkinsonian disorder). Neuroanatomically, the FTD spectrum is characteristically associated with dysfunction and neuronal loss in the frontal and temporal lobes, but more widespread cortical, subcortical, cerebellar and brainstem involvement is now recognised. Genetically, around a third of FTD is familial with autosomal dominant mutations in three genes accounting for most of the inheritance: progranulin (*GRN*), chromosome 9 open reading frame 72 (*C9orf72*) and microtubule-associated protein tau (*MAPT*).[2] Lastly, pathologically, cellular inclusions containing abnormal forms of tau, TDP-43 or FET proteins are found in the majority of people with an FTD syndrome. The interaction between clinical phenotype, neuroanatomy, genotype and pathology is complex (figure 1) and means that FTD can be hard to diagnose (particularly its specific pathological form during life) and difficult to track over time.

One way that researchers have aimed to solve some of these outstanding issues in the FTD field has been to develop fluid biomarkers, and there has been a growing literature in recent years investigating new cerebrospinal fluid (CSF) or blood measures in people with FTD. This review aimed to set out what has been done so far, where we are at present and what we still need to achieve in the future within the FTD fluid biomarker research world. This is particularly important in a time when potential therapies have now been developed and clinical trials have started.

CLASSIFICATION AND USE OF BIOMARKERS IN FTD

Fluid biomarkers, measured typically in CSF, serum or plasma (box 1) using a variety of different techniques (box 2), are objective indicators of normal or pathological biological processes or pharmacological responses to a therapeutic intervention. In FTD, biomarkers can be classified in a number of ways:

- *Diagnostic*, including distinguishing FTD versus non-neurodegenerative disorders and FTD versus Alzheimer's disease (AD) or other

Box 4 Measures of hypothalamic function

Excessive eating and dietary changes are well recognised in frontotemporal dementia (FTD) and can be used to differentiate behavioural frontotemporal dementia (bvFTD) from Alzheimer's disease (AD).[S25-S27] Studies investigating eating behaviour have shown altered metabolism,[S27-S30] and so studies have begun to focus on molecular measures of appetite, and therefore also the hypothalamus, as it acts as a key control centre in modulating appetite through various highly interconnected nuclei that communicate through neuropeptides.[S26,S31-S34] Lower levels of ghrelin and cortisol, but higher levels of insulin, have been observed in bvFTD compared with controls and AD.[S35] In the same study, higher levels of leptin were found in those with marked hyperphagia.[S35] These metabolic patterns are consistent with a state of satiety, which suggests the eating disturbances observed in FTD result from loss of inhibitory signals; however, the compensatory hormonal responses cannot reverse these symptoms. The first study investigating cerebrospinal fluid (CSF) agouti-related peptide (AgRP) in FTD found a sevenfold increase in people with TDP-43 pathology compared with people with tau pathology.[45] A further study detected increased levels of AgRP in bvFTD and semantic variant PPA compared with controls,[S27] also showing that both AgRP and leptin were predictors of body mass index.[S27] These findings of elevated AgRP, which stimulates appetite, suggest that neuropeptides may directly be involved in modulating eating behaviour in FTD and promote excessive eating. In a further study, decreased neuropeptide Y levels were found in people with FTD compared with controls and patients with amyotrophic lateral sclerosis (ALS).[S36] They also showed increased levels of leptin and insulin resistance in people with bvFTD and ALS,[S36] further complicating the picture of metabolic changes underlying eating disturbances in FTD. Few metabolic studies have focused on specific forms of FTD so far. However, in one study focused on *GRN*-related FTD, serum C-peptide, resistin and ghrelin were all increased.[S37] Lastly, hypothalamic proteins have been studied in relation to sleep in FTD, with CSF orexin levels being correlated to daytime somnolence[S38] in one study, and plasma orexin being lower in people with FTD compared with controls in another.[S39]

Box 5 Staging of frontotemporal dementia (FTD) and proximity markers

Unlike in Alzheimer's disease, few markers of disease stage have been identified in FTD so far. 'Preclinical' FTD may be defined by the onset of detectable pathological changes, but at present, we have no biomarkers of tau, TDP-43 or FET that might theoretically become abnormal quite a number of years prior to symptom onset. While we do have markers of dipeptide repeat proteins (DPRs) in chromosome 9 open reading frame 72 expansion-related disease, further studies of when these become abnormal are required (as it is likely that in this group DPR pathology precedes TDP-43 pathology[S40]). 'Prodromal' FTD may be defined by the onset of mild symptoms that do not meet diagnostic criteria, that is, mild behavioural, cognitive (and/or motor) impairment. During or just prior to this stage, we may define 'proximity measures', that is, those identifying a period prior to phenoconversion, and for FTD both neurofilament light chain,[S13,S19] and glial fibrillary acidic protein in *GRN* mutation carriers[41] are candidates for these.

Box 6 Frontotemporal dementia (FTD) cohorts

As FTD is a rare disease with multiple phenotypes and pathogenetic causes, a substantial number of prior biomarker studies have involved relatively small sample sizes, reducing the power and likelihood of finding abnormalities. In recent years, however, larger, deeply phenotyped cohorts have been developed that have facilitated biomarker studies of increased size. In genetic FTD, these include the Genetic FTD Initiative (www.genfi.org)[S41] in Europe and Eastern Canada, and the ARTFL/LEFFTDS (now ALLFTD: https://www.allftd.org) cohort in the USA and Western Canada,[S42,S43] which collaborate through the FTD Prevention Initiative (www.thefpi.org).[S44] The ALLFTD cohort also includes sporadic FTD, and while there are no large multinational cohorts in Europe, large studies of sporadic FTD with biomarker analyses include the German FTLD consortium[S45] and DZNE FTD DESCRIBE cohort.[S46] Specific atypical parkinsonian cohorts include the UK PROSPECT study[S47] and the US 4RTNI cohort.[S48]

imperative to look beyond the fluid biomarker field to consider multimodal combinations of measures that cross biofluids, MRI and PET, making use of new data science methods, including machine and deep learning. It is likely that such studies will take place within the context of large cohort studies (see box 6), avoiding the problems of small sample sizes that are inherent to so many of the published papers so far. Such studies will also pave the way for larger omics studies which have so far been relatively small and focused on proteomics despite initial evidence for abnormalities in metabolomics[75–77] and lipidomics[78–80] in FTD as well.

CONCLUSION

The past of FTD biomarkers as described here is a wide range of different markers, some more promising than others, but many examined in small single centre cohorts. The present is the recent introduction of more sensitive blood-based biomarker methods and the availability of larger sample collections from the well-characterised multinational genetic FTD cohorts, with great promise for markers such as NfL and GFAP. The future is trials and within this, the validation of multiplex biomarker panels targeted at specific pathogenetic forms of FTD, and eventually to individuals, providing a more personalised approach to outcome measures in upcoming trials.

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ORIGINAL ARTICLE

Disease-specific plasma levels of mitokines FGF21, GDF15, and Humanin in type II diabetes and Alzheimer's disease in comparison with healthy aging

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Abstract Fibroblast Growth Factor 21 (FGF21), Growth Differentiation Factor 15 (GDF15), and Humanin (HN) are mitochondrial stress-related mitokines, whose role in health and disease is still debated. In this study, we confirmed that their plasma levels are positively correlated with age in healthy subjects. However, when looking at patients with type 2 diabetes (T2D) or Alzheimer's disease (AD), two age-related diseases sharing a mitochondrial impairment, we found that GDF15 is elevated in T2D but not in AD and represents a risk factor for T2D complications, while FGF21 and HN are lower in AD but not in T2D. Moreover, FGF21 reaches the highest levels in centenarian' offspring, a model of successful aging. As a whole, these data indicate that (i) the adaptive mitokine response observed in healthy aging is lost in age-related diseases, (ii) a common expression pattern of mitokines does not emerge in T2D and AD, suggesting an unpredicted complexity and disease-specificity, and (iii) FGF21 emerges as a candidate marker of healthy aging.

Keywords GDF15 · FGF21 · Humanin · Aging · AD · T2D

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Disease-related cortical thinning in presymptomatic granulin mutation carriers

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Progression of Behavioral Disturbances and Neuropsychiatric Symptoms in Patients With Genetic Frontotemporal Dementia

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Abstract

IMPORTANCE Behavioral disturbances are core features of frontotemporal dementia (FTD); however, symptom progression across the course of disease is not well characterized in genetic FTD.

OBJECTIVE To investigate behavioral symptom frequency and severity and their evolution and progression in different forms of genetic FTD.

DESIGN, SETTING, AND PARTICIPANTS This longitudinal cohort study, the international Genetic FTD Initiative (GENFI), was conducted from January 30, 2012, to May 31, 2019, at 23 multicenter specialist tertiary FTD research clinics in the United Kingdom, the Netherlands, Belgium, France, Spain, Portugal, Italy, Germany, Sweden, Finland, and Canada. Participants included a consecutive sample of 232 symptomatic FTD gene variation carriers comprising 115 with variations in *C9orf72*, 78 in *GRN*, and 39 in *MAPT*. A total of 101 carriers had at least 1 follow-up evaluation (for a total of 400 assessments). Gene variations were included only if considered pathogenetic.

MAIN OUTCOMES AND MEASURES Behavioral and neuropsychiatric symptoms were assessed across disease duration and evaluated from symptom onset. Hierarchical generalized linear mixed models were used to model behavioral and neuropsychiatric measures as a function of disease duration and variation.

RESULTS Of 232 patients with FTD, 115 (49.6%) had a *C9orf72* expansion (median [interquartile range (IQR)] age at evaluation, 64.3 [57.5-69.7] years; 72 men [62.6%]; 115 White patients [100%]), 78 (33.6%) had a *GRN* variant (median [IQR] age, 63.4 [58.3-68.8] years; 40 women [51.3%]; 77 White patients [98.7%]), and 39 (16.8%) had a *MAPT* variant (median [IQR] age, 56.3 [49.9-62.4] years; 25 men [64.1%]; 37 White patients [94.9%]). All core behavioral symptoms, including disinhibition, apathy, loss of empathy, perseverative behavior, and hyperorality, were highly expressed in all gene variant carriers (>50% patients), with apathy being one of the most common and severe symptoms throughout the disease course (51.7%-100% of patients). Patients with *MAPT* variants showed the highest frequency and severity of most behavioral symptoms, particularly disinhibition (79.3%-100% of patients) and compulsive behavior (64.3%-100% of patients), compared with *C9orf72* carriers (51.7%-95.8% of patients with disinhibition and 34.5%-75.0% with compulsive behavior) and *GRN* carriers (38.2%-100% with disinhibition and 20.6%-100% with compulsive behavior). Alongside behavioral symptoms, neuropsychiatric symptoms were very frequently reported in patients with genetic FTD: anxiety and depression were most common in *GRN* carriers (23.8%-100% of patients) and *MAPT* carriers (26.1%-77.8% of patients); hallucinations,

(continued)

Key Points

Question Do behavioral and neuropsychiatric symptoms evolve differently in patients with distinct genetic variations for frontotemporal dementia?

Findings In this cohort study of 232 patients with genetic frontotemporal dementia, patients with *MAPT* variants showed the highest frequency and severity of most behavioral symptoms compared with *C9orf72* and *GRN* carriers. Anxiety and depression were most common in *GRN* and *MAPT* carriers; hallucinations, particularly auditory and visual, were most common in *C9orf72* carriers.

Meaning These findings suggest that behavioral and neuropsychiatric disturbances differ between the common frontotemporal dementia gene variations and have different trajectories through the course of disease.

+ Supplemental content

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related to aphasia and comprehension deficits. Nevertheless, we evaluated the core symptoms that define the criteria for behavioral variant FTD and that are virtually always encountered during the disease. Considering the wide variability of symptoms in patients with genetic FTD, even within individuals with the same gene variation in the same family, generalization of these results to single patients could be misleading. The current analysis does, however, represent one of the largest and best characterized studies in monogenic FTD to our knowledge. Further studies should assess the actual correspondence between these models and the observed symptoms during the natural history of the disease.

Conclusions

In conclusion, the results of this cohort study suggest that behavioral and neuropsychiatric disturbances differ between the common FTD gene variations and have different trajectories through the course of the disease. This finding has crucial implications for counseling patients and caregivers and is very important for the design of disease-modifying treatment trials in genetic FTD.

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RESEARCH ARTICLE

Impairment of episodic memory in genetic frontotemporal dementia: A GENFI study

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Analysis of *C9orf72* Intermediate Alleles in a Retrospective Cohort of Neurological Patients: Risk Factors for Alzheimer's Disease?

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Abstract.
Background: *C9orf72* hexanucleotide *GGGGCC (G4C2)* large repeat expansions within the first intron of the gene are a major cause of familial frontotemporal dementia, but also of apparently sporadic cases. Alleles with > 30 repeats are often considered pathogenic, but the repeat length threshold is still undefined. It is also unclear if *C9orf72* intermediate alleles (9–30 repeats) have clinically significant effects.
Objectives: We correlated the presence of *C9orf72* intermediate alleles with clinical diagnoses in a perspective cohort referred to a secondary memory clinic.
Methods: All samples were genotyped with AmplideXPCR/CE C9ORF72 Kit (Asuragen, Inc), an optimized *C9orf72* PCR amplification reagent.
Results: We showed that in patients with Alzheimer's disease (AD) the frequency of the intermediate repeat alleles was significantly increased versus controls (34/54, 63% AD versus 16/39, 41% CTRLs, $^{*}p = 0.01$, OR 2.91 CI 95% 1.230–6.077), whereas no significant differences ($p > 0.05$) were observed when comparing all other dementias with non-demented individuals.
Conclusion: Our findings suggest that *C9orf72* intermediate repeat units may represent a genetic risk factor, contributing to the occurrence of AD. Nevertheless, further longitudinal studies, including larger cohort of subjects with intermediate alleles with long-term follow-up, would be needed to confirm these results.

Keywords: Alzheimer's disease, C9orf72, intermediate repeats, risk factor, two-mode multiplexed PCR chemistry

INTRODUCTION

C9orf72 hexanucleotide GGGGCC (G4C2) large repeat expansions within the first intron of the gene are a major cause of autosomal dominant frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS), but also of apparently sporadic cases [1, 2]. A repeat length of > 30 units is defined as pathogenic in accordance with one of the original studies about *C9orf72* expansions [1]. Healthy individuals have 2–30 repeat units on both alleles, commonly 2, 5, and 8 units [3]. Nevertheless, it is important to underline that an exact cut-off to dis-

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Differential early subcortical involvement in genetic FTD within the GENFI cohort

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ARTICLE OPEN ACCESS CLASS OF EVIDENCE

Plasma Neurofilament Light for Prediction of Disease Progression in Familial Frontotemporal Lobar Degeneration

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Abstract

Objective

We tested the hypothesis that plasma neurofilament light chain (NfL) identifies asymptomatic carriers of familial frontotemporal lobar degeneration (FTLD)–causing mutations at risk of disease progression.

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Criteria for rating therapeutic and diagnostic studies
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From the University of California, San Francisco (J.C.R., P.W., A.M.S., Y.C., A.W., S.-Y.M.G., P.A.L., H.W.H., J.C.F., J.B.T., A.M.K., L.L.M., J.K., J.H.K., B.L.M., H.J.S., A.L.B.); UK Dementia Research Centre (C.H., D.M.C., R.S.C., M.B., M.F., C.V.G., G.P., L.R., I.S., E.T., J.D.R.), UCL Institute of Neurology, Queen Square, London; Quanterix Corp (E.V., L.S., A.J., D.H.), Lexington; Novartis Institutes for Biomedical Research Inc (L.Y., A. Khinikar, R.S.), Cambridge, MA; Novartis Pharma AG (A. Kieloch, M.-A.V.), Basel, Switzerland; Bluefield Project to Cure Frontotemporal Dementia (L.L.M., R.P.), San Francisco, CA; Mayo Clinic (K.K., D.S.K., B.F.B.), Rochester, MN; Mayo Clinic (N.G.-R., L.P., R.R.), Jacksonville, FL; University of Pennsylvania (D.J.I., M.G.), Philadelphia; University of California, Los Angeles (E.M.R., G.C., M.F.M., Y.B.); Harvard University/Massachusetts General Hospital (B.D.C.), Boston, MA; Washington University (N.G.), St. Louis, MO; Columbia University (E.D.H.), New York, NY; University of British Columbia (I.R.M., G.-Y.R.H.), Vancouver, Canada; Case Western Reserve University (B.S.A.), Cleveland, OH; University of Washington (K.D.-R.), Seattle; Laboratory of Neuroimaging (A.W.T.), University of Southern California, Los Angeles; Northwestern University (S.W.), Chicago, IL; University of North Carolina (D.I.K.), Chapel Hill; Texas Health Presbyterian Hospital Dallas (D.K.); University of California, San Diego (I.L.); Johns Hopkins Hospital (C.U.O., A.P.), Baltimore, MD; University of Alabama at Birmingham (E.D.R.); University of Toronto (M.C.T., M.M.), Ontario, Canada; Indiana University School of Medicine (T.F.), Indianapolis; Biogen Inc (W.C., J.C., D.L.G.), Cambridge, MA; Erasmus Medical Centre (J.C.v.S.), Rotterdam, the Netherlands; University of Brescia (B.B.), Italy; University of Barcelona (R.S.-V.); Donostia University Hospital (F.M.), San Sebastian, Gipuzkoa, Spain; Clinique Interdisciplinaire de Mémoire (R.L.), Département des Sciences Neurologiques, CHU de Québec; Faculté de Médecine (R.L.), Université Laval, Quebec, Canada; Center for Alzheimer Research (C.G.), Division of Neurogeriatrics, Department of Neurobiology, Care Sciences and Society, Bioclinicum, Karolinska Institutet; Unit for Hereditary Dementias (C.G.), Theme Aging, Karolinska University Hospital, Solna, Sweden; University of Tübingen (M.S.); Center for Neurodegenerative Diseases (DZNE) (M.S.), Tübingen, Germany; Fondazione IRCCS Ospedale Policlinico (D.G.); University of Milan (D.G.), Centro Dino Ferrari, Italy; Department of Clinical Neurosciences and Cambridge University Hospital (J.B.R.), University of Cambridge, UK; University of Western Ontario (E.F.), London, Canada; KU Leuven (R.V.), Belgium; Neurology Service (R.V.), University Hospitals Leuven, Belgium; University of Lisbon (A.d.M.), Portugal; Fondazione IRCCS Istituto Neurologico Carlo Besta (F.T.), Milan, Italy; University of Coimbra (I.S.), Portugal; McGill University (S.D.), Montreal, Québec, Canada; University of Oxford (C.R.B.); Wolfson Molecular Imaging Centre (A.G.), University of Manchester, UK; University of Duisburg-Essen (A.G.), Duisberg; Ludwig-Maximilians-Universität München (J.L., A.D.); German Center for Neurodegenerative Diseases (J.L.), Munich Cluster for Systems Neurology (SyNergy); University of Ulm (M.O.), Germany; and Department of Neuroscience, Psychology, Drug Research and Child Health (S.S.), University of Florence, and IRCCS Fondazione Don Carlo Gnocchi, Florence, Italy.

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Review Article

The distinct roles of monoamines in multiple sclerosis: A bridge between the immune and nervous systems?

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ABSTRACT

The monoaminergic neurotransmitters dopamine, noradrenaline, and serotonin are pivotal actors of the interplay between the nervous and the immune system due to their ability of binding to cell-receptors of both systems, crucially regulating their function within the central nervous system and the periphery. As monoamines are dysfunctional in many neurological and psychiatric diseases, they have been successfully used as pharmacological targets. Multiple sclerosis (MS) is one of the best examples of neurological disease caused by an altered interaction between the nervous and immune system and emerging evidence supports a dysregulation of monoaminergic systems in the pathogenesis of MS, secondary to both inflammation-induced reduction of monoamines' synthesis and structural damage to monoaminergic pathways within the brain. Here we review the evidence for monoamines being key mediators of neuroimmune interaction, affecting MS pathogenesis and course. Moreover, we discuss how the reduction/dysfunction of monoamines in MS may contribute to some clinical features typical of the disease, particularly fatigue and depression. Finally, we summarize different drugs targeting monoamines that are currently under evaluation for their potential efficacy to treat MS, as well as to alleviate fatigue and depression in MS.

1. Introduction

Multiple Sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), and the leading cause of non-traumatic disability in young adults in the developed world (Murray, 2006). The complex pathogenesis of MS is still largely unknown with respect to dysregulation of the immune system that pathologically targets the CNS myelin and oligodendrocytes (Weiner, 2008). Both adaptive and innate immune systems are involved to different extents during the course of the disease. The early, relapsing-remitting (RR) stage of MS is associated with antigen-specific T and B cell-mediated adaptive immune responses, whereas the progressive (P) phase is associated with innate immune responses characterized by chronic inflammation and microglial activation (Weiner, 2008). Alongside inflammation, neurodegeneration and axonal loss are also early pathophysiological processes occurring to MS brains, eventually leading to the accumulation of brain atrophy and irreversible disability (Ellwardt and Zipp, 2014).

In recent years, the investigation of MS pathogenesis has focused on the reciprocal interactions between the immune and the nervous systems (Melnikov et al., 2018). Monoamines are crucial for these

Abbreviations: 5HT, serotonin; 5HTR, 5HT receptors; AR, adrenoreceptor; BDNF, brain-derived neurotropic factors; cAMP, cyclic adenosine monophosphate; CNS, central nervous system; DA, dopamine; DAT, dopamine transporter; DCs, dendritic cells; E, epinephrine; EAE, experimental autoimmune encephalomyelitis; FC, functional connectivity; HC, healthy controls; IDO, indoleamine-2,3-dioxygenase; LC, *locus coeruleus*; LPS, lipopolysaccharide; MAO, monoamine oxidase; MBrN, monoaminergic brainstem nuclei; MS, multiple sclerosis; MSF, MS fatigue; NA, noradrenaline; NFkB, nuclear factor kappa-b; P, progressive; PBMC, peripheral blood mononuclear cells; PFC, prefrontal cortex; RR, relapsing-remitting; RS-fMRI, resting-state functional MRI; SSRIs, selective serotonin reuptake inhibitors; T_{reg}, T-regulatory cells; VTA, ventral tegmental area; WM, white matter.

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Disruption of brainstem monoaminergic fibre tracts in multiple sclerosis as a putative mechanism for cognitive fatigue: a fixel-based analysis

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ABSTRACT

In multiple sclerosis (MS), monoaminergic systems are altered as a result of both inflammation-dependent reduced synthesis and direct structural damage. Aberrant monoaminergic neurotransmission is increasingly considered a major contributor to fatigue pathophysiology. In this study, we aimed to compare the integrity of the monoaminergic white matter fibre tracts projecting from brainstem nuclei in a group of patients with MS ($n = 68$) and healthy controls ($n = 34$), and to investigate its association with fatigue. Fibre tracts integrity was assessed with the novel fixel-based analysis that simultaneously estimates axonal density, by means of 'fibre density', and white matter atrophy, by means of fibre 'cross section'. We focused on ventral tegmental area, locus coeruleus, and raphe nuclei as the main source of dopaminergic, noradrenergic, and serotoninergic fibres within the brainstem, respectively. Fourteen tracts of interest projecting from these brainstem nuclei were reconstructed using diffusion tractography, and compared by means of the product of fibre-density and cross-section (FDC). Finally, correlations of monoaminergic axonal damage with the modified fatigue impact scale scores were evaluated in MS. Fixel-based analysis revealed significant axonal damage – as measured by FDC reduction – within selective monoaminergic fibre-tracts projecting from brainstem nuclei in MS patients, in comparison to healthy controls; particularly within the dopaminergic-mesolimbic pathway, the noradrenergic-projections to prefrontal cortex, and serotoninergic-projections to cerebellum. Moreover, we observed significant correlations between severity of cognitive fatigue and axonal damage within the mesocorticolimbic tracts projecting from ventral tegmental area, as well as within the locus coeruleus projections to prefrontal cortex, suggesting a potential contribution of dopaminergic and noradrenergic pathways to central fatigue in MS. Our findings support the hypothesis that axonal damage along monoaminergic pathways contributes to the reduction/dysfunction of monoamines in MS and add new information on the mechanisms by which monoaminergic systems contribute to MS pathogenesis and fatigue. This supports the need for further research into monoamines as therapeutic targets aiming to combat and alleviate fatigue in MS.

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CASE REPORT
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Case Report: Efficacy of Rituximab in a Patient With Familial Mediterranean Fever and Multiple Sclerosis

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Familial Mediterranean Fever (FMF) is a genetic autoinflammatory disease characterized by recurrent episodes of fever and serositis caused by mutations in the MEFV gene, while Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the CNS with genetic and environmental etiology. The two diseases rarely occur in association with relevant implications for clinical management and drug choice. In this paper, we present the case of a 53-year-old male with an autosomal dominant FMF since childhood who presented acute paresthesia at the right part of the body. He performed a brain and spinal cord MRI, which showed multiple brain lesions and a gd-enhancing lesion in the cervical spinal cord, and then received a diagnosis of MS. He then started Interferonβ-1a which was effective but not tolerated and caused hepatotoxicity, and then shifted to Rituximab with 3-month clinical and neuroradiological efficacy.

Keywords: multiple sclerosis, rituximab, hepatotoxicity, case report, familal mediterranean fever

BACKGROUND

Familial Mediterranean Fever (FMF) is an inherited disease caused by mutations in *MEFV* (Mediterranean fever) gene, which encodes the pyrin protein, an important modulator of innate immunity. *MEFV* gene is localized on chromosome 16p13.3 and consists of 10 exons. Five founder mutations (M680I, M694V, M694I, V726A, E148Q) account for over 85% of Mediterranean-origin based FMF cases, with M694V mutation being the most common (1), associated with worse prognosis and higher risk of comorbidity.

FMF is commonly reported in the Mediterranean region, with a prevalence of 1:150–1:10.000 (2). The reason is likely the so called "founder effect," which is the migration of a small group of people, in this case of Jewish origin, from a larger population to go settling in another environment.

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Diogenes syndrome in dementia: a case report

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Background

Diogenes syndrome is a neurobehavioural syndrome characterised by domestic squalor, hoarding and lack of insight. It is an uncommon but high-mortality condition, often associated with dementia.

Aims

To describe the clinical features and treatment of Diogenes syndrome secondary to behavioural variant frontotemporal dementia (bvFTD).

Method

We describe a case of bvFTD in a 77-year-old man presenting with Diogenes syndrome.

Results

The patient's medical and psychiatric histories were unremarkable, but in recent years he had begun packing his flat with 'art pieces'. Mental state examination revealed confabulation and more structured delusions. Neuropsychological evaluation outlined an impairment in selective attention and letter verbal fluency, but no semantic impairment, in the context of an overall preserved mental functioning. Brain magnetic resonance imaging and positron emission tomography (PET) with fluorodeoxyglucose showed mild bilateral temporo-insular atrophy and hypometabolism in the left-superior temporal gyrus respectively. An amyloid PET scan and genetic analysis covering the dementia spectrum were normal. A diagnosis of bvFTD was made.

Conclusions

The clinical framing of behavioural symptoms of dementia such as hoarding poses a diagnostic challenge. This case illustrates the importance of a deeper understanding of Diogenes syndrome, leading to timelier diagnosis and effective therapeutic strategies.

Keywords

Diogenes syndrome; frontotemporal dementia; hoarding; collecting.

Diogenes syndrome – named after the Greek philosopher and cynic – is a neurobehavioural syndrome characterised by severe domestic squalor, pathological hoarding and lack of insight into the condition,[1] the latter preventing the majority of patients from seeking medical help. First cases of elderly patients with self-neglect and extreme lack of hygiene were published by Dupré in 1925[2] and Stevens in 1963[3] and then more precisely described as a syndrome in 1966 by Macmillan & Shaw,[4] who called this condition 'senile breakdown'. Halliday et al in 2000 proposed domestic squalor, evidence of self-neglect, living alone, tendency to hoard and lack of concern for surroundings as the five defining features of the syndrome and they also developed the Environmental Cleanliness and Clutter Scale to assess squalor and hoarding.[5] However, even in their study only 22% of individuals met all items on the scale and diagnostic criteria still lack consensus.[5]

The majority of cases occur in older adults (average age of 79 years) who live alone, although rare cases have been described in siblings and married couples.[6] The current incidence of the full syndrome is not well-known: a retrospective observational French study found 1.6 cases per 10 000 inhabitants; 25% of patients had the complete syndrome and 75% had the partial syndrome.[7] Cipriani et al estimate an approximate annual incidence of 0.05% in people over the age of 60.[8] In all probability, our society will be faced with more cases in the future owing to increased life expectancy and a consequent higher number of elderly individuals living alone.

Despite being an uncommon condition, diagnosis is paramount since Diogenes syndrome has been associated with increased morbidity and a 46% 5-year mortality rate,[9] with death commonly due to physical illnesses subsequent to self-neglect. Moreover, Diogenes syndrome poses ethical questions and legal challenges, such as finding a balance between autonomy and beneficence.[10]

Although the syndrome may occur as a single entity – so-called primary Diogenes syndrome – it is usually secondary to an underlying neuropsychiatric condition. Overall, Diogenes syndrome tends to be associated with psychosis, alcohol-induced disorder, affective disorder or obsessive–compulsive disorder (OCD) in younger individuals, whereas it is frequently associated with dementia in the elderly: clinical hoarding behaviour, often associated with self-neglect, and Diogenes syndrome are described in 23%[11] and 15%[12] respectively of older individuals with dementia. The unique combination of behavioural symptoms of behavioural variant frontotemporal dementia (bvFTD) may predispose to a high likelihood of developing Diogenes syndrome, which in fact occurs in up to 36% of people with bvFTD.[13] We describe here a case of bvFTD presenting with Diogenes syndrome and subsequent follow-up of the patient.

Ethics statement

Informed written consent approved by the local Institutional Review Board was obtained from the patient, in accordance with specific national laws and the ethics standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Case report

A 77-year-old dextral man was referred to the emergency department of our hospital by the police because he was found on the landing outside his flat, unkempt and dishevelled in personal appearance, after reportedly having lived there for 10 days. When questioned about this behaviour, he claimed that he was waiting for his next-door neighbour, who held a spare set of his flat keys, since he had accidentally locked his set in his garage. However, while he believed her to

impulse control disorders and the approved use of SSRIs in the treatment of OCD, a therapeutic trial with molecules from one of these classes may be justified.

In fact, Finney et al reported some effect of high-dose sertraline in reducing collecting behaviours in two of their patients.[23] Other authors found benefit on hoarding from other SSRIs[26] and a combination of valproic acid and quetiapine in treating patients with bvFTD and secondary Diogenes syndrome.[27] Lithium significantly improved one patient with long-standing Diogenes syndrome, although probably secondary to bipolar disorder,[28] and risperidone reduced hoarding in an individual with Diogenes syndrome, but caused significant motor side-effects.[29] Although not specifically tried for hoarding, trazodone has proven beneficial for behavioural symptoms in FTD.[30]

Our patient needed typical antipsychotics to manage agitation but quitting hoarding was mainly due to hospital admission and no specific treatment was tried. Admitting patients to hospital or moving them to another location is sometimes mandatory, but out-patient treatment through community care should be privileged if there is little risk to the patient or neighbours. Management should be conducted sensitively lest patients return to previous living conditions even more reluctant to receive medical aid.

Implications

This case, while illustrating the importance of suspecting Diogenes syndrome in elderly patients presenting with squalor and hoarding, especially in the context of dementia, supports recent evidence about the importance of the left temporal lobe in compulsive collecting pathogenesis. A deeper understanding of this condition, facilitating a timelier diagnosis, may lead to more effective pharmacological and non-pharmacological interventions, which in turn may reduce acute and chronic physical illness and improve social health outcomes.

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Data availability

The study data are available on reasonable request.

Author contributions

L.S. made substantial contributions to the acquisition of data and drafted the manuscript; ER., S.P., A.F., G.S., C.M., C.C., G.G.F., T.C., A.M.P., D.G.G., E.A.S., M.C. and P.B. made substantial contributions to the acquisition of data and revised the work; F.T. and G.M. made substantial contributions to the acquisition and interpretation of data and revised the work; A.A. made substantial contributions to the acquisition of data and drafted and revised the manuscript. All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work.

Declaration of interest

None.

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COVID-19

Facing the digital divide into a dementia clinic during COVID-19 pandemic: caregiver age matters

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Abstract

Background The coronavirus disease 2019 (COVID-19) pandemic has dramatically stressed the health care system and has provoked changes in population use of digital technologies. Digital divide is any uneven distribution in Information and Communications Technologies between people.

Aims The purpose of this work was to describe the digital divide of a population of patients with dementia contacted by telemedicine during Italian lockdown for COVID-19 pandemic.

Method One hundred eight patients with cognitive impairment were contacted by video call to perform a telemedicine neurological evaluation. Information on patients and caregivers attending the televisit were recorded.

Results Seventy-four patients connected with neurologist (successful televisit, 68.5%) and 34 patients were not able to perform televisit and were contacted by phone (failed televisit, 31.5%). No significant differences were observed among the two groups concerning age, gender, and education, but the prevalence of successful televisit was higher in the presence of younger caregivers: televisits performed in the presence of subjects of younger generation (sons and grandsons) had a successful rate higher (86% successful, 14% failed) than the group without younger generation caregiver (49% successful, 51% failed). This difference is mainly due to the ability of technological use among younger people.

Discussion The most impacting factors on digital divide in our population are the social support networks and the experience with the technology: the presence of a digital native caregiver. The COVID-19 pandemic is unmasking an emerging form of technology-related social inequalities: political and community interventions are needed to support the most socially vulnerable population and prevent social health inequalities.

Keywords Dementia · Caregiver · Telemedicine · Digital divide · Digital native

Introduction

Over the past few months, the coronavirus disease 2019 (COVID-19) pandemic has dramatically stressed the health care system. The rapid rate of COVID-19-related patient illnesses has caused the Ministry of Health to recommend postponing or canceling outpatient visits of patients with chronic diseases. Therefore, clinicians had to find strategies to deal with the management of their patients' problems during the lockdown: the rate at which medical practices have had to transition to telemedicine visits is just as fast as the rate at which COVID-19 is surging through the world [1]. From a technological perspective, the COVID-19 pandemic has provoked massive, immediate, and unprecedented changes in population use of digital technologies and media [2]. Online technologies became the privileged channel for governments and supranational entities such as the World Health Organization to convey their messages and recommendations. More importantly, technology is becoming central to maintain active social interactions [3]. This rapid transition has made it difficult for physicians and patients to anticipate barriers to successfully implement telemedicine visits, facing with digital divide.

Digital divide is any uneven distribution in the access to, use of, or impact of Information and Communications

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Alzheimer's Research & Therapy

RESEARCH Open Access

The Revised Self-Monitoring Scale detects early impairment of social cognition in genetic frontotemporal dementia within the GENFI cohort

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Abstract

Background: Although social cognitive dysfunction is a major feature of frontotemporal dementia (FTD), it has been poorly studied in familial forms. A key goal of studies is to detect early cognitive impairment using validated measures in large patient cohorts.

Methods: We used the Revised Self-Monitoring Scale (RSMS) as a measure of socioemotional sensitivity in 730 participants from the genetic FTD initiative (GENFI) observational study: 269 mutation-negative healthy controls, 193 *C9orf72* expansion carriers, 193 *GRN* mutation carriers and 75 *MAPT* mutation carriers. All participants underwent the standardised GENFI clinical assessment including the 'CDR® plus NACC FTLD' scale and RSMS. The RSMS total score and its two subscores, socioemotional expressiveness (EX score) and modification of self-presentation (SP score) were measured. Volumetric T1-weighted magnetic resonance imaging was available from 377 mutation carriers for voxel-based morphometry (VBM) analysis.

Results: The RSMS was decreased in symptomatic mutation carriers in all genetic groups but at a prodromal stage only in the *C9orf72* (for the total score and both subscores) and *GRN* (for the modification of self-presentation subscore) groups. RSMS score correlated with disease severity in all groups. The VBM analysis implicated an overlapping network of regions including the orbitofrontal cortex, insula, temporal pole, medial temporal lobe and striatum.

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all mutation groups, with *C9orf72* carriers also exhibiting an association with superior temporal gyrus and *GRN* and *MAPT* carriers showing a correlation with inferior temporal gyri specifically. Our results also show an association of the basal ganglia, particularly the caudate and putamen, in all genetic groups. These subcortical regions are also known to be implicated in emotion recognition [36–38], an integral factor in an individual's performance on the RSMS.

Overall, there appears to be a network of brain regions associated with impairment of socioemotional sensitivity in FTD that includes frontal, temporal, insula and striatal areas, including significant crossover with areas involved in the salience network, thus supporting the established role of aberrant saliency detection in FTD-related social cognitive dysfunction.

Limitations

These data should be interpreted in light of some limitations. Despite the large nature of GENFI in comparison to other FTD studies, one limitation lies in the relatively small numbers in some of the groups once stratified. Future studies should aim to replicate these findings in larger cohorts, as well as investigate longitudinal changes in socioemotional sensitivity over time.

Another limitation lies in the design of the RSMS, due to the inclusion of reverse scoring. While every effort is taken to ensure the informant understands how to answer correctly, we cannot eliminate the chance of misinterpretation.

Although the RSMS has been examined in a number of studies previously, and the data presented here suggests it could potentially be included as an outcome measure in genetic FTD trials, there has been limited validation of the questionnaire so far and more work will be necessary e.g. investigation of test-retest reliability.

Lastly, while global CDR® plus NACC FTLD scoring is a validated and robust tool used to measure disease severity in FTD, the assessment of motor and neuropsychiatric symptoms is not included. With FTD representing a diverse spectrum of symptomatic profiles, a limitation of this study lies in possible mis-categorisation of individuals who might be at a more advanced stage of their disease but present with symptoms that are not specifically addressed by this scale.

Conclusions

In summary, this study describes the ability of the RSMS to detect early changes in socioemotional behaviour in distinct genetic cohorts of FTD and illustrates the neural correlates of self-monitoring in these populations. Whilst further studies will be needed to validate the RSMS and explore how it changes over time, the present data suggests it may well serve as a useful outcome measure in future clinical trials.

Abbreviations

FTD: Frontotemporal dementia; RSMS: Revised Self-Monitoring Scale; GENFI: Genetic FTD initiative; *C9orf72*: Chromosome 9 open-reading frame 72; *GRN*: Progranulin; *MAPT*: Microtubule-associated protein tau; CDR® plus NACC FTLD: CDR® Dementia Staging Instrument with National Alzheimer Coordinating Centre Frontotemporal Lobar Degeneration component; VBM: Voxel-based morphometry; bvFTD: Behavioural variant FTD; CDR® plus NACC FTLD-SB: CDR® plus NACC FTLD sum of boxes; EX: RSMS socio-emotional expressiveness subscore; SP: RSMS modification of self-presentation subscore; SPM: Statistical parametric mapping; GM: Grey matter; WM: White matter; CSF: Cerebrospinal fluid; DARTEL: Fast-diffeomorphic image registration algorithm; MNI: Montreal Neurological Institute; TIV: Total intracranial volume; FWE: Family-Wise Error

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-021-00865-w.

Additional file 1: Figure S1. RSMS EX scores in each genetic carrier group, stratified by Global CDR® plus NACC FTLD scores. Significant differences from controls and within each carrier group are starred. Differences between carrier groups are not shown. **Figure S2.** RSMS SP scores in each genetic carrier group, stratified by Global CDR® plus NACC FTLD scores. Significant differences from controls and within each carrier group are starred. Differences between carrier groups are not shown. **Figure S3.** Negative correlations between RSMS total and CDR® plus FTLD NACC SOB scores were observed across all mutation carrier groups: C9orf72 ($r = -0.67$, $p < 0.001$), GRN ($r = -0.59$, $p < 0.001$), MAPT ($r = -0.53$, $p < 0.001$). Each dot represents one mutation carrier. **Table S1.** RSMS total test scores (mean and SD) in healthy controls split by age group. **Table S2.** Cumulative frequency of RSMS total test scores in healthy controls. **Table S3.** Adjusted mean differences in RSMS EX scores between the genetic groups stratified by Global CDR® plus NACC FTLD scores with 95% bias-corrected confidence intervals (significant values in bold). **Table S4.** Adjusted mean differences in RSMS SP scores between the genetic groups stratified by Global CDR® plus NACC FTLD scores with 95% bias-corrected confidence intervals (significant values in bold). **Table S5.** Correlation of RSMS total test score with cognitive tests. Significant results are in bold. **Table S6.** Positive neuroanatomical correlates of grey matter volume with the RSMS total score in each genetic group.

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Distinct patterns of MRI lesions in MOG antibody disease and AQP4 NMOSD: a systematic review and meta-analysis

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ABSTRACT

Background: the distinct MRI features of MOG-antibody disease (MOG-AD) and AQP4-NMOSD are still poorly defined. We performed a systematic review and meta-analysis to identify specific patterns of MRI abnormalities able to discriminate between MOG-AD and AQP4-NMOSD.

Methods: fourteen case-series (1028 patients) were included. Outcomes were MRI lesion patterns in optic nerve (ON), brain and spinal cord (SC) that were selected after a systematic literature review and analysed separately as the event rate for individual MRI lesions in MOG-AD (experimental group) and AQP4-NMOSD (control group) by using a random effect model.

Results: MOG-AD showed a higher number of MRI lesions than AQP4-NMOSD patients in the retrobulbar ON (OR=5.67; 95%CI=2.11–15.24; p=0.0006) with ON head swelling (OR=8.20; 95%CI=4.13–16.28; p<0.00001), corpus callosum (OR=2.30; 95%CI=1.11–4.76; p=0.02), pons (OR=2.87; 95%CI=1.45–5.67; p=0.002), and lumbar/conus SC (OR=3.47; 95%CI=1.66–7.24; p=0.0009). Conversely, lesions in the canalicular (OR=0.42; 95%CI=0.18–0.98; p=0.05) and intracranial ON (OR=0.30; 95%CI=0.11=0.84; p=0.02), area postrema (OR=0.12; 95%CI=0.02–0.61; p=0.01), medulla (OR=0.40; 95%CI=0.20–0.78; p=0.007), and cervical SC (OR=0.29; 95%CI=0.09–0.92; p=0.04) were prominent in patients with AQP4-NMOSD. Participants' age was found to be a source of heterogeneity across studies.

Conclusion: our study provides further evidence that MOG-AD and AQP4-NMOSD have distinct MRI features that may help clinicians for an early differential diagnosis.

Abbreviations

4thVL	4th ventricle lesions
AQP4	aquaporin-4
CI	confidence intervals
DWM	deep white matter
EAE	experimental autoimmune encephalomyelitis
GM	gray matter
LETM	longitudinally-extended transverse myelitis
MOG	myelin oligodendrocyte glycoprotein
MOG-AD	MOG-antibody disease
MS	multiple sclerosis
NMOSD	neuromyelitis optica spectrum disorders
ON	optic nerve
OR	odds-ratio
PVL	periventricular lesions
SC	spinal cord
WM	white matter

1. Introduction

Neuromyelitis-optica spectrum disorders (NMOSD) and myelin oligodendrocyte glycoprotein (MOG)-antibody disease (MOG-AD) are demyelinating disorders of the CNS that have been recently recognized

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Research Report

More Atypical than Atypical Alzheimer's Disease Phenotypes: A Treviso Dementia (TREDEM) Registry Case Report

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Abstract.
Background: A 57-year-old right-handed man was admitted to the Treviso Memory Clinic due to the presence of memory forgetfulness, repetition of the same questions, episodes of confusion, initial difficulties in performing complex tasks and easy distraction over the past two years, as well as recurrent and never-happened-before car accidents.
Objective: We report a peculiar case of an early onset Alzheimer's disease (AD) with an unusual symptomatology, apparently not fitting in any of the categorized atypical forms of AD nor being representative of a typical amnestic AD.
Methods: The patient underwent a neuropsychological, structural, and metabolic cerebral evaluation by MRI and ^{18}F-FDG PET, together with the search for cerebral amyloid (amyloid PET), a genetic testing for dementia related genes and the dosage of CSF protein biomarkers of neurodegenerative conditions.
Results: We observed a convergence of predominant frontal (dysexecutive, verbal disinhibition) and posterior (visuospatial) features of cognitive impairment. Structural MRI sequences showed subarachnoid spaces of the vault enlarged in the fronto-parietal region with anterior and posterior cortical atrophy. The hippocampus appeared preserved. The ^{18}F-FDG PET scans showed hypometabolism in the prefrontal, lateral temporal, posterior parietal, and occipital regions bilaterally. The ^{18}F-Flutemetamol scan showed a diffused uptake of the amyloid tracer at the cerebral cortex. CSF biomarkers were compatible with Alzheimer's disease (AD).
Conclusion: This case report presented with clinical phenotypic aspects atypical of AD, both frontal and posterior, never described as concomitant in the most accredited criteria for atypical AD, and appeared therefore more atypical than each of the atypical AD phenotypes already reported.

Keywords: Alzheimer's disease, frontal variant, magnetic resonance, neurodegenerative diseases, PET scan, posterior cortical atrophy, TREDEM

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RESEARCH ARTICLE OPEN ACCESS

Characterizing the Clinical Features and Atrophy Patterns of *MAPT*-Related Frontotemporal Dementia With Disease Progression Modeling

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Abstract

Background and Objective

Mutations in the *MAPT* gene cause frontotemporal dementia (FTD). Most previous studies investigating the neuroanatomical signature of *MAPT* mutations have grouped all different mutations together and shown an association with focal atrophy of the temporal lobe. The variability in atrophy patterns between each particular *MAPT* mutation is less well-characterized. We aimed to investigate whether there were distinct groups of *MAPT* mutation carriers based on their neuroanatomical signature.

Methods

We applied Subtype and Stage Inference (SuStaIn), an unsupervised machine learning technique that identifies groups of individuals with distinct progression patterns, to characterize patterns of regional atrophy in *MAPT*-associated FTD within the Genetic FTD Initiative (GENFI) cohort study.

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ARTICLE

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A multicentre validation study of the diagnostic value of plasma neurofilament light

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Increased cerebrospinal fluid neurofilament light (NfL) is a recognized biomarker for neurodegeneration that can also be assessed in blood. Here, we investigate plasma NfL as a marker of neurodegeneration in 13 neurodegenerative disorders, Down syndrome, depression and cognitively unimpaired controls from two multicenter cohorts: King's College London ($n = 805$) and the Swedish BioFINDER study ($n = 1,464$). Plasma NfL was significantly increased in all cortical neurodegenerative disorders, amyotrophic lateral sclerosis and atypical parkinsonian disorders. We demonstrate that plasma NfL is clinically useful in identifying atypical parkinsonian disorders in patients with parkinsonism, dementia in individuals with Down syndrome, dementia among psychiatric disorders, and frontotemporal dementia in patients with cognitive impairment. Data-driven cut-offs highlighted the fundamental importance of age-related clinical cut-offs for disorders with a younger age of onset. Finally, plasma NfL performs best when applied to indicate no underlying neurodegeneration, with low false positives, in all age-related cut-offs.

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Common variants in Alzheimer's disease and risk stratification by polygenic risk scores

Genetic discoveries of Alzheimer's disease are the drivers of our understanding, and together with polygenetic risk stratification can contribute towards planning of feasible and efficient preventive and curative clinical trials. We first perform a large genetic association study by merging all available case-control datasets and by-proxy study results (discovery $n = 409{,}435$ and validation size $n = 58{,}190$). Here, we add six variants associated with Alzheimer's disease risk (near *APP, CHRNE, PRKD3/NDUFAF7, PLCG2* and two exonic variants in the *SHARPIN* gene). Assessment of the polygenic risk score and stratifying by *APOE* reveal a 4 to 5.5 years difference in median age at onset of Alzheimer's disease patients in *APOE* ε4 carriers. Because of this study, the underlying mechanisms of *APP* can be studied to refine the amyloid cascade and the polygenic risk score provides a tool to select individuals at high risk of Alzheimer's disease.

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Molecular Neurodegeneration

RESEARCH ARTICLE Open Access

A panel of CSF proteins separates genetic frontotemporal dementia from presymptomatic mutation carriers: a GENFI study

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Abstract

Background: A detailed understanding of the pathological processes involved in genetic frontotemporal dementia is critical in order to provide the patients with an optimal future treatment. Protein levels in CSF have the potential to reflect different pathophysiological processes in the brain. We aimed to identify and evaluate panels of CSF proteins with potential to separate symptomatic individuals from individuals without clinical symptoms (unaffected), as well as presymptomatic individuals from mutation non-carriers.

Methods: A multiplexed antibody-based suspension bead array was used to analyse levels of 111 proteins in CSF samples from 221 individuals from families with genetic frontotemporal dementia. The data was explored using LASSO and Random forest.

Results: When comparing affected individuals with unaffected individuals, 14 proteins were identified as potentially important for the separation. Among these, four were identified as most important, namely neurofilament medium polypeptide (NEFM), neuronal pentraxin 2 (NPTX2), neurosecretory protein VGF (VGF) and aquaporin 4 (AQP4). The combined profile of these four proteins successfully separated the two groups, with higher levels of NEFM and AQP4 and lower levels of NPTX2 in affected compared to unaffected individuals. VGF contributed to the models, but the levels were not significantly lower in affected individuals. Next, when comparing presymptomatic *GRN* and *C9orf72* mutation carriers in proximity to symptom onset with mutation non-carriers, six proteins were identified with a potential to contribute to a separation, including progranulin (GRN).

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Conclusion

In conclusion, by using multivariate statistical methods to explore CSF levels of 111 proteins, we have identified a panel of four proteins (NEFM, AQP4, NPTX2 and VGF) which successfully distinguish most affected individuals from unaffected individuals. However, these four proteins were not able to separate between the different genes (mutation groups) or between the different clinical phenotypes. Furthermore, when focusing on PMC *GRN* and *C9orf72* close to expected symptom onset, we have identified five proteins (TARDBP, KNG1, HBEGF, MBP, CLSTN1) in addition to GRN, with the potential to contribute to the separation between PMC and NC. Continued exploration of these proteins, in independent cohorts, is needed in order to elucidate their potential association to FTD pathology.

Abbreviations

AD: Alzheimer disease; ALS: Amyotrophic lateral sclerosis; AMC: Affected mutation carriers; AMPH: Amphiphysin; ANOVA: Analysis of variance; APOA1: Apolipoprotein A1; APOE4: Apolipoprotein E isoform 4; AQP4: Aquaporin 4; AUC: Area under curve; bvFTD: Behavioural variant FTD; C4A/B: Complement C4B, complement C4A; C9orf72: Chromosome 9 open reading frame 72; CD14: Monocyte differentiation antigen CD14; CLSTN1: Calsyntenin-1; CSF: Cerebrospinal fluid; CTSS: Cathepsin S; D-NOS: Dementia not otherwise specified; FTD: Frontotemporal dementia; GENFI: Genetic Frontotemporal dementia Initiative; GRN: Progranulin; HBEGF: Heparin binding EGF like growth factor; KNG1: Kininogen 1; LASSO: Least absolute shrinkage and selection operator; MAPT: Microtubule associated protein tau; MBP: Myelin basic protein; NC: Non-carriers; NEFH/NfH: Neurofilament heavy polypeptide; NEFL/NfL: Neurofilament light polypeptide; NEFM/NfM: Neurofilament medium polypeptide; NPTX2: Neuronal pentraxin 2; OOB: Out-of-bag; PC: Principal component; PCA: Principal component analysis; PMC: Presymptomatic mutation carriers; PPA: Primary progressive aphasia; PRM: Parallel reaction monitoring; PSP: Progressive supranuclear palsy; PTPRN2: Protein tyrosine phosphatase, receptor type N2; ROC: Receiver operating characteristic; SEC63: Translocation protein SEC63 homolog; SERPINA3: Serpin family A member 3; SPP1: Secreted phosphoprotein 1; TARDBP/TDP-43: TAR DNA-binding protein 43; TBK1: TANK-binding kinase 1; VGF: Neurosecretory protein VGF

Supplementary Information

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Additional file 1.

Additional file 2.

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Differences in Sex Distribution Between Genetic and Sporadic Frontotemporal Dementia

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Abstract.
Background: Reported sex distributions differ between frontotemporal dementia (FTD) cohorts. Possible explanations are the evolving clinical criteria of FTD and its subtypes and the discovery of FTD causal genetic mutations that has resulted in varying demographics.
Objective: Our aim was to determine the sex distribution of sporadic and genetic FTD cases and its subtypes in an international cohort.
Methods: We included 910 patients with behavioral variant frontotemporal dementia (bvFTD; $n = 654$), non-fluent variant primary progressive aphasia (nfvPPA; $n = 99$), semantic variant primary progressive aphasia (svPPA; $n = 117$), and right temporal variant frontotemporal dementia (rtvFTD; $n = 40$). We compared sex distribution between genetic and sporadic FTD using χ^2-tests.
Results: The genetic FTD group consisted of 51.2% males, which did not differ from sporadic FTD (57.8% male, $p = 0.08$). In the sporadic bvFTD subgroup, males were predominant in contrast to genetic bvFTD (61.6% versus 52.9% males, $p = 0.04$). In the other clinical FTD subgroups, genetic cases were underrepresented and within the sporadic cases the sex distribution was somewhat equal.

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Dissemination in time and space in presymptomatic *granulin* mutation carriers: a GENFI spatial chronnectome study

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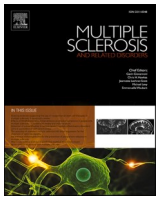

In vivo evidence of functional disconnection between brainstem monoaminergic nuclei and brain networks in multiple sclerosis

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ABSTRACT

Background: : brainstem monoaminergic (dopaminergic, noradrenergic, and serotoninergic) nuclei (BrMn) contain a variety of ascending neurons that diffusely project to the whole brain, crucially regulating normal brain function. BrMn are directly affected in multiple sclerosis (MS) by inflammation and neurodegeneration. Moreover, inflammation reduces the synthesis of monoamines. Aberrant monoaminergic neurotransmission contributes to the pathogenesis of MS and explains some clinical features of MS. We used resting-state functional MRI (RS-fMRI) to characterize abnormal patterns of BrMn functional connectivity (FC) in MS.

Methods: : BrMn FC was studied with multi-echo RS-fMRI in $n = 68$ relapsing-remitting MS patients and $n = 39$ healthy controls (HC), by performing a seed-based analysis, after producing standard space seed masks of the BrMn. FC was assessed between ventral tegmental area (VTA), locus coeruleus (LC), median raphe (MR), dorsal raphe (DR), and the rest of the brain and compared between MS patients and HC. Between-group comparisons were carried out only within the main effect observed in HC, setting $p<0.05$ family-wise-error corrected ($_{FWE}$).

Results: : in HC, VTA displayed FC with the core regions of the default-mode network. As compared to HC, MS patients showed altered FC between VTA and posterior cingulate cortex ($p<0.05_{FWE}$). LC displayed FC with core regions of the executive-control network with a reduced functional connection between LC and right prefrontal cortex in MS patients ($p<0.05_{FWE}$). Raphe nuclei was functionally connected with cerebellar cortex, with a significantly lower FC between these nuclei and cerebellum in MS patients, as compared to HC ($p<0.05_{FWE}$).

Conclusions: : our study demonstrated in MS patients a functional disconnection between BrMn and cortical/subcortical efferent targets of central brain networks, possibly due to a loss or a dysregulation of BrMn neurons. This adds new information about how monoaminergic systems contribute to MS pathogenesis and suggests new potential therapeutic targets.

Abbreviations: BA, Brodmann area; BrMn, brainstem monoaminergic nuclei; CNS, central nervous system; DMN, default mode network; DR, dorsal raphe; ECN, executive control network; EDSS, expanded disease status score; EPI, echo-planar imaging; ESS, epworth sleepiness scale; FC, functional connectivity; GM, grey matter; HADS-D, hospital anxiety and depression scale; HC, healthy controls; ICA, independent component analysis; LC, locus coeruleus; ME-ICA, multi-echo independent component analysis; MNI, montreal neurological institute; MR, median raphe; MS, multiple sclerosis; PCC, posterior cingulate cortex; PFC, prefrontal cortex; RR-MS, relapsing-remitting MS; RS-fMRI, resting-state functional magnetic resonance imaging; SDMT, symbol digit modalities test; SPM, Statistical Parametric Mapping; TE, echo-time; TIV, total intracranial volume; VBM, voxel-based morphometry; VTA, ventral tegmental area; WM, white matter; WM-LL, WM lesion-load.

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Niemann-Pick Type C 1 (*NPC1*) and *NPC2* Gene Variability in Demented Patients with Evidence of Brain Amyloid Deposition

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Abstract.
Background: Variants in Niemann-Pick Type C genes (*NPC1* and *NPC2*) have been suggested to play a role as risk or disease modifying factors for Alzheimer's disease (AD).
Objective: The aim of this study was to analyze *NPC1* and *NPC2* variability in demented patients with evidence of brain amyloid-β 1–42 (Aβ) deposition and to correlate genetic data with clinical phenotypes.
Methods: A targeted Next Generation Sequencing panel was customized to screen *NPC1*, *NPC2*, and main genes related to neurodegenerative dementias in a cohort of 136 demented patients with cerebrospinal fluid (CSF) low Aβ levels or positive PET with Aβ tracer and 200 non-demented geriatric subjects.
Results: Seven patients were carriers of *NPC* variants in heterozygosis. Four of them displayed pathogenic variants previously found in NPC patients and one AD patient had a novel variant. The latter was absent in 200 non-demented elderly subjects. Five of seven patients (70%) exhibited psychiatric symptoms at onset or later as compared with 43% in non-carriers ($p > 0.05$).
Conclusion: The frequency of *NPC1* and *NPC2* heterozygous variants in patients with CSF evidence of Aβ deposition is higher than in the general population.

Keywords: Amyloid, cerebrospinal fluid, *NPC1*, *NPC2*, Niemann-Pick Type C, psychiatric onset, variability

INTRODUCTION

Niemann-Pick Type C (NPC) is a rare neurovisceral disease characterized by abnormal lysosomal storage of lipids. It is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in two genes involved in the cholesterol trafficking: *NPC1* (95% of cases) and *NPC2*, encoding for late-endosomal and lysosomal protein, respectively [1]. The disruption in the lipidic metabolism leads to intracellular accumulation of unesterified cholesterol mainly in spleen, liver, and brain, leading to visceral and neurological symptoms [2]. NPC presents with a highly heterogeneous phenotype for both age of onset and clinical features [3]. Some conditions are indeed considered "clinical niches" for NPC, where patients with symptoms related to the pathology go overlooked for the presence of more relevant or more recognizable features, i.e., movement disorders and early-onset cognitive decline [4].

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widely investigated *NPC1* genetic variability in a large cohort of PD patients aiming to find novel genetic association with the disease. However, the study concluded that both common and rare variants in *NPC* genes were not associated with PD.

As already pointed out, all patients recruited had low CSF Aβ levels or evidence of Aβ deposition at PET [18], thus suggesting amyloid deposition in the brain. Nevertheless, after the complete workup, seven patients were diagnosed with other dementias rather than AD. To explain the observed low amyloid levels in patients diagnosed with other neurodegenerative diseases than AD, different hypotheses may be raised, including: 1) the accuracy of the test is not 100% and the definition of the threshold for defining CSF/PET Aβ levels "normal" is debatable; 2) amyloid deposition may co-occur with other pathologies. For instance, low CSF amyloid levels have been previously reported in carriers of the *C9ORF72* expansion [28], associated with TDP-43 deposition in the brain, or in patients with LBD, associated with synuclein deposition.

An unexpected result of the study was the presence of two causal mutations, one for AD (*APP*) and another for FTD (*GRN*). Regarding the former, the genetic counselling was not considered at time of diagnosis in light of current literature, suggesting an onset in the fifth decade of life and a complete penetrance [29]. The patient worsened over two years from diagnosis and was then lost at follow up. Regarding the latter, it is known that symptoms at presentation as well as the age at onset are very heterogeneous [30], and presentation with memory disturbances has been reported previously [31]. Despite *GRN* mutations are associated with behavioral disturbances, the patient never developed such symptoms over time (until the last visit, three years prior to death), never meeting current criteria for FTD [32].

In conclusion, this is the first study of *NPC1* and *NPC2* variability in a cohort of demented patients with evidence of brain Aβ deposition. We showed that the frequency of *NPC1* and *NPC2* heterozygous variants in patients with CSF or amyloid-PET evidence of amyloid deposition is higher than in the general population and is associated with behavioral and psychiatric symptoms. Even though NPC is a recessive inherited disorder, growing evidence highlighted the possible pathogenicity of heterozygous mutations. As speculated by Bauer et al., heterozygous mutation in *NPC* could have a dominant effect with reduced penetrance [12]. The high proportion of neurodegenerative diseases among NPC families further support this hypothesis. Nevertheless, further studies are needed to highlight the connection between NPC, amyloid deposition, and clinical phenotype.

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BRAIN COMMUNICATIONS

Cognitive fatigue in multiple sclerosis is associated with alterations in the functional connectivity of monoamine circuits

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Fatigue is a highly prevalent and debilitating symptom in multiple sclerosis, but currently the available treatment options have limited efficacy. The development of innovative and efficacious targeted treatments for fatigue in multiple sclerosis has been marred by the limited knowledge of the underlying mechanisms. One of the hypotheses postulates that multiple sclerosis pathology might cause reduced monoaminergic release in the central nervous system with consequences on motivation, mood and attention. Here, we applied the recently developed Receptor-Enriched Analysis of Functional Connectivity by Targets method to investigate whether patients with high and low fatigue differ in the functional connectivity (FC) of the monoamine circuits in the brain. We recruited 55 patients with multiple sclerosis, which were then classified as highly fatigued or mildly fatigued based on their scores on the cognitive sub-scale of the Modified Fatigue Impact scale. We acquired resting-state functional MRI scans and derived individual maps of connectivity associated with the distribution of the dopamine, noradrenaline and serotonin transporters as measured by positron emission tomography. We found that patients with high fatigue present decreased noradrenaline transporter (NAT)-enriched connectivity in several frontal and prefrontal areas when compared to those with lower fatigue. The NAT-enriched FC predicted negatively individual cognitive fatigue scores. Our findings support the idea that alterations in the catecholaminergic functional circuits underlie fatigue in multiple sclerosis and identify the NAT as a putative therapeutic target directed to pathophysiology.

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Parkinsonism and ataxia

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ABSTRACT

Ataxia is not a common feature in Parkinson's disease. Nevertheless, some rare forms of parkinsonism have ataxia as one of the main features in their clinical picture, especially those with juvenile or early-onset.

On the other side, in cerebellar degenerative diseases, parkinsonism might accompany the typical symptoms and even become predominant in some cases.

Many disorders involving different neurological systems present with a movement phenomenology reflecting the underlying pattern of pathological involvement, such as neurodegeneration with brain iron accumulation, neurodegeneration associated with calcium deposition, and metabolic and mitochondrial disorders. The prototype of sporadic disorders that present with a constellation of symptoms due to the involvement of multiple Central Nervous System regions is multiple system atrophy, whose motor symptoms at onset can be cerebellar ataxia or parkinsonism. Clinical syndromes encompassing both parkinsonian and cerebellar features might represent a diagnostic challenge for neurologists. Recognizing acquired and potentially treatable causes responsible for complex movement disorders is of paramount importance, since an early diagnosis is essential to prevent permanent consequences. The present review aims to provide a pragmatic overview of the most common diseases characterized by the coexistence of cerebellar and parkinsonism features and suggests a possible diagnostic approach for both inherited and sporadic disorders.

This article is part of the Special Issue "Parkinsonism across the spectrum of movement disorders and beyond" edited by Joseph Jankovic, Daniel D. Truong and Matteo Bologna.

1. Introduction

Ataxia is not a common feature in Parkinson's disease (PD). Nevertheless, some rare forms of parkinsonism have ataxia as one of the main features in their clinical picture, especially those with juvenile or early-onset.

On the other side, in cerebellar degenerative diseases, parkinsonism might accompany the typical symptoms and even become predominant in some cases.

Many disorders involving different neurological systems present with a movement phenomenology reflecting the underlying pattern of pathological involvement, such as neurodegeneration with brain iron accumulation (NBIA), neurodegeneration associated with calcium deposition, and metabolic and mitochondrial disorders. The prototype of sporadic disorders that present with a constellation of symptoms due to the involvement of multiple Central Nervous System (CNS) regions is multiple system atrophy (MSA), whose motor symptoms at onset can be cerebellar ataxia (MSA-C) or parkinsonism (MSA-P).

Clinical syndromes encompassing both parkinsonian and cerebellar features might represent a significant diagnostic challenge for neurologists.

The number of genetic loci associated with inherited ataxias is rapidly growing and constitute a whole set of heterogeneous diseases. Nevertheless, some peculiar anamnestic, clinic or radiologic features may guide the correct diagnosis and, finally, might be of substantial support in defining the prognosis.

Recognizing acquired and sometimes potentially treatable causes responsible for complex movement disorders combination is of paramount importance, since an early diagnosis is essential to prevent permanent consequences.

The present review aims to provide a pragmatic overview of the most common diseases characterized by the coexistence of cerebellar and parkinsonism features and suggests a possible diagnostic approach for both inherited and sporadic disorders.

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Review

Unravelling Genetic Factors Underlying Corticobasal Syndrome: A Systematic Review

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Abstract: Corticobasal syndrome (CBS) is an atypical parkinsonian presentation characterized by heterogeneous clinical features and different underlying neuropathology. Most CBS cases are sporadic; nevertheless, reports of families and isolated individuals with genetically determined CBS have been reported. In this systematic review, we analyze the demographical, clinical, radiological, and anatomopathological features of genetically confirmed cases of CBS. A systematic search was performed using the PubMed, EMBASE, and Cochrane Library databases, included all publications in English from 1 January 1999 through 1 August 2020. We found forty publications with fifty-eight eligible cases. A second search for publications dealing with genetic risk factors for CBS led to the review of eight additional articles. *GRN* was the most common gene involved in CBS, representing 28 out of 58 cases, followed by *MAPT*, *C9ORF72*, and *PRNP*. A set of symptoms was shown to be significantly more common in *GRN*-CBS patients, including visuospatial impairment, behavioral changes, aphasia, and language alterations. In addition, specific demographical, clinical, biochemical, and radiological features may suggest mutations in other genes. We suggest a diagnostic algorithm to help in identifying potential genetic cases of CBS in order to improve the diagnostic accuracy and to better understand the still poorly defined underlying pathogenetic process.

Keywords: corticobasal syndrome; corticobasal degeneration; CBS; atypical parkinsonism; genetics

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1. Introduction

Corticobasal syndrome (CBS) is a rare neurological disorder characterized by a combination of asymmetric akinetic–rigid parkinsonism, fixed dystonic postures, pyramidal signs, and cognitive deficits, such as behavioral changes, speech and language alterations, apraxia, visuospatial impairment, and other cortical signs, including alien-limb phenomena, myoclonus, and cortical sensory loss [1].

Typically, levodopa responsiveness is limited or absent. The clinical features correlate with neuroimaging evidence of asymmetric atrophy and hypometabolism, particularly in the striatum and parietal lobes [2]. CBS has a reported prevalence of 4.9 to 7.3 cases per 100,000 individuals [3]; symptoms usually appear between the fifth and the seventh decade of life [4], and death occurs within 6 or 7 years after the symptoms' onset [4,5].

Different diagnostic criteria for CBS have been proposed over time, including those advanced by Lang and Bergeron (Toronto) [6], Boeve et al. (Mayo clinic) [7], and Bak and Hodges (Cambridge) [8], as well as the latest by Armstrong et al. in 2013 [9]. Despite extensive efforts in developing more specific diagnostic criteria, the clinical diagnosis of CBS does not always match with neuropathological evidence of corticobasal degeneration

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Review

Targeting the Autonomic Nervous System for Risk Stratification, Outcome Prediction and Neuromodulation in Ischemic Stroke

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Abstract: Ischemic stroke is a worldwide major cause of mortality and disability and has high costs in terms of health-related quality of life and expectancy as well as of social healthcare resources. In recent years, starting from the bidirectional relationship between autonomic nervous system (ANS) dysfunction and acute ischemic stroke (AIS), researchers have identified prognostic factors for risk stratification, prognosis of mid-term outcomes and response to recanalization therapy. In particular, the evaluation of the ANS function through the analysis of heart rate variability (HRV) appears to be a promising non-invasive and reliable tool for the management of patients with AIS. Furthermore, preclinical molecular studies on the pathophysiological mechanisms underlying the onset and progression of stroke damage have shown an extensive overlap with the activity of the vagus nerve. Evidence from the application of vagus nerve stimulation (VNS) on animal models of AIS and on patients with chronic ischemic stroke has highlighted the surprising therapeutic possibilities of neuromodulation. Preclinical molecular studies highlighted that the neuroprotective action of VNS results from anti-inflammatory, antioxidant and antiapoptotic mechanisms mediated by α7 nicotinic acetylcholine receptor. Given the proven safety of non-invasive VNS in the subacute phase, the ease of its use and its possible beneficial effect in hemorrhagic stroke as well, human studies with transcutaneous VNS should be less challenging than protocols that involve invasive VNS and could be the proof of concept that neuromodulation represents the very first therapeutic approach in the ultra-early management of stroke.

Keywords: stroke; autonomic nervous system; heart rate variability; vagus nerve stimulation; risk stratification

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1. Introduction

Cerebrovascular disease ranks as one of the leading causes of disability and mortality worldwide [1,2]. Acute ischemic stroke (AIS), induced by abnormal cerebral perfusion due to sudden rupture or occlusion of cerebral vessels, is the most common type of stroke, accounting for 70% of all stroke cases [1]. In the general population, acute ischemic stroke has been found to be more frequent in men than in women [3]. However, women experience more severe strokes and have longer hospitalizations than men, resulting in higher percentages of permanent disability and mortality [4,5].

Autonomic nervous system (ANS) dysfunction and ischemic stroke have an intricate and deep interconnection. The disruption of autonomic regulatory pathways may determine clinical complications during both the acute and chronic phases of stroke, leading to a

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IM - CASE RECORD

A 79-year-old man with unexplained recurrent syncope and severe orthostatic hypotension

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Presentation of case

Dr. Montinaro: a 79-year-old man was admitted to our hospital, because of diarrhoea and pre-renal acute kidney injury AKI. The gastrointestinal syndrome developed 3 days before the admission. The symptoms were not responsive to common antimotility agents, and the patient developed severe fatigue and syncopal prodromes in orthostatism.

The patient's medical history included paucisymptomatic idiopathic pulmonary fibrosis IPF with usual interstitial pneumonia pattern treated with protein-kinase inhibitor Nintedanib, chronic kidney disease, bilateral nephrolithiasis, benign prostatic hyperplasia and previous cholecystectomy.

His medical record was also notable for a 2-year history of relapsing episodes of transient loss of consciousness (T-LOC) that did not have specific triggers, such as defecation, cough or sneeze, post-exercise, or emotional triggers. Some episodes lead to falls with severe traumatic outcomes, and in one case, the patient had been hospitalized for post-traumatic subarachnoid haemorrhage and the c5 vertebrae fracture. At that time, sick sinus syndrome was identified, and the patient was implanted with a bicameral pacemaker (DDDR) and discharged with calcium channel blockers. However, runs of sustained atrial tachycardia were seen, and the patient was started on low-dose beta-blockers and anticoagulation with a direct oral anticoagulant. Despite the listed interventions, the patient kept on experiencing severe traumatic falls due to syncopal T-LOC, but pacemaker interrogation did not reveal any further rhythm anomalies.

On arrival at the emergency department, the patient was unable to keep the upright position because of asthenia. The axillary temperature was 36 °C, the heart rate (HR) 78 beats per minute (bpm), the blood pressure (BP) in clinostatism was 120/90 mm Hg, and the oxygen saturation 98% while breathing in ambient air. The lungs presented bilateral crackles at the middle and lower fields, which were fibrosis-related findings previously described at pneumological follow-up. The heart was normal on auscultation, with rhythmic heart sounds and without detectable heart murmurs. He had diffused abdominal discomfort with point tenderness on palpation of the left flank, without signs of peritonism. Peripheral pulses were valid and symmetrical, and the skin was normally perfused.

Electrocardiography (ECG) showed sinus rhythm at 78 bpm, left anterior fascicular block, and some premature ventricular contractions, and was comparable to previous ECGs. On blood testing, a moderate deterioration in the baseline renal function was noted, with increased levels of serum creatinine and blood urea nitrogen, suggesting a pre-renal origin of AKI. There was a rise in acute phase proteins and international normalized ratio (INR) was prolonged. Serum electrolytes and blood levels of transaminases, alkaline phosphatase, bilirubin, lipase, and complete blood count were normal (Table 1). Urine dipstick was positive for leukocytes, erythrocytes, and protein.

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ORIGINAL ARTICLE

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Leukoencephalopathy with calcifications and cysts: Genetic and phenotypic spectrum

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BRAIN
CLINICAL TRIAL

Motor and cognitive outcomes of cerebello-spinal stimulation in neurodegenerative ataxia

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Cerebellar ataxias represent a heterogeneous group of disabling disorders characterized by motor and cognitive disturbances, for which no effective treatment is currently available.

In this randomized, double-blind, sham-controlled trial, followed by an open-label phase, we investigated whether treatment with cerebello-spinal transcranial direct current stimulation (tDCS) could improve both motor and cognitive symptoms in patients with neurodegenerative ataxia at short and long-term. Sixty-one patients were randomized in two groups for the first controlled phase. At baseline (T0), Group 1 received placebo stimulation (sham tDCS) while Group 2 received anodal cerebellar tDCS and cathodal spinal tDCS (real tDCS) for 5 days/week for 2 weeks (T1), with a 12-week (T2) follow-up (randomized, double-blind, sham controlled phase). At the 12-week follow-up (T2), all patients (Group 1 and Group 2) received a second treatment of anodal cerebellar tDCS and cathodal spinal tDCS (real tDCS) for 5 days/week for 2 weeks, with a 14-week (T3), 24-week (T4), 36-week (T5) and 52-week follow-up (T6) (open-label phase). At each time point, a clinical, neuropsychological and neurophysiological evaluation was performed. Cerebellar-motor cortex connectivity was evaluated using transcranial magnetic stimulation.

We observed a significant improvement in all motor scores (scale for the assessment and rating of ataxia, international cooperative ataxia rating scale), in cognition (evaluated with the cerebellar cognitive affective syndrome scale), in quality-of-life scores, in motor cortex excitability and in cerebellar inhibition after real tDCS compared to sham stimulation and compared to baseline (T0), both at short and long-term. We observed an addon-effect after two repeated treatments with real tDCS compared to a single treatment with real tDCS. The improvement at motor and cognitive scores correlated with the restoration of cerebellar inhibition evaluated with transcranial magnetic stimulation.

Cerebello-spinal tDCS represents a promising therapeutic approach for both motor and cognitive symptoms in patients with neurodegenerative ataxia, a still orphan disorder of any pharmacological intervention.

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Keywords: cerebellar ataxia; cognition; motor; transcranial direct current stimulation; cerebello-spinal stimulation

Abbreviations: CCAS = cerebellar cognitive affective syndrome; CCASS = cerebellar cognitive affective syndrome scale; ICARS = international cooperative ataxia rating scale; MEP = motor evoked potential; RMT = resting motor threshold; SARA = scale for the assessment and rating of ataxia; SCA = spinocerebellar ataxia; SF-36 = short-form health survey 36; tDCS = transcranial direct current stimulation; TMS = transcranial magnetic stimulation

Introduction

Neurodegenerative ataxias represent a heterogeneous group of disabling progressive diseases characterized by limb and gait ataxia, oculomotor deficits, dysarthria, and kinetic tremor.[1–3] Often these disorders result in the cerebellar cognitive affective syndrome (CCAS), whose hallmark features include deficits in executive function, visual spatial processing, linguistic skills and emotion regulation.[4,5] The genetic causes of ataxias involve a growing list of over 100 genes whose products are implicated into mitochondrial dysfunction, oxidative stress, abnormal mechanisms of DNA repair, possible protein misfolding, and abnormalities in cytoskeletal proteins.[6]

No effective treatment is currently available for most of these diseases, with the exception of the symptomatic, physical and occupational therapies, treatments in immune-mediated cerebellar ataxias, and specific supplementation as in vitamin E deficiency and spinocerebellar ataxia 38 (SCA38).[7,8] For this reason, there is an increasing interest in finding innovative approaches to reduce clinical symptoms. In this view, cerebellar transcranial direct current stimulation (tDCS) is a non-invasive treatment which promotes neuroplasticity and has been shown to improve motor symptoms in patients with neurodegenerative cerebellar ataxias.[9–19]

Our previous pilot study has supported the clinical efficacy of cerebello-spinal tDCS in these disorders.[20] The results were accomplished by the restoration of cerebellar inhibition, a neurophysiological measure which reflects the modulation of cerebellar excitability, using a transcranial magnetic stimulation (TMS) paired-pulse protocol.[20]

Despite the fact our findings were promising, several unmet issues still need to be addressed, and were the objective of this work: (i) to evaluate if treatment with cerebello-spinal tDCS may also improve cognitive symptoms, such as those related to CCAS; (ii) to verify whether two repeated tDCS treatments are superior to a single tDCS treatment in improving both motor and cognitive symptoms; (iii) to study when the effects tDCS treatments wane over time to define the best timing for repeated tDCS treatments; (iv) to evaluate if the effects on cerebellar excitability are long-lasting and if they correlate with motor and cognitive improvement; and (v) to increase sample size in order to improve generalizability of these results to specific groups of ataxias.

To perform these tasks, we conducted a double-blind, randomized, sham-controlled tDCS trial (5 days/week for 2 weeks) followed, after a 3-month washout period, by an open-label phase (5 days/week for 2 weeks).

Materials and methods

Standard protocol approvals, registrations, and patient consents

Written informed consent was obtained from all participants according to the Declaration of Helsinki. The study protocol was approved by the local ethics committee (Brescia Hospital), #NP3244. This trial has been registered at ClinicalTrials.gov (NCT04153110).

Participants

Sixty-one patients with neurodegenerative ataxia were recruited from the Centre for Ageing Brain and Neurodegenerative Disorders, Neurology Unit, University of Brescia, Italy and entered the study. Inclusion criteria consisted of: patients $\geqslant$18 years old with a cerebellar syndrome and quantifiable cerebellar or spinal cord atrophy on MRI. We excluded cases with severe head trauma in the past, history of seizures, stroke or intracranial haemorrhage, intracranial expansive process, pacemaker, metal implants in the head/neck region, severe comorbidity (i.e. cancer in the past 5 years, non-controlled hypertension), use of illegal drugs, or pregnancy.

Twenty-four had a genetic form of spinocerebellar ataxia (five SCA1, 12 SCA2, one SCA14, one SCA28, five SCA38),[21] 10 had multiple system atrophy with cerebellar phenotype (MSA-C),[22] seven had Friedreich's ataxia,[23] 17 had a sporadic adult-onset ataxia (SAOA),[3] and three had cerebellar ataxia with neuropathy and

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VPS13C-associated Parkinson's disease: Two novel cases and review of the literature

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ABSTRACT

VPS13C is a protein-coding gene involved in the regulation of mitochondrial function through the endolysosomal pathway in neurons. Homozygous and compound heterozygous *VPS13C* mutations are etiologically associated with early-onset Parkinson's disease (PD). Moreover, recent studies linked biallelic *VPS13C* mutations with the development of dementia with Lewy bodies (DLB). Neuropathological studies on two mutated subjects showed diffuse Lewy body disease. In this article, we report the clinical and genetic findings of two subjects affected by early-onset PD carrying three novel *VPS13C* mutations (i.e., one homozygous and one compound heterozygous), and review the previous literature on the genetic and clinical findings of *VPS13C*-mutated patients, contributing to the knowledge of this rare genetic alpha-synucleinopathy.

VPS13C is a protein-coding gene known to be involved in mitochondrial homeostasis through Pink1/Parkin-mediated mitophagy in response to mitochondrial depolarization [1]. Biallelic *VPS13C* mutations cause a distinct form of early-onset Parkinson's disease (PD), characterized by rapid and severe disease progression, early cognitive decline, dystonic features, pyramidal signs, and neuropathological findings consistent with diffuse Lewy body disease [1]. In addition, recent studies suggested that rare biallelic *VPS13C* variants are also a genetic cause of Dementia with Lewy Bodies (DLB) [2,3]. Here we aim to describe two cases of early-onset PD carrying novel *VPS13C* mutations and review the existing literature on genetic and clinical features of *VPS13C*-associated alpha-synucleinopathy.

The first case is a 55-year-old female, daughter of consanguineous parents (Fig. 1A). The eldest brother of the proband was affected by rapidly worsening parkinsonism, which started when he was 44 and was complicated by cognitive deterioration, hallucinations, severe psychomotor agitation, and violent behaviour. Institutionalized and bedridden, he died of pneumonia when he was 52. At the age of 42, the proband manifested hyposmia and slightly progressive bradykinesia of the left limbs. She performed a 123I-ioflupane SPECT, which showed severe symmetrical dopaminergic denervation (Fig. 1B). A dopamine agonist (pramipexole) was initiated and it was initially effective and well-tolerated, however, it was soon discontinued due to drug-induced visual hallucinations. Levodopa was then started with good initial motor benefit but with rapid development of motor fluctuations and dyskinesias. In addition, she developed urinary urgency, symptomatic orthostatic hypotension, and frequent falls. A bilateral sensorineural hypoacusia became apparent at that age. On neurological examination (Video part 1) she showed continuous vocalizations and echolalia. Hypomimia, limitation of the downward vertical gaze, and oculomotor apraxia were also appreciated. Vertical eye movements were conserved when prompted by Doll's eyes maneuver, suggesting a supranuclear origin of the gaze palsy. Plastic hypertonia of the neck and limbs was present. Cortical release reflexes, such as snout and palmo-mental, as well as masseter reflex were elicitable. Pull test was positive. The gait was unsteady, wide-based, and slow. Sub-continuous choreodystonic dyskinetic movements of the hands were observed, associated with lips self-mutilations. The proband underwent an extensive assessment, including a brain MRI scan, displaying only a moderate frontal cortical atrophy without midbrain atrophy, an FDG-PET (normal), and neuropsychological evaluation, which disclosed an important ideomotor slowing with memory, attention, and executive deficits, associated with oculomotor and ideomotor apraxia. A lumbar puncture was performed, revealing normal levels of Tau, Phospho-Tau, Aβ1-42, and 14-3-3 proteins. The parkinsonism progressed and at last examination she showed a stuporous, progressive supranuclear palsy-like face, with a complete downward vertical gaze paralysis and worsening of oculomotor and limbs apraxia (Video part 2). Genetic analysis showed the presence of a novel homozygous frameshift *VPS13C* mutation c.860_866dupATATACC predicted to code a highly deleterious early protein truncation (p.Pro290Tyrfs*45) (NM_020821) (Fig. 1C).

The second case is a 43-years-old man without family history of movement disorders (Fig. 1D). Past medical history showed hearing impairment from the age of 18 years. He presented with painful dystonic dorsal flexion of the right big toe after moderate physical activity. One year after he showed bradykinesia affecting his right arm, micrography, and mild depression. At the age of 45 years, he started taking levodopa with good control of motor symptoms, except for foot dystonia. At the age of 48 years, he underwent the following investigations: 123I-ioflupane SPECT, which disclosed significant bilateral reduction in dopamine in the putamen and caudate; brain MRI, which showed only mild cortical cerebellar atrophy and mild parietal cortical atrophy in the left cerebral hemisphere; Mini Mental State Examination (MMSE), which was within the normal range (28/30). At the age of 49 years, he reported progression of his symptoms, with nocturnal akinesia, hypomimia, Pisa syndrome, wearing off, and forgetfulness. Rapid Eye Movement Sleep

; MRI, Magnetic Resonance Imaging; SPECT, Single Photon Emission Computed Tomography; FDG-PET, F-fluorodeoxyglucose Positron Emission Tomography; STN DBS, Deep Brain Stimulation of the Subthalamic Nucleus; PSP, Progressive Supranuclear Palsy.

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Behaviour Disorder (RBD), snoring and daytime sleepiness appeared. Urine and faecal urgency became manifest. Neuropsychological assessment disclosed severe deficits in language, memory, and executive functions (Supplementary Table 1). He was treated with rivastigmine and memantine with only temporary and subjective benefits. At 55, he was no longer able to stand and walk independently and he needed a wheelchair. At the age of 58, he was bedridden, unable to speak, and a percutaneous endoscopic gastrostomy (PEG) tube was placed due to severe dysphagia. Genetic analysis identified three rare variants: c.532delA (p.Lys178=fs*12), c.4669G>C (p.Ala1557Pro), and c.7806C>G (p.Tyr2602*) (Fig. 1E). The c.7806C>G and c.532delA are novel, while the c. 4669G > C is a known extremely rare variant of unknown significance (rs201577653). The frameshift substitution (c.532delA) is expected to lead to a premature stop codon (p.Lys178=fs*12). Conversely, the c.7806C > G is predicted to trunk the VPS13C protein at the amino acid position 2602 (p.Tyr2602*). Segregation analysis showed that the c.532delA (p.Lys178=fs*12) and c.4669G>C (p.Ala1557Pro) were associated in cis and derived from the father, while the c.7806C>G (p.Tyr2602*) originated from the mother.

To date, only 16 clinically described cases of *VPS13C*-related PD cases have been reported in the literature [1,4,2,3,5–7] (Supplementary Table 2, Fig. 1F). From the review of the literature and the two cases described here, it emerges clearly that *VPS13C*-related parkinsonism is characterized, with only few exceptions [2], by the classical motor (bradykinesia, rigidity, rest tremor, freezing, postural instability) and non-motor clinical features of PD (dysautonomia, cognitive decline, visual hallucinations, and hyposmia). The clinical response to dopaminergic therapy appears to be favourable in most cases. Motor fluctuations and levodopa-induced dyskinesias are common. A single *VPS13C*-mutated patient underwent STN DBS, with clinical benefit. The age at onset is earlier in comparison to the idiopathic form (mean age at onset: 37.5 ± 10.5 years). The clinical progression appears to be generally faster. In addition, several associated motor features can be present, such as dystonia and, less frequently, pyramidal signs. Progressive cognitive deterioration is present in most cases. Brain MRI can show symmetrical or asymmetrical lobar atrophic changes without a clear basal ganglia involvement. 123I-ioflupane SPECT shows features compatible with dopaminergic denervation, often in an asymmetrical fashion.

The two probands described here exhibited some peculiar phenotypic findings, such as hearing impairment (both subjects), oculomotor disturbances (subject 1), and self-mutilating behaviour (subject 1). Interestingly, the presence of supranuclear gaze palsy, cognitive dysfunction and postural instability in case 1 suggested a PSP-like phenotype, especially in the last years of clinical follow-up. In conclusion, we presented two novel cases and reviewed the existing literature on the clinical and genetic features of *VPS13C*-associated PD, contributing to the knowledge of this rare monogenic alpha-synucleinopathy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.11.031.

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Massive cerebral venous thrombosis due to vaccine-induced immune thrombotic thrombocytopenia

Since the breakthrough of coronavirus disease (COVID-19) more than 3 million people died worldwide[1] and different vaccines were developed, tested in phase III clinical trials and used in the general population. Few reports of moderate-to-severe thrombocytopenia and thromboses (especially cerebral-venous and splanchnic-vein thromboses) developing approximately 4-14 days after vaccination were reported. These events were related to the adenovirus vector–based DNA vaccines ChAdOx1 nCoV-19 (Oxford–AstraZeneca)[2,3,4] or Ad26.COV2.S (Johnson&Johnson/Janssen).[5] Recently, this new rare autoimmune syndrome that mimics heparin-induced thrombocytopenia (HIT)[3] was defined as vaccine-induced immune thrombotic thrombocytopenia (VITT).[4] Even though details on the pathophysiology are still scanty, diagnostic and therapeutic recommendations were proposed by international scientific organizations.[6-8] No definite data on risk factors are reported and it is unknown whether or not the therapeutic options currently adopted for HIT are also valid for VITT.

With this background, we describe an Italian case of severe VITT-related cerebral venous thrombosis (CVT) and bi-hemispheric hemorrhage, which was successfully treated with argatroban, intravenous immunoglobulin (IVIG) and corticosteroids. The case report is described according to CARE (CAse REport)-statement and checklist.[9]

A previously healthy 26-year-old female presented to the emergency department 14 days after the first injection of ChAdOx1 nCoV-19 vaccine with a headache non-responsive to anti-inflammatory drugs. On admission, she had right-sided weakness and visual disturbances. She has been on combined (estrogen-progestogen) contraceptives for more than 10 years but her past medical history was otherwise unremarkable and there was no prior exposure to heparin.

While general examination and vital signs were normal, neurological examination found a severe right-sided weakness but no visual field defects. Computerised tomography (CT) scan at admission showed a hyperdense rectus sinus and vein of Galen (Figure 1A). Magnetic resonance imaging (MRI) venography showed multifocal venous thrombosis with bilateral occlusion of parietal cortical veins, straight sinus, vein of Galen, internal cerebral veins and inferior sagittal sinus. Transverse sinuses were also partially involved but still patent (Figure 1B). At the right parietal and left frontoparietal lobes an extensive venous infarction with hemorrhagic transformation was present (Figure 1C). D-dimer was dramatically raised to 12,204 μg/L (reference value <500 μg/L) and the platelet count was $134x10^9$/L. Given her recent exposure to ChAdOx1 nCoV-19 and clinical presentation, she was first treated with fondaparinux (5 mg subcutaneously) and admitted her to the intensive care unit. Her clinical condition rapidly deteriorated with decreased consciousness, right-sided hemiplegia and complete Balint syndrome.

In order to perform an extensive hemostasis laboratory work-up before and after therapies, blood was collected at different time points (T0=April 13; T1=April 15, and T2=April 20, 2021) into vacuum-tubes containing 1/10 volumes of trisodium-citrate 0.109 M, K-EDTA or plain tubes. Activated partial thromboplastin time (aPTT), prothrombin time (PT), D-dimer, fibrinogen and factor (F)VIII were obtained. Platelet-factor 4 (PF4)–heparin IgG antibodies (aPF4) were evaluated by a commercially-available enzyme-linked immunosorbant assay (ELISA) (Immucor, Waukesha, WI, USA). Platelet-activating antibodies were evaluated by a platelet-activation test (PAT).[2,10] Platelet function was also evaluated by using the Total Thrombus-Formation Analysis device (T-TAS®, Zacros, Fujimori-Kogyo, Tokyo, Japan),[11-12] a flow-chamber device that assesses platelet-mediated thrombus formation in capillary channels by means of the following parameters: area under the flow-pressure curve (AUC), occlusion start-time (OST) and occlusion time (OT). Thrombin generation (TG) was measured in platelet-poor plasma (PPP).[13] Controls were plasma samples from subjects negative for aPF4 and normal TG.

PT, aPTT and fibrinogen were within the normal range; FV-Leiden and G20210A-prothrombin mutations were absent; antithrombin and protein C/S were normal; lupus anticoagulant and antiphospholipid antibodies were negative. Patient serum (T1) was positive to aPF4-heparin ELISA (OD=1.918, reference value <0.4) and was inhibited (OD<0.5) by 100 U/mL heparin. Patient serum (T1) showed strong platelet activation on PPP from two controls in the presence and absence of low-dose heparin, whereas control serum showed no platelet activation. Five days afterwards (T2), the patient serum showed significant reduction of aPF4 reactivity (OD=0.6) and no longer did activate platelets (Figure 2A to C). At T0, platelet thrombus formation was impaired, AUC was smaller and OT longer than the reference range. In contrast, at T1 and T2 thrombus stability improved and T-TAS parameters as well as platelet count also improved (Figure 2D to F). Results at the time of hospital admission (T1) showed a marked state of hypercoagulability when compared to control, as indicated by short lag-time (8.5 minutes [min] *vs.* 21.3 min), increased thrombin-peak (289 nM *vs.* 115 nM), short time-to-peak (11.8 min *vs.* 26.2min), increased ETP (2,158 nM/min *vs.* 1,684 nM/min) and ETP-TM ratio (0.99 *vs.* 0.79) (Figure 3). FVIII, one of the most potent procoagulants, was higher (200 U/dL) than the upper limit of the reference range (<150 U/dL). In contrast PC, the physiological inhibitor to activated FVIII was normal (88 U/dL). The imbalance between FVIII and PC corresponded to an increased FVIII/PC ratio (2.3), considerably greater than the expected unity and consistently with the hypercoagulability shown by TG. There are potential limitations of the TG assessment. First, measurements (owing to assay complexity and limited blood volumes) were performed only in PPP. Therefore, the potential role of procoagulant platelets in supporting TG could not be assessed. Second, TG could not be assessed on samples obtained during the time course of the disease because soon after the onset of the symptoms and the preliminary diagnosis the patient was treated with anticoagulants, so that TG results would have been unreliable.

Considering the clinical conditions and laboratory results, IVIG (1 g/kg o.d. for 2 days) and dexamethasone (40 mg/day, for 4 days) were started.[6-8] Owing to the possible need for a sudden decompressive neurosurgical intervention, anticoagulation with fondaparinux was replaced by the short-acting drug argatroban (starting dosage 1 μg/kg/min with an aPTT-ratio [patient/normal] target of 1.5-2.0). Argatroban was subsequently increased to 3 μg/kg/min.

The patient's neurological conditions improved in the next few days. She was awake and fully responsive to stimuli with a progressive recovery of right upper-limb strength, partial optic ataxia and regression of apraxia.

thrombosis at unusual sites, even when apparent prothrombotic risk factors are identified (oral contraceptives in our case) and irrespective of the baseline platelet count. Indeed, this patient had mild thrombocytopenia on admission, but historical testing carried out before VITT recorded a platelet count of 275x10^9/L. Thus, the platelet count had decreased by approximately 50%, in agreement with HIT and VITT diagnostic criteria. Aware of the possible diagnosis of VITT, we initially avoided a potentially detrimental heparin treatment, and this decision is likely to have played a major role in determining the positive outcome.[2,3] Another important decision was to promptly start immune modulating therapy which caused the reduction of aPF4 titer and D-dimer. The causative prothrombotic mechanism in the reported patient is likely to be due to the antibodies to PF4 that induced a strong platelet activation even in absence of heparin exposure. The fact that the patient started to improve soon after the antibody titer decreased strongly supports this mechanism. One of the potential mechanisms that can explain the loss of serum activity in the functional assays (PAT) could be the IVIG blockade of FCγ platelet receptors and/or the antibody suppression. Interestingly, platelets' ability to promote thrombus formation *in vitro* was greatly reduced at admission, probably as a consequence of *in vivo* platelet activation and the formation of exhausted platelets as observed in other pathological conditions such as disseminated intravascular coagulation or sepsis. Laboratory tests correlated well with the clinical and radiological course. All in all, our experience supports the application of an early and multidisciplinary therapeutic approach in cases of VITT, with the possibility to avoid fatalities and obtain a resolution of the syndrome as in this case.

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Contributions: SB managed the clinical case, collected clinical data and wrote the clinical part of the manuscript; AA, GS, CG and GPC managed the clinical case, collected clinical data and critically reviewed the manuscript; AL performed the laboratory assays, interpreted the results and contributed to writing the manuscript; SLM, LP and MC performed the laboratory assays; AT organized the hemostatic assays, interpreted the results and contributed to writing the manuscript; FP designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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Behavioral Variant of Frontotemporal Dementia and Homicide in a Historical Case

Stefano Zago, PhD, Cristina Scarpazza, PhD, Teresa Difonzo, PsyD, Andrea Arighi, MD, Dounia Hajhajate, PsyD, Yvan Torrente, MD, PhD, and Giuseppe Sartori, PhD

Criminal behavior is a clinical feature of the behavioral variant of frontotemporal dementia (bvFTD), ranging from socially inappropriate behavior and minor offenses (such as shoplifting, driving-related violations, housebreaking, trespassing) to the more extreme acts of sex crimes and violence. To our knowledge, no homicide case involving bvFTD is well illustrated in the scientific literature, and only a few anecdotal annotations are available about bvFTD and homicide. This is surprising considering the inclination of individuals with bvFTD to lack impulse control, to manifest disinhibition, to display diminished emotional awareness and loss of empathy, and to show behavior indicative of disordered moral reasoning. Here, we describe the 19th-century homicide case of Benjamin Reynaud, a man whose clinical characteristics suggest the bvFTD diagnosis. Reynaud's case may represent a rare instance of homicide committed by an individual with bvFTD and provide a basis for some reflections regarding the relationship between homicidal behavior and bvFTD.

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Key words: behavioral variant frontotemporal dementia; criminal behavior; homicide; murder

A significant inclination to crime has been described in individuals with a diagnosis of a behavioral variant of frontotemporal dementia (bvFTD), the most common clinical phenotype of frontotemporal lobe degeneration.[1,2] Persons with bvFTD often demonstrate a slow decline in social interpersonal conduct and regulation of personal behavior along with a lack of insight and emotional blunting.[3,4] Execution-related cognitive deficits in inhibitory control, flexibility, abstract reasoning, decision-making, and goal-oriented behavior are often evident in individuals with bvFTD.[4] Structural and functional imaging studies have shown that bvFTD involves a large network of structures including the dorsolateral prefrontal and orbitofrontal cortex, anterior insula, anterior cingulate and adjacent medial prefrontal cortices, amygdala, striatum, and thalamus.[2] To date, a diagnosis of bvFTD is based primarily on clinical diagnostic criteria, while brain-imaging and genetic data are considered supportive criteria.[4] In 2011, the International Behavioral Variant of Frontotemporal Dementia Criteria Consortium established the criteria for diagnosis of bvFTD.[4] Currently, there are no known treatments to stop or reverse the progression of bvFTD. Some medications have been useful in regulating and controlling behavior, including antidepressant and antipsychotic medications and even intranasal oxytocin.[5–7]

In the last decade, the literature indicates that transgression of social norms and criminal behavior

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biomedicines

MDPI

Review

The Immune System in Duchenne Muscular Dystrophy Pathogenesis

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Abstract: Growing evidence demonstrates the crosstalk between the immune system and the skeletal muscle in inflammatory muscle diseases and dystrophic conditions such as Duchenne Muscular Dystrophy (DMD), as well as during normal muscle regeneration. The rising of inflammation and the consequent activation of the immune system are hallmarks of DMD: several efforts identified the immune cells that invade skeletal muscle as CD4+ and CD8+ T cells, Tregs, macrophages, eosinophils and natural killer T cells. The severity of muscle injury and inflammation dictates the impairment of muscle regeneration and the successive replacement of myofibers with connective and adipose tissue. Since immune system activation was traditionally considered as a consequence of muscular wasting, we recently demonstrated a defect in central tolerance caused by thymus alteration and the presence of autoreactive T-lymphocytes in DMD. Although the study of innate and adaptive immune responses and their complex relationship in DMD attracted the interest of many researchers in the last years, the results are so far barely exhaustive and sometimes contradictory. In this review, we describe the most recent improvements in the knowledge of immune system involvement in DMD pathogenesis, leading to new opportunities from a clinical point-of-view.

Keywords: Duchenne Muscular Dystrophy; innate and adaptive immune system; inflammation

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1. Introduction

Muscular dystrophies (MDs) are a heterogeneous group of genetic diseases caused by mutations in proteins that mainly constitute the sarcolemma and the cytosol of the muscle fibers. Even if inflammation is a shared feature among MDs, differences exist in the molecular and cellular pathways involved in cellular infiltrates, suggesting inflammatory milieu differences in each form of MDs [1]. Duchenne Muscular Dystrophy (DMD) is a devastating X-linked disease caused by mutations in the dystrophin gene. The asynchronous cycles of muscle fibre degeneration in DMD exacerbate the muscle infiltration of macrophages and lymphocytes and their secretion of pro-inflammatory cytokines. The severity of muscle injury and the inflammation dictate the impairment of muscle regeneration and the successive replacement of myofibers with connective and adipose tissue [2]. No cure exists for DMD: corticosteroids are largely in routine use although limited by side effects [3]. In the last two decades, research efforts have been trying to identify small molecules able to bypass the wide range of dystrophin mutations, or cell populations with high in vivo homing capacity. The use of mini- and micro-dystrophin with recombinant AAV vectors showed promising results in terms of muscle force and the rescue of pathological phenotype, but it was limited by the worsening of clinical parameters and the activation of immune responses, namely the complement system [4]. Reducing the inflammatory features of muscular pathologies could represent a potential field able to bypass immune responses determined by AAV vectors [5].

cells

MDPI

Article

Flavonoids and Omega3 Prevent Muscle and Cardiac Damage in Duchenne Muscular Dystrophy Animal Model

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Abstract: Nutraceutical products possess various anti-inflammatory, antiarrhythmic, cardiotonic, and antioxidant pharmacological activities that could be useful in preventing oxidative damage, mainly induced by reactive oxygen species. Previously published data showed that a mixture of polyphenols and polyunsaturated fatty acids, mediate an antioxidative response in mdx mice, Duchenne muscular dystrophy animal model. Dystrophic muscles are characterized by low regenerative capacity, fibrosis, fiber necrosis, inflammatory process, altered autophagic flux and inadequate anti-oxidant response. FLAVOmega β is a mixture of flavonoids and docosahexaenoic acid. In this study, we evaluated the role of these supplements in the amelioration of the pathological phenotype in dystrophic mice through in vitro and in vivo assays. FLAVOmega β reduced inflammation and fibrosis, dampened reactive oxygen species production, and induced an oxidative metabolic switch of myofibers, with consequent increase of mitochondrial activity, vascularization, and fatigue resistance. Therefore, we propose FLAVOmega β as food supplement suitable for preventing muscle weakness, delaying inflammatory milieu, and sustaining physical health in patients affected from DMD.

Keywords: muscle homeostasis; muscle regeneration; satellite cells; inflammatory response; Duchenne muscular dystrophy; food supplement

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1. Introduction

Dietary supplementation is an important source of vitamins, minerals, herbs, or products made from plants, animals, algae, seafood, or yeasts, intended to complement the common diet in physiological or pathological conditions. In fact, food supplements may be useful for the maintenance of a proper nutrient supply to promote health span and improve physical performance of healthy subjects, in case of nutritional deficiencies and as an adjuvant tool for the management of multiple disease conditions.

Neuromuscular disorders, such as Duchenne muscular dystrophy (DMD), present important secondary pathologic features that fulfil the requirements for dietary supplementation. DMD is an X-linked recessive disease that affects muscular function and strength. It is caused by mutations of the dystrophin gene, which result in null expression of the structural protein, leading to instability of the dystrophin-associated glycoprotein complex and sarcolemma fragility. DMD muscles are characterized by low regenerative capacity, fibrosis, fiber necrosis [1], inflammatory infiltrates, intracellular Ca^{2+} dysregulation, aberrant cellular signaling, mitochondrial malfunction and overproduction of reactive oxygen species (ROS), which outweigh a physiological antioxidant response [2]. Heart complications and respiratory disorders are manifested as the pathology progresses and are the major causes of death in DMD patients [3]. Among cardiac complications, dilated cardiomyopathy (DCM) leads to increased ventricular chamber size, coupled with loss of contractile function (ejection fraction < 40%) [4], progressive myocardial fibrosis and decreased cardiac function [5].

frontiers in Neurology

ORIGINAL RESEARCH
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Clinical Determinants of Disease Progression in Patients With Beta-Sarcoglycan Gene Mutations

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Background: Limb-girdle muscular dystrophy 2E (LGMD 2E), recently renamed as autosomal recessive limb-girdle muscular dystrophy-4 (LGMDR4), is characterized by the lack of beta-sarcoglycan, normally expressed in skeletal muscles and cardiomyocytes. We hypothesized that progressive respiratory and left ventricular (LV) failure in LGMDR4 could be associated with the age and interrelated phenomena of the disease's natural history.

Methods: We conducted a retrospective review of the records of 26 patients with LGMDR4. Our primary objective was to compare the rates of decline among creatine phosphokinase (CPK) values, pulmonary function test (PFT) measures, and echocardiographic estimates and to relate them to patients' age.

Results: The rates of decline/year of CPK, PFTs, and LV function estimates are significatively bound to age, with the LV ejection fraction (EF) being the strongest independent variable describing disease progression. Moreover, the rate of decline of CPK, PFTs, and LV differed in patients grouped according to their genetic mutations, demonstrating a possible genotype–phenotype correlation. The parallel trend of decline in CPK, PFT, and EF values demonstrates the presence in LGMDR4 of a simultaneous and progressive deterioration in muscular, respiratory, and cardiac function.

Conclusions: This study expands the current knowledge regarding the trend of CPK values and cardiac and respiratory impairment in patients with LGMDR4, to optimize the monitoring of these patients, to improve their quality of life, and to provide clinical indices capable of quantifying the effects of any new gene or drug therapy.

Keywords: limb-girdle muscular dystrophy, type 2E, creatine kinase, respiratory function tests, echocardiography, disease progression

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BACKGROUND

Limb-girdle muscular dystrophy 2E (LGMD 2E), recently reclassified as limb-girdle muscular dystrophy recessive type 4 (LGMDR4) (1), is a rare type of recessive muscular dystrophy caused by the lack of beta-sarcoglycan. Sarcoglycans (SGs) count four transmembrane proteins: alpha (a-), beta (b-), gamma (c-), and delta-sarcoglycan (d-SG), organized in a tetramer that stabilizes the

nature COMMUNICATIONS

ARTICLE

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Defective dystrophic thymus determines degenerative changes in skeletal muscle

Andrea Farini[1,8], Clementina Sitzia[2,8], Chiara Villa[1], Barbara Cassani[3,4], Luana Tripodi[1], Mariella Legato[1], Marzia Belicchi[1], Pamela Bella[1], Caterina Lonati[5], Stefano Gatti[5], Massimiliano Cerletti[6,7] & Yvan Torrente[1✉]

In Duchenne muscular dystrophy (DMD), sarcolemma fragility and myofiber necrosis produce cellular debris that attract inflammatory cells. Macrophages and T-lymphocytes infiltrate muscles in response to damage-associated molecular pattern signalling and the release of TNF-α, TGF-β and interleukins prevent skeletal muscle improvement from the inflammation. This immunological scenario was extended by the discovery of a specific response to muscle antigens and a role for regulatory T cells (Tregs) in muscle regeneration. Normally, autoimmunity is avoided by autoreactive T-lymphocyte deletion within thymus, while in the periphery Tregs monitor effector T-cells escaping from central regulatory control. Here, we report impairment of thymus architecture of mdx mice together with decreased expression of ghrelin, autophagy dysfunction and AIRE down-regulation. Transplantation of dystrophic thymus in recipient nude mice determine the up-regulation of inflammatory/fibrotic markers, marked metabolic breakdown that leads to muscle atrophy and loss of force. These results indicate that involution of dystrophic thymus exacerbates muscular dystrophy by altering central immune tolerance.

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Research Article

Functionalized Scintillating Nanotubes for Simultaneous Radio- and Photodynamic Therapy of Cancer

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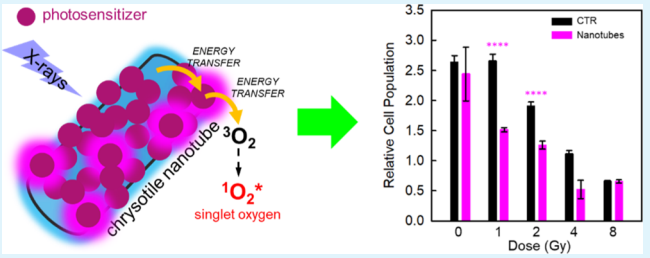
ABSTRACT: As a model radio-photodynamic therapy (RPDT) agent, we developed a multicomponent nanomaterial by anchoring conjugated chromophores on the surface of scintillating chrysotile nanotubes. Its ultimate composition makes the system a scintillation-activated photosensitizer for the singlet oxygen production. This nanomaterial shows a remarkable ability to enhance the production of singlet oxygen in an aqueous environment, under X-ray irradiation, boosting its production by almost 1 order of magnitude. Its efficiency as a coadjutant for radiotherapy has been tested *in vitro*, showing a striking efficacy in enhancing both the prompt cytotoxicity of the ionizing radiation and the long-term cytotoxicity given by radiation-activated apoptosis. Notably, the beneficial activity of the RPDT agent is prominent at low levels of delivered doses comparable to the one employed in clinical treatments. This opens the possibility of effectively reducing the therapy exposure and consequently undesired collateral effects due to prolonged exposure of patients to high-energy radiation.



KEYWORDS: *nanomaterials, radiotherapy, singlet oxygen, photodynamic therapy, scintillating nanoparticles*

INTRODUCTION

Over the past few years, biomedical science has recognized the crucial role that nanotechnology can play in the field thanks to the development and use of nanoparticles in theranostics, which allows a deeper investigation of biological processes, faster diagnosis of diseases, accurate monitoring of specific injured tissues or organs, and, importantly, the improvement of some traditional therapeutic treatments.[1−5] Due to their benefits with respect to larger systems, such as a high surface-to-volume ratio; facile surface functionalization; and tailorable optical, magnetic, and structural properties crucial for the adaptability to satisfy specific targets, nanomaterials are indeed ideal carriers for chemo- and phototherapeutic agents or radiosensitizers across several physiological barriers.[6,7] Therefore, nowadays, a plethora of nanoscale materials, such as metallic and semiconductor nanoparticles, fluorites, and metal/lanthanide oxides, as well as organic and hybrid systems, are successfully exploited in advanced diagnostic and imaging techniques or innovative therapeutic approaches against cancer and other deadly diseases,[8−11] as demonstrated by the more and more increasing number of nanosystems approved by the Food and Drug Administration (FDA) agency.[8,12]

In particular, biomedicine is moving toward the use of radioluminescent nanoparticles, that is, nanoscintillators, which are able to absorb and convert the ionizing radiation (X- or γ-rays) into a large number of UV/visible (UV/vis) photons exploitable to boost the efficacy of diagnosis routes, in nuclear medicine for preclinical mapping and intraoperative imaging and radiation dosimetry, and as coadjutants in oncological therapies.[13−16] The search for innovative therapies overtaking state-of-the-art oncological treatments is challenging. Conventional cancer treatment options—chemotherapy, radiotherapy (RT), and surgery—are still associated with systemic side effects, disease recurrence, and drug/radio resistance of malignant cells. In particular, ionizing radiation is used in approximately 50% of all cancer treatments to stop the rapid proliferation of cancer cells directly by damaging their DNA and by thermal shock or indirectly by forming cytotoxic free radicals, that is, reactive oxygen species (ROS) such as hydroxyl radicals and singlet oxygen, upon interaction with the intracellular aqueous environment.[17,18] However, RT is limited by the maximum radiation dose that can be given to a tumor mass without incurring significant injuries to the adjacent tissues or organs.[19] In order to maximize the therapeutic

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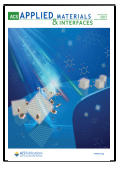

Instruments, a pulse width of 150 ps) as a light source. Data were obtained with an Edinburgh Instruments FLS-980 spectrophotometer, with a 5 nm bandwidth and a time resolution of 0.1 ns.

Singlet Oxygen Relative Concentration Measurement. The optical probe SOSG has been purchased from Thermo Fisher and used as is. The SOSG powered has been diluted in a 1:10 solution of dimethyl sulfoxide (DMSO) and PBS, which has been used to disperse the NTs. The intensity of the SOSG fluorescence, which is directly proportional to the concentration of singlet oxygen in the environment, has been monitored under *cw* laser light excitation at 473 nm (Figure S5).

Cell Culture. Human primary glioblastoma cells U-87 were purchased from ATCC (HTB-14) and thawed in pre-warmed Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 15% fetal bovine serum (FBS, Gibco). Cells were seeded in a 75 cm^2 flask and incubated at 37 °C and 5% CO_2 until 90% of confluence was reached. The cell culture medium was changed every 2 days.

Confocal Microscopy. U-87 cells were seeded onto rounded coverslips housed into 12-well plates at a density of 30.000 cells/cm^2 in DMEM supplemented with 15% FBS and 50 μg/mL gentamicin (Gibco). After 24 h, 20, 40, and 60 $\mu g\ cm^{-2}$ NT-PEO-Por* were added to cells and incubated overnight. Briefly, 25 mg/mL NT-PEO-Por* stock solution was prepared in distillated sterile water and sonicated by means of an ultrasound bath for 30 min to break big aggregates. NT-PEO-Por* dilutions were calculated accordingly to final concentrations and mixed directly into the complete cell medium. For fluorescence imaging, NT-PEO-Por*-labeled U-87 cells were washed twice with PBS (without Ca^{2+} and Mg^{2+}, Gibco), fixed with 4% paraformaldehyde, and permeabilized with 0.1% Triton X-100 in PBS for 15 min. 1% BSA solution in PBS was then added for 45 min to reduce not specific background staining. Alexa Fluor 488 phalloidin (Thermo Fisher Scientific) was diluted in PBS according to the manufacturer's instructions, added to cells, and incubated at room temperature for 45 min. Cells were then washed twice in PBS, and coverslips were removed from the multiwells and mounted onto glass slides with the Fluoromount-G medium (Thermo Fisher Scientific). Z-stack images were obtained with a confocal microscope Leica TCS SP8 with a white light laser.

Cell Viability. For cell viability experiments, cells were seeded in 96-well plates at a density of 3×10^3 cells/well ($n = 6$ for each condition); after 24 h, NT-PEO-Por* was added to the complete cell medium, as previously described. U-87 cells were washed twice in PBS, and the MTT test was performed (methylthiazolyldiphenyl-tetrazolium bromide, Sigma) at 24, 48, and 72 h from NT-PEO-Por* labeling according to the manufacturer's instructions. Briefly, a 50 μg/mL MTT solution was added to the samples; after 3 h of incubation at 37 °C, the medium was removed, the converted dye was solubilized with DMSO (Sigma), and the absorbance was measured at 560 nm (GloMax Discover, Promega). Not labeled cells in the complete medium were used as control conditions.

Viability Test under Irradiation (Trypan Blue Exclusion Assay and MTT). U-87 cells were seeded in 35 mm cell culture dishes at a density of 3000 cells cm^{-2} in DMEM (Gibco) supplemented with 15% FBS and 50 μg/mL gentamicin (Gibco). 25 mg/mL NT-PEO and NT-PEO-Por* were resuspended in distillated sterile water and sonicated for 30 min. After 24 h from seeding, cells were treated with 20 $\mu g\ cm^{-2}$ of NT-PEO or NT-PEO-Por* and incubated overnight. Untreated cells were used as the control. Cells were washed with PBS and after X-ray exposure at different doses (0, 1, 2, 4, 8, and 12 Gy) were detached by 0.25% trypsin−ethylenediaminetetraacetate (EDTA) (Thermo Fisher Scientific). In order to distinguish dead cells, 20 μL of a U-87 suspension was stained with an equal volume of Trypan blue 0.4% (Thermo Fisher Scientific) and counted using a Fast Read 102 chamber. The cell concentration was determined by the formula cells/mL = $[(\sum$cells counted in 5 squares/5) × dilution factor × $10^4]$. The percentage of death cells was calculated by the following formula: % dead cells = (number of blue cells ÷ number of total cells) × 100. All the experiments were performed in triplicate. MTT assessment was performed as already described with minimum modifications. Immediately after X-ray exposure, the MTT solution was directly added to dishes housing the X-ray-treated cells and incubated for 3 h. In order to avoid the loss of stressed cells detaching from dishes during the assay, supernatants and cells were collected and centrifuged at 1200 rpm for 5 min before adding DMSO.

Caspase 3/7 Activity Detection. For the evaluation of caspase, we performed the Caspase-Glo 3/7 assay (Promega), which measures caspase-3 and caspase-7 activities through a luminescence signal, following the manufacturer's instructions. Briefly, unstained and NT-PEO-Por*-stained U-87 that were exposed to escalating doses of X-rays were detached by 0.25% trypsin−EDTA, counted, and seeded into a white-walled 96-well plate at 3000/cm^2. Cells were then incubated in 100 μL of the fresh medium for 24 h to let them adhere. 100 μL of the Caspase-Glo 3/7 Reagent was then added to the medium and incubated for 3 h at 37 °C, and the luminescence was recorded with Glomax multiplate readers. The Caspase-Glo 3/7 Reagent was also added to the fresh medium without cells in order to measure the background luminescence. Before apoptosis evaluation, representative images of seeded cells were obtained using a Leica DMi8 inverted microscope.

Flow Cytometry. The induction of apoptosis and necrosis of U-87 was measured 4 h after X-ray exposure by flow cytometry. X-ray-exposed unstained and NT-PEO-Por*-stained U-87 were detached from dishes by 0.25% trypsin−EDTA, centrifuged at 1200 rpm for 5 min, and resuspended in 100 μL of the annexin buffer (BD) and 5 μL of Annexin V BV421 (BD), which binds to phosphatidylserin (PS) residues and allows the identification of apoptotic cells. Cells were incubated a 4 °C for 20 min, washed in the annexin buffer, and centrifuged. The viability dye 7-aminoactinomycin D (7-AAD, BD) was added to cell pellets to stain non-viable cells and recognize late-apoptotic fractions. For fluorescence-activated cell sorting (FACS) characterization, data were obtained using a FACSAria Fusion cell sorter, equipped with five lasers, and analyzed with FACSDiva software (ver. 8.0, BD). At least 10×10^3 events were recorded for each condition. Debris events were excluded from the analysis by morphological gating (side scatter vs forward scatter dot plot).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.1c02504.

RL emission band assignment discussion, time-resolved data analysis, FT-IR data, and singlet oxygen measurement raw data (PDF)

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Article

Guide Cells Support Muscle Regeneration and Affect Neuro-Muscular Junction Organization

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Abstract: Muscular regeneration is a complex biological process that occurs during acute injury and chronic degeneration, implicating several cell types. One of the earliest events of muscle regeneration is the inflammatory response, followed by the activation and differentiation of muscle progenitor cells. However, the process of novel neuromuscular junction formation during muscle regeneration is still largely unexplored. Here, we identify by single-cell RNA sequencing and isolate a subset of vessel-associated cells able to improve myogenic differentiation. We termed them 'guide' cells because of their remarkable ability to improve myogenesis without fusing with the newly formed fibers. In vitro, these cells showed a marked mobility and ability to contact the forming myotubes. We found that these cells are characterized by CD44 and CD34 surface markers and the expression of Ng2 and Ncam2. In addition, in a murine model of acute muscle injury and regeneration, injection of guide cells correlated with increased numbers of newly formed neuromuscular junctions. Thus, we propose that guide cells modulate de novo generation of neuromuscular junctions in regenerating myofibers. Further studies are necessary to investigate the origin of those cells and the extent to which they are required for terminal specification of regenerating myofibers.

Keywords: guide cells; neuro-muscular junctions; mesoangioblasts; muscle injury; scRNA-seq

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1. Introduction

Unraveling the regenerative processes in skeletal and cardiac muscles represents an intriguing and ambitious frontier. In order to define novel therapeutic strategies, one fundamental step is to understand the properties of plasticity in post-natal progenitor cells.

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RESEARCH PAPER

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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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frontiers in Genetics

REVIEW
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Myogenic Cell Transplantation in Genetic and Acquired Diseases of Skeletal Muscle

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This article will review myogenic cell transplantation for congenital and acquired diseases of skeletal muscle. There are already a number of excellent reviews on this topic, but they are mostly focused on a specific disease, muscular dystrophies and in particular Duchenne Muscular Dystrophy. There are also recent reviews on cell transplantation for inflammatory myopathies, volumetric muscle loss (VML) (this usually with biomaterials), sarcopenia and sphincter incontinence, mainly urinary but also fecal. We believe it would be useful at this stage, to compare the same strategy as adopted in all these different diseases, in order to outline similarities and differences in cell source, pre-clinical models, administration route, and outcome measures. This in turn may help to understand which common or disease-specific problems have so far limited clinical success of cell transplantation in this area, especially when compared to other fields, such as epithelial cell transplantation. We also hope that this may be useful to people outside the field to get a comprehensive view in a single review. As for any cell transplantation procedure, the choice between autologous and heterologous cells is dictated by a number of criteria, such as cell availability, possibility of *in vitro* expansion to reach the number required, need for genetic correction for many but not necessarily all muscular dystrophies, and immune reaction, mainly to a heterologous, even if HLA-matched cells and, to a minor extent, to the therapeutic gene product, a possible antigen for

frontiers in Immunology

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Role of Immunoglobulins in Muscular Dystrophies and Inflammatory Myopathies

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Muscular dystrophies and inflammatory myopathies are heterogeneous muscular disorders characterized by progressive muscle weakness and mass loss. Despite the high variability of etiology, inflammation and involvement of both innate and adaptive immune response are shared features. The best understood immune mechanisms involved in these pathologies include complement cascade activation, auto-antibodies directed against muscular proteins or *de-novo* expressed antigens in myofibers, MHC-I overexpression in myofibers, and lymphocytes-mediated cytotoxicity. Intravenous immunoglobulins (IVIGs) administration could represent a suitable immunomodulator with this respect. Here we focus on mechanisms of action of immunoglobulins in muscular dystrophies and inflammatory myopathies highlighting results of IVIGs from pre-clinical and case reports evidences.

Keywords: muscular dystrophies, immunoglobulins, autoimmunity, inflammatory myopathies, muscle inflammation, autoantibodies

INTRODUCTION

Growing evidences support the role of the immune system in different pathological conditions of the skeletal muscle. Immune cell infiltrate following muscle injury contributes to the pathology of various muscular dystrophies (MDs), whereas autoimmune responses specific for defined or yet undefined muscle antigens are suggested as the cause of some idiopathic inflammatory myopathies (IIMs). The initial immune response to muscle damage consists of innate immunity in which phagocytic, cytolytic, and secretory inflammatory cells (mainly macrophages and neutrophils) are rapidly mobilized and activated to identify, kill, and remove invading infectious organisms during infectious events or remove muscle fiber debris and promote muscle repair following disruption of muscle homeostasis. Innate immunity predates the adaptive immune system through the activation of professional antigen-presenting cells (APCs) that process and present muscle antigens to T-effector cells (mainly T-CD4+ and T-CD8+ cells) toward major histocompatibility complex (MHC) leading to intensive secretion of pro-inflammatory cytokines and muscle fiber necrosis (1). The MDs constitute a group of genetically muscle diseases characterized by progressive muscle weakness and degeneration. The most frequently occurring MDs involve damage to the muscle fiber membrane, which can lead to the release of Danger Associated Molecular Patterns (DAMPs) in

molecules

MDPI

Article

Shotgun Proteomics of Isolated Urinary Extracellular Vesicles for Investigating Respiratory Impedance in Healthy Preschoolers

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Abstract: Urine proteomic applications in children suggested their potential in discriminating between healthy subjects from those with respiratory diseases. The aim of the current study was to combine protein fractionation, by urinary extracellular vesicle isolation, and proteomics analysis in order to establish whether different patterns of respiratory impedance in healthy preschoolers can be characterized from a protein fingerprint. Twenty-one 3–5-yr-old healthy children, representative of 66 recruited subjects, were selected: 12 late preterm (LP) and 9 full-term (T) born. Children underwent measurement of respiratory impedance through Forced Oscillation Technique (FOT) and no significant differences between LP and T were found. Unbiased clustering, based on proteomic signatures, stratified three groups of children (A, B, C) with significantly different patterns of respiratory impedance, which was slightly worse in group A than in groups B and C. Six proteins (Tripeptidyl peptidase I (TPP1), Cubilin (CUBN), SerpinA4, SerpinF1, Thy-1 membrane glycoprotein (THY1) and Angiopoietin-related protein 2 (ANGPTL2)) were identified in order to type the membership of subjects to the three groups. The differential levels of the six proteins in groups A, B and C suggest that proteomic-based profiles of urinary fractionated exosomes could represent a link between respiratory impedance and underlying biological profiles in healthy preschool children.

Keywords: extracellular vesicle; urine fractionation; proteomics; forced oscillation technique; preschooler healthy children

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1. Introduction

Physiological changes throughout childhood characterize lung function, which is at least in part influenced by perinatal factors, including prematurity. Significantly, Late Preterm (LP, 34–36 weeks' gestational age, GA) children without clinical lung disease may show deficits in lung function that may persist throughout infancy [1]. Indeed, increased respiratory impedance was reported in healthy children aged 3–7 years born LP in comparison with age-matched healthy term-born children [2]. Studying the underlying biological

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ORIGINAL ARTICLE

A nationwide survey on clinical neurophysiology education in Italian schools of specialization in neurology

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Abstract

Introduction Clinical neurophysiology deals with nervous system functions assessed with electrophysiological and ultrasound-based imaging techniques. Even though the need for highly specialized neurophysiologists has increased, residency training rarely takes today's requirements into account. This study aimed to snapshot the neurophysiological training provided by Italian specialization schools in neurology.

Methods A single-page web-based survey comprising 13 multiple-choice categorical and interval scale questions was sent via e-mail to neurology specialization school directors. The survey addressed the programs' structural neurophysiology organization, time dedicated to each clinical neurophysiology subspecialty, and descriptors assessing the discipline's importance (e.g., residents who attempted residential courses, gained certifications, or awards gained).

Results The most studied neurophysiological techniques were electroencephalography (EEG) and electromyography (EMG). Most specialization schools devoted less than 3 months each to multimodal evoked potentials (EPs), ultrasound sonography (US), and intra-operative monitoring. Of the 35 specialization schools surveyed, 77.1% reported that four students, or fewer, participated in the Italian Society of Clinical Neurophysiology Examination in Neurophysiology. Of the 35 specialization centers surveyed, 11.4% declared that the final evaluation required students to discuss a neurophysiological test.

Discussion Our survey underlined the poorly standardized technical requirements in postgraduate neurology specialization schools, wide variability among training programs, and limited training on multi-modal evoked potentials, intraoperative monitoring, and sonography. These findings underline the need to reappraise and improve educational and training standards for clinical neurophysiology during postgraduate specialization schools in neurology with an international perspective.

Keywords Medical education · Clinical neurophysiology · Specialization in neurology · Training in neurophysiology

Introduction

Clinical neurophysiology (CN) according to the International Federation of Clinical Neurophysiology (IFCN) is a "medical specialty concerned with function and dysfunction of the nervous system caused by disorders of the brain, spinal cord, peripheral nerve and muscle, using physiological and imaging techniques to measure nervous system activity" (http://www.ifcn.info).

Conventional neurophysiological techniques include two main areas: studies investigating brain activity: electroencephalography (EEG) and those investigating the peripheral nervous system: nerve conduction studies (NCS) and electromyography (EMG). In the modern era, neurophysiological methods have greatly expanded to include techniques traditionally used in daily clinical practice (EEG, NCS, EMG, evoked potential studies, polysomnography and assessment

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Next-generation sequencing application to investigate skeletal muscle channelopathies in a large cohort of Italian patients

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Abstract

Non-dystrophic myotonias and periodic paralyses are a heterogeneous group of disabling diseases classified as skeletal muscle channelopathies. Their genetic characterization is essential for prognostic and therapeutic purposes; however, several genes are involved. Sanger-based sequencing of a single gene is time-consuming, often expensive; thus, we designed a next-generation sequencing panel of 56 putative candidate genes for skeletal muscle channelopathies, codifying for proteins involved in excitability, excitation-contraction coupling, and metabolism of muscle fibres. We analyzed a large cohort of 109 Italian patients with a suspect of NDM or PP by next-generation sequencing. We identified 24 patients mutated in *CLCN1* gene, 15 in *SCN4A*, 3 in both *CLCN1* and *SCN4A*, 1 in *ATP2A1*, 1 in *KCNA1* and 1 in *CASQ1*. Eight were novel mutations: p.G395Cfs*32, p.L843P, p.V829M, p.E258E and c.1471+4delTCAAGAC in *CLCN1*, p.K1302R in *SCN4A*, p.L208P in *ATP2A1* and c.280–1G>C in *CASQ1* genes. This study demonstrated the utility of targeted next generation sequencing approach in molecular diagnosis of skeletal muscle channelopathies and the importance of the collaboration between clinicians and molecular geneticists and additional methods for unclear variants to make a conclusive diagnosis.

Keywords: Next-generation sequencing; Non-dystrophic myotonias; Periodic paralyses; Skeletal muscle channelopathies; *CLCN1* gene; *SCN4A* gene.

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Systematic Review

Emotional Processing and Experience in Amyotrophic Lateral Sclerosis: A Systematic and Critical Review

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Abstract: Even though increasing literature describes changes in emotional processing in Amyotrophic Lateral Sclerosis (ALS), efforts to summarize relevant findings are lacking in the field. A systematic literature review was performed to provide a critical and up-to-date account of emotional abilities in ALS. References were identified by searches of PubMed, Web of Science and Scopus (1980–2021, English literature), with the following key terms: ("Amyotrophic Lateral Sclerosis" or "Primary Lateral Sclerosis" or "Motor Neuron") and "Emotion*" and ("Processing" or "Attribution" or "Elaboration" or "Perception" or "Recognition"). Studies concerning only caregivers, pseudobulbar affect, and social cognition were excluded. Forty-one articles were included, all concerning ALS, and seven topics were identified: Emotion recognition, Emotional responsiveness, Emotional reactivity, Faces approachability rating, Valence rating, Memory for emotional materials and Alexithymia. The majority of these aspects have only been sparsely addressed. The evidence confirms altered emotional processing in ALS. The most consistent findings regard the recognition of facial expressions for negative emotions, but also alterations in the subjective responsiveness to emotional stimuli (arousal, valence and approachability), in psychophysiological and cerebral reactivity and in emotional memory, together with alexithymia traits, were reported. According to this evidence, emotional abilities should be included in the clinical assessment and therapeutic interventions.

Keywords: amyotrophic lateral sclerosis; motor neuron diseases; emotions; alexithymia

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ORIGINAL ARTICLE

Compensating for verbal-motor deficits in neuropsychological assessment in movement disorders: sensitivity and specificity of the ECAS in Parkinson's and Huntington's diseases

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Abstract

Introduction The study aims at investigating psychometric properties of the *Edinburgh cognitive and behavioural ALS screen* (ECAS) in Parkinson's (PD) and Huntington's (HD) diseases. The sensitivity and specificity of the ECAS in highlighting HD and PD cognitive-behavioural features and in differentiating between these two populations and from healthy controls (HC) were evaluated. Moreover, correlations between the ECAS and traditional cognitive measures, together with core clinical features, were analysed.

Methods Seventy-three PD patients, 38 HD patients, and 49 education-matched healthy participants were enrolled. Participants were administered the ECAS, together with other cognitive screening tools and psychological questionnaires. Patients' behavioural assessment was also carried out with carers.

Results The ECAS distinguished between HD patients and HC and between the two clinical syndromes with high sensitivity and specificity. Even if the diagnostic accuracy of the ECAS in distinguishing between PD and HC was low, the PD cognitive phenotype was very well described by the ECAS performances. Convergent validity of the ECAS against other traditional cognitive screening was observed, as well as correlations with psychological aspects and typical clinical features, especially for the HD group.

Conclusions The ECAS represents a rapid and feasible tool, useful also in other neurodegenerative disorders affecting verbal-motor abilities than the amyotrophic lateral sclerosis such as PD and HD. Clinical applications in these neurodegenerative conditions require further investigations and, probably, some adaptations of the original test.

Keywords Parkinson's disease · Huntington's disease · ECAS · Cognitive assessment · Movement disorders · Psychological symptoms

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ORIGINAL RESEARCH
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Identification of the Raman Salivary Fingerprint of Parkinson's Disease Through the Spectroscopic–Computational Combinatory Approach

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Despite the wide range of proposed biomarkers for Parkinson's disease (PD), there are no specific molecules or signals able to early and uniquely identify the pathology onset, progression and stratification. Saliva is a complex biofluid, containing a wide range of biological molecules shared with blood and cerebrospinal fluid. By means of an optimized Raman spectroscopy procedure, the salivary Raman signature of PD can be characterized and used to create a classification model. Raman analysis was applied to collect the global signal from the saliva of 23 PD patients and related pathological and healthy controls. The acquired spectra were computed using machine and deep learning approaches. The Raman database was used to create a classification model able to discriminate each spectrum to the correct belonging group, with accuracy, specificity, and sensitivity of more than 97% for the single spectra attribution. Similarly, each patient was correctly assigned with discriminatory power of more than 90%. Moreover, the extracted data were significantly correlated with clinical data used nowadays for the PD diagnosis and monitoring. The preliminary data reported highlight the potentialities of the proposed methodology that, once validated in larger cohorts and with multi-centered studies, could represent an innovative minimally invasive and accurate procedure to determine the PD onset, progression and to monitor therapies and rehabilitation efficacy.

Keywords: Parkinson's disease, saliva, Raman spectroscopy, classification model, deep learning

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RESEARCH ARTICLE

Progression of cognitive and behavioral disturbances in motor neuron diseases assessed using standard and computer-based batteries

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Abstract

Objective: Detecting and monitoring cognitive and behavioral deficits in motor neuron diseases (MND) is critical due to their considerable clinical impact. In this scenario, computer-based batteries may play an important role. In this study, we investigated the progression of cognitive and behavioral deficits in MND patients using both standard and computer-based neuropsychological batteries.

Methods: This is a retrospective study on 74 MND patients (52 amyotrophic lateral sclerosis [ALS], 12 primary lateral sclerosis [PLS], and 10 progressive muscular atrophy [PMA]) who were followed up for 12 months and underwent up to three cognitive/behavioral assessments, 6 months apart, including standard and/or computerized based (the Test of Attentional Performance [TAP]) batteries. Behavioral/cognitive changes were investigated over time using generalized linear model for longitudinal data accounting for time and revised-ALS Functional Rating Scale.

Results: Over 12 months, ALS patients showed a global cognitive decline (Mini Mental State Examination) at the standard battery and reduced performance in the alertness, sustained and divided attention, go/nogo, cross-modal and incompatibility TAP tasks. Most of these findings remained significant when ALSFRS-R changes over time were included as covariate in the analyses. ALS patients did not show significant behavioral abnormalities over time. No cognitive and behavioral changes were found in PLS and PMA cases.

Conclusions: Computer-based neuropsychological evaluations are able to identify subtle cognitive changes in ALS, unique to this condition. This study highlights the need of specific, accurate and well-tolerated tools for the monitoring of cognitive deficits in MND.

Keywords: *Motor neuron disease, amyotrophic lateral sclerosis, cognitive decline, computer-based neuropsychological evaluation, test of attentional performance*

Introduction

In amyotrophic lateral sclerosis (ALS), cognitive and behavioral disturbances are observed in about 50% of patients (1). Detecting and monitoring even subtle cognitive deficits in ALS is critical due to their considerable clinical impact: patients who present with cognitive impairment at the early stage show a faster progression of motor impairment (2), and the occurrence of executive dysfunction during the disease course has been associated with a reduced survival (3). Furthermore, the presence of dysexecutive symptoms in ALS impacts on patient and caregiver psychological well-being, adherence to treatment, decision-making, and ability to benefit from non-pharmacological interventions (4). However, the

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Impaired recognition of disgust in amyotrophic lateral sclerosis is related to basal ganglia involvement

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ABSTRACT

In the present study we investigated emotion recognition in pure motor amyotrophic lateral sclerosis (ALS) patients and its relationship with the integrity of basal ganglia, hippocampus and amygdala. Twenty ALS patients without either cognitive or behavioural impairment, and 52 matched healthy controls performed a neuropsychological assessment including the Comprehensive Affect Testing System (CATS) investigating emotion recognition. All participants underwent also a 3T brain MRI. Volumes of basal ganglia, hippocampus and amygdala bilaterally were measured using FIRST in FSL. Sociodemographic, cognitive and MRI data were compared between groups. In ALS patients, correlations between CATS significant findings, brain volumes, cognition, mood and behaviour were explored. ALS patients showed altered performances at the CATS total score and, among the investigated emotions, patients were significantly less able to recognize disgust compared with controls. No brain volumetric differences were observed between groups. In ALS patients, a lower performance in disgust recognition was related with a reduced volume of the left pallidum and a lower performance on the Edinburgh Cognitive and Behavioural ALS Screen. Cognitively/behaviourally unimpaired ALS patients showed impaired disgust recognition, which was associated with pallidum volume. The association with cognitive alterations may suggest impaired disgust recognition as an early marker of cognitive decline.

1. Introduction

Alterations of socioemotional behaviour are important early features of frontotemporal lobar degeneration (FTLD), particularly of the behavioural variant of frontotemporal dementia (FTD) and the semantic variant of primary progressive aphasia (Werner et al., 2007). Social cognition deficits have been reported also in amyotrophic lateral sclerosis (ALS) patients as alterations in theory of mind and emotion processing (Girardi et al., 2011). The first studies on emotion processing in ALS are dated back to 2005 (Lule et al., 2005; Papps et al., 2005), and up to date several investigations reported emotion perception impairment in ALS (Aho-Özhan et al., 2016; Andrews et al., 2017; Crespi et al., 2014; Crespi et al., 2016; Girardi et al., 2011; Lule et al., 2005; Oh et al., 2016; Palmieri et al., 2010; Zimmerman et al., 2007), such as emotion recognition (both facial and prosodic), emotion attribution, and reduced psychophysiological excitability to emotional stimuli (Benbrika et al., 2019). According to a recent *meta*-analysis, the most frequent alteration in ALS patients is in facial recognition for disgust and surprise (Bora, 2017), followed by inability in recognizing anger (Andrews et al., 2017; Crespi et al., 2014; Girardi et al., 2011; Oh et al., 2016; Savage et al., 2014; Zimmerman et al., 2007) and sadness (Aho-Özhan et al., 2016; Andrews et al., 2017; Oh et al., 2016; Zimmerman et al., 2007). On the other hand, recognition of fear and happiness seems to be relatively spared (Aho-Özhan et al., 2016; Girardi et al., 2011). Although emotion

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BRAIN
CLINICAL TRIAL

The unfolded protein response in amyotrophic later sclerosis: results of a phase 2 trial

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Strong evidence suggests that endoplasmic reticulum stress plays a critical role in the pathogenesis of amyotrophic lateral sclerosis (ALS) through altered regulation of proteostasis. Robust preclinical findings demonstrated that guanabenz selectively inhibits endoplasmic reticulum stress-induced eIF2α-phosphatase, allowing misfolded protein clearance, reduces neuronal death and prolongs survival in *in vitro* and *in vivo* models. However, its safety and efficacy in patients with ALS are unknown.

To address these issues, we conducted a multicentre, randomized, double-blind trial with a futility design. Patients with ALS who had displayed an onset of symptoms within the previous 18 months were randomly assigned in a 1:1:1:1 ratio to receive 64 mg, 32 mg or 16 mg of guanabenz or placebo daily for 6 months as an add-on therapy to riluzole. The purpose of the placebo group blinding was to determine safety but not efficacy. The primary outcome was the proportion of patients progressing to higher stages of disease within 6 months as measured using the ALS Milano-Torino staging system, compared with a historical cohort of 200 patients with ALS. The secondary outcomes were the rate of decline in the total revised ALS functional rating scale score, slow vital capacity change, time to death, tracheotomy or permanent ventilation and serum light neurofilament level at 6 months.

The primary assessment of efficacy was performed using intention-to-treat analysis. The treatment arms using 64 mg and 32 mg guanabenz, both alone and combined, reached the primary hypothesis of non-futility, with the proportions of patients who progressed to higher stages of disease at 6 months being significantly lower than that expected under the hypothesis of non-futility and a significantly lower difference in the median rate of change in the total revised ALS functional rating scale score.

This effect was driven by patients with bulbar onset, none of whom (0/18) progressed to a higher stage of disease at 6 months compared with those on 16 mg guanabenz (4/8; 50%), the historical cohort alone (21/49; 43%; $P = 0.001$) or plus placebo (25/60; 42%; $P = 0.001$). The proportion of patients who experienced at least one adverse event was higher in any guanabenz arm than in the placebo arm, with higher dosing arms having a significantly higher proportion of drug-related side effects and the 64 mg arm a significantly higher drop-out rate. The number of serious adverse events did not significantly differ between the guanabenz arms and the placebo. Our findings indicate that a larger trial with a molecule targeting the unfolded protein response pathway without the alpha-2 adrenergic related side-effect profile of guanabenz is warranted.

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Keywords: amyotrophic lateral sclerosis; unfolded protein response; guanabenz

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = revised ALS functional rating scale; eIF = eukaryotic translation initiation factor; ER = endoplasmic reticulum; MITOS = Milano-Torino staging; sVC = slow vital capacity

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal disease hallmarked by the non-cell-autonomous degeneration of motor neurons in the cortex, medulla and spinal cord and the inclusion of cytoplasmic misfolded proteins in degenerating neuronal and non-neuronal cells, occurring both in familial and sporadic cases.[1–6] The misfolded protein overload triggers pathological signalling and induces abnormal interactions with native membrane proteins.[7] This can lead to the diffusion of misfolded proteins in the extracellular space and cell-to-cell propagation of the disease.[8–11] Such impairment in the homeostasis and propagation of proteins is a recognized pathological pathway in ALS,[12–20] possibly driven also by disease-related genes encoding adapter proteins.[6]

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FEATURED ARTICLE

Clinical reporting following the quantification of cerebrospinal fluid biomarkers in Alzheimer's disease: An international overview

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Abstract

Introduction: The current practice of quantifying cerebrospinal fluid (CSF) biomarkers as an aid in the diagnosis of Alzheimer's disease (AD) varies from center to center. For a same biochemical profile, interpretation and reporting of results may differ, which can lead to misunderstandings and raises questions about the commutability of tests.
Methods: We obtained a description of (pre-)analytical protocols and sample reports from 40 centers worldwide. A consensus approach allowed us to propose harmonized comments corresponding to the different CSF biomarker profiles observed in patients.
Results: The (pre-)analytical procedures were similar between centers. There was considerable heterogeneity in cutoff definitions and report comments. We therefore identified and selected by consensus the most accurate and informative comments regarding the interpretation of CSF biomarkers in the context of AD diagnosis.
Discussion: This is the first time that harmonized reports are proposed across worldwide specialized laboratories involved in the biochemical diagnosis of AD.

KEYWORDS
Alzheimer's disease, cerebrospinal fluid biomarkers, clinical report, consensus approach, harmonization

1 | INTRODUCTION

Alzheimer's disease (AD) has gradually become one of the major global public health issues due to its prevalence, which increases with age and life expectancy, and the economic cost of caring for patients whose cognitive decline progressively leads to loss of functional autonomy.[1]

The diagnosis of AD is based on a multidisciplinary approach, involving, among other things, evaluation of the medical history together with clinical symptoms and signs, neuropsychological tests, and neuroimaging. The quantification of cerebrospinal fluid (CSF) core biomarkers (amyloid beta peptides [A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$], total tau [t-tau] and its phosphorylated form on threonine 181 [p-tau(181)]) has progressively proven useful for the diagnosis of AD and its prodromal forms.[1] CSF biomarkers are now included in international guidelines for the diagnosis of AD in research settings and clinical practice[2,3] and the Alzheimer's Association appropriate use criteria for the use of lumbar puncture and CSF testing in the diagnosis of AD have been published.[4] Such biochemical diagnostics are currently implemented in many specialized centers around the world. Different methods of analysis have been developed over the last decade and each laboratory has implemented the one best suited to its own practice. Related to this diversity there are also variations in pre-analytical and analytical conditions (such as sample tubes, storage, dilution of the biological sample, definition of cut-off values) between centers. The subsequent interpretation of the analytical results may depend on the calculation of ratios (such as t-tau/A$\beta_{1\text{-}42}$ or A$\beta_{1\text{-}42}$/A$\beta_{1\text{-}40}$[5–7]), the use of scales (PLM,[8] Erlangen[9] scores), or on additional experiments (eg, dilution if t-tau is above the limit for detection[10]). Some laboratories mentioned the use of the A/T/N[11] classification, which is, however, based on data additional to CSF biomarkers, and is used more in the research setting than in the clinic. Depending on the laboratory, the type of report sent back to physicians (prescribing or referring physicians, and general practitioners) varies greatly, which may raise questions about the commutability of the tests and cause misunderstanding. It is therefore very important to harmonize comments on the reporting of results, so that the conclusions are similar regardless of where the analysis is performed.

Our work provides an overview of the procedures used in 40 centers worldwide performing CSF analysis to support AD diagnosis. For

brain sciences

MDPI

Article

Long-Lasting Cognitive Abnormalities after COVID-19

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Abstract: Considering the mechanisms capable of causing brain alterations in COVID-19, we aimed to study the occurrence of cognitive abnormalities in the months following hospital discharge. We recruited 38 (aged 22–74 years; 27 males) patients hospitalized for complications of SARS-CoV-2 infection in nonintensive COVID units. Participants underwent neuropsychological testing about 5 months after hospital discharge. Of all patients, 42.1% had processing speed deficits, while 26.3% showed delayed verbal recall deficits. Twenty-one percent presented with deficits in both processing speed and verbal memory. Bivariate analysis revealed a positive correlation between the lowest arterial oxygen partial pressure (PaO_2) to fractional inspired oxygen (FiO_2) (P/F) ratio during hospitalization and verbal memory consolidation performance (SRT-LTS score, $r = 0.404$, $p = 0.027$), as well as a positive correlation between SpO_2 levels upon hospital arrival and delayed verbal recall performance (SRT-D score, $r_s = 0.373$, $p = 0.042$). Acute respiratory distress syndrome (ARDS) during hospitalization was associated with worse verbal memory performance (ARDS vs. no ARDS: SRT-LTS mean score = 30.63 ± 13.33 vs. 44.50 ± 13.16, $p = 0.007$; SRT-D mean score = 5.95 ± 2.56 vs. 8.10 ± 2.62, $p = 0.029$). Cognitive abnormalities can frequently be found in COVID-19 patients 5 months after hospital discharge. Increased fatigability, deficits of concentration and memory, and overall decreased cognitive speed months after hospital discharge can interfere with work and daily activities.

Keywords: COVID-19; cognition; processing speed; acute respiratory distress syndrome

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1. Introduction

COVID-19 was initially considered almost exclusively a respiratory syndrome, but increasing evidence indicates that SARS-CoV-2 infection also affects other body districts and functions [1]. More specifically, studies have shown that SARS-CoV-2 is capable of invading the central nervous system (CNS) and causing neurological symptoms [2–6]. Indeed, many coronaviruses are capable of altering the structure and function of the nervous system [7,8]. Additionally, they have been shown to cause nervous system alterations not only through direct infection pathways (both neuronal and circulatory), but also through secondary hypoxia, immune-mediated tissue damage, procoagulative and prothrombotic states, and other mechanisms [9,10].

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ORIGINAL COMMUNICATION

A preliminary comparison between ECAS and ALS-CBS in classifying cognitive–behavioural phenotypes in a cohort of non-demented amyotrophic lateral sclerosis patients

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Abstract

To define the presence and type of frontotemporal dysfunction in amyotrophic lateral sclerosis (ALS), different screening tools have been created. Currently, the most used screening tests are the Edinburgh cognitive and behavioural ALS screen (ECAS) and the ALS cognitive behavioural screen (ALS-CBS). The objective of this study was to compare the ability of ECAS and ALS-CBS in classifying non-demented ALS patients according to Strong criteria. One-hundred and fifty-four in- and out-patients with an age > 18 and a definite or probable ALS diagnosis were recruited between September 2019 and February 2020 at NeMO Clinical Centre and at Istituto Auxologico Italiano in Milan and underwent the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) and the ALS Cognitive Behavioural Screen (ALS-CBS). Exclusion criteria involved patients with a diagnosis of FTD, with a severe cognitive deterioration and/or an important behavioural impairment, with a significant psychiatric disorder or with the co-presence of another significant illness. The distribution of patients according to Strong criteria was different for ECAS and ALS-CBS and the degree of agreement between the two tests in terms of Cohen's Kappa coefficient resulted equal to 0.2047 with a 95% confidence limits interval between 0.1122 and 0.2973. This study for the first time compares the ability of ECAS and ALS-CBS in stratifying ALS patients. Further studies will be conducted to better understand the reasons underlying the differences between these two tests in classifying the different subtypes of fronto-temporal dysfunction in ALS.

Keywords Amyotrophic lateral sclerosis · ECAS · ALS-CBS · Strong criteria

Lucia Catherine Greco, Andrea Lizio, Barbara Poletti, and Christian Lunetta have contributed equally to the manuscript.

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Original research

Comparison of CSF and serum neurofilament light and heavy chain as differential diagnostic biomarkers for ALS

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ABSTRACT

Objective Elevated levels of neurofilament light (NfL) and heavy (NfH) chain in amyotrophic lateral sclerosis (ALS) cerebrospinal fluid (CSF) and serum reflect neuro-axonal degeneration and are used as diagnostic biomarkers. However, studies comparing the differential diagnostic potential for ALS of all four parameters are missing. Here, we measured serum NfL/NfH and CSF NfL/NfH in a large cohort of ALS and other neurological disorders and analysed the differential diagnostic potential.

Methods In total CSF and serum of 294 patients were analysed. The diagnostic groups comprised: ALS (n=75), frontotemporal lobar degeneration (FTLD) (n=33), Alzheimer's disease (n=20), Parkinson's disease (dementia) (n=18), Creutzfeldt-Jakob disease (n=11), non-neurodegenerative controls (n=77) (Con) and 60 patients who were seen under the direct differential diagnosis of a patient with ALS (Con.DD).

Results CSF and serum NfL and NfH showed significantly increased levels in ALS (p<0.0001) compared with Con and Con.DD. The difference between ALS and FTLD was markedly stronger for NfH than for NfL. CSF and serum NfL demonstrated a stronger correlation (r=0.84 (95% CI 0.80 to 0.87), p<0.001) than CSF and serum NfH (r=0.68 (95% CI 0.61 to 0.75), p<0.0001). Comparing ALS and Con.DD, receiver operating characteristic analysis revealed the best area under the curve (AUC) value for CSF NfL (AUC=0.94, 95% CI 0.91 to 0.98), followed by CSF NfH (0.93, 95% CI 0.88 to 0.98), serum NfL (0.93, 95% CI 0.89 to 0.97) and serum NfH (0.88, 95% CI 0.82 to 0.94).

Conclusion Our results demonstrate that CSF NfL and NfH as well as serum NfL are equally suited for the differential diagnosis of ALS, whereas serum NfH appears to be slightly less potent.

INTRODUCTION

Neurofilaments as cytoskeletal proteins of neurons are widely accepted as markers of axonal damage in various diseases including amyotrophic lateral sclerosis (ALS).[1–4] ALS, a severe neurodegenerative disease characterised by the dysfunction and death of the upper and lower motor neurons, affects approximately 2.6–3.0 in 100 000 individuals and leads to death on average 3 years after first clinical symptoms.[5 6] In patients with ALS cerebrospinal fluid (CSF) and serum levels of neurofilament light (NfL) and heavy (NfH) chain are elevated compared with most other neurological disorders.[7] Furthermore, neurofilaments in CSF and serum of patients with ALS are elevated early in the disease, which allows the diagnosis to be supported at a stage when possible treatment strategies could still be disease modifying.[8] Hence, at present, neurofilaments represent the most promising biomarker candidates to enter the clinical routine supporting the differential diagnosis and prognosis of ALS, the stratification of patients in clinical trials and the monitoring of therapeutic effects.[9–13] However, so far analyses investigating and comparing the differential diagnostic potential of CSF NfL and NfH as well as serum NfL and NfH in ALS in a single study are missing. Here, we apply the same microfluidic system for the analysis of all four markers in a group of patients with neurological disorders including ALS, frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD), Parkinson's disease (PD) and PD with dementia (PDD), Creutzfeldt-Jakob disease (CJD), a cohort of non-ALS patients whose initial differential diagnosis included ALS (Con.DD) as well as non-neurodegenerative control patients (Con). Furthermore, we perform correlations and compare by receiver operating characteristic (ROC) analysis the individual potential of the four neurofilament parameters for the discrimination of ALS from Con and Con.DD.

METHODS

Patients

All CSF and serum specimen examined in this study were from patients of the Department of Neurology Ulm (between 2014 and 2020) with the exception of patients with CJD, which were seen in the unit for transmissible spongiform encephalopathies of the Department of Neurology in Göttingen (1997–2003).

Neurofilaments were measured in CSF and serum of seven different diagnostic groups comprising ALS, FTLD, PD/PDD, AD, CJD, Con.DD and Con.

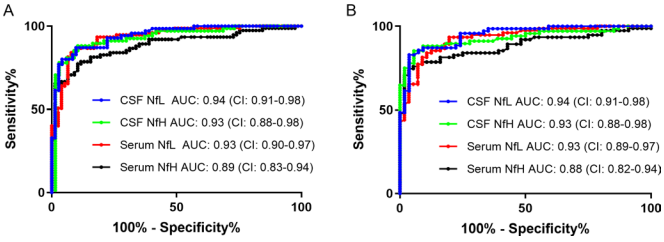

Figure 3 Comparison of neurofilament ROC analyses of ALS versus Con and Con.DD. (A) ROC curves for the discrimination of ALS and Con for CSF and serum NfL and NfH. (B) ROC curves for the discrimination of ALS and Con.DD for CSF and serum NfL and NfH. ALS, amyotrophic lateral sclerosis; AUC, area under the curve; Con, non-neurodegenerative control patients; Con.DD, control patients with initial diagnostic suspicion of ALS but final diagnosis of different condition; NfL, neurofilament light chain; NfH, neurofilament heavy chain; ROC, receiver operating characteristic.

less power for serum NfH in the (differential) diagnosis of ALS. These results confirm studies on either NfH or NfL in ALS which also reported a better discrimination for CSF NfH compared with blood NfH[34 40] as well as similar good results for the NfLs.[32 33] One possible explanation for the slightly worse performance of serum NfH and the weaker correlation of CSF and serum NfH might be that the heavily phosphorylated NfH in the blood stream is more prone to changes of its post-translational modifications and/or masking of its epitopes leading to a slightly lower affinity of the detecting antibodies. In contrast to our results, one study using ELISAs for analysis found a slightly better potential of CSF NfH compared with CSF NfL in discriminating ALS from disease mimics.[41] The same study also reported a better potential of CSF NfH compared with CSF NfL in discriminating ALS from disease controls. As the disease control group of the colleagues also comprised neurodegenerative patients, in fact many FTLD cases, this result, however, is not necessarily contradictory to our findings as we compared the ALS neurofilament levels to non-neurodegenerative controls. If anything the results could underline the higher potential of CSF NfH in the discrimination of ALS and FTLD as we describe above. The combinations of CSF NfL and NfH as well as serum NfL and NfH levels did not prominently improve the differential potential (data not shown). However, as our findings demonstrate, a complementary use of NfL and NfH could be beneficial in certain differential diagnostic questions and merits further investigation.

To conclude, we here propose that for the diagnosis and differential diagnosis of ALS, CSF and serum NfL as well as CSF NfH are equally well suited. For the discrimination between ALS and bvFTD our data suggests CSF NfH to be preferable, however, more research is needed for example, on the clearance mechanism of NfH to better understand possible differences regarding neurofilaments between the two diseases.

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Contributors All authors made substantial contributions to conception and design, and/or acquisition of data, and/or analysis and interpretation of data. All authors gave final approval of the version to be submitted and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.Conception and design of the study: SH, PS and MO; Sample collection and data management: SH, PS, FV, JW, PO, CVA, JD, EF, BM, AR, VS, ACL and MO; Study management and coordination: SH, MO; Statistical methods and analysis: SH, PS, BM and MO; Interpretation of results: SH, PS, FV, JW, PO, CVA, JD, EF, BM, AR, VS, ACL and MO; Manuscript writing (first draft): SH and MO; Critical revision of the manuscript: SH, PS, FV, JW, PO, CVA, JD, EF, BM, AR, VS, ACL and MO.

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Competing interests SH, PS, FV, JW, PO, JD, EF, BM, AR, VS and ACL report no competing interests. CVA received honoraria from serving on the scientific advisory board of Biogen, Roche, and Willmar Schwabe & Co. KG and has received funding for travel and speaker honoraria from Lilly GmbH, Daiichi Sankyo, Biogen, Roche diagnostics AG and Willmar Schwabe GmbH &Co. KG and has received research support from Roche diagnostics AG. MO gave scientific advice for Fujirebio, Roche, Biogen and Axon.

Patient consent for publication Not required.

Ethics approval The study was approved by local Ethics Commitees (approval numbers: Ulm 20/10, Göttingen 100305) and conducted following the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

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Table 3 Sensitivity and specificity of CSF and serum NfL and NfH for discrimination between ALS and con and between ALS and Con.DD

ALS vs Con ALS vs Con.DD	Discrimination	Calculated cut-off (pg/mL)	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	Positive likelihood ratio (95% CI) (%)
CSF NfL	vs Con	>1324	87 (77 to 94)	90 (81 to 96)	9 (4 to 18)
	vs Con.DD	>1599	83 (72 to 91)	96 (87 to 99)	22 (6 to 88)
CSF NfH	vs Con	>1598	88 (78 to 95)	90 (80 to 96)	8 (4 to 17)
	vs Con.DD	>1754	85 (75 to 92)	94 (85 to 98)	15 (5 to 46)
Serum NfL	vs Con	>45	87 (77 to 93)	90 (81 to 95)	8 (4 to 16)
	vs Con.DD	>34	93 (85 to 98)	80 (68 to 90)	5 (3 to 8)
Serum NfH	vs Con	>529	79 (68 to 87)	88 (79 to 95)	6 (4 to 13)
	vs Con.DD	>677	75 (63 to 84)	96 (88 to 99)	21 (5 to 82)

ALS, amyotrophic lateral sclerosis; CI, confidence interval; Con, non-neurodegenerative control patients; Con.DD, patients with initial diagnostic suspicion of ALS but final diagnosis of different condition; CSF, cerebrospinal fluid; NfH, neurofilament heavy chain; NfL, neurofilament light chain.

BRAIN COMMUNICATIONS

SCFD1 expression quantitative trait loci in amyotrophic lateral sclerosis are differentially expressed

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Evidence indicates that common variants found in genome-wide association studies increase risk of disease through gene regulation via expression Quantitative Trait Loci. Using multiple genome-wide methods, we examined if Single Nucleotide Polymorphisms increase risk of Amyotrophic Lateral Sclerosis through expression Quantitative Trait Loci, and whether expression Quantitative Trait Loci expression is consistent across people who had Amyotrophic Lateral Sclerosis and those who did not. In combining public expression Quantitative Trait Loci data with Amyotrophic Lateral Sclerosis genome-wide association studies, we used Summary-data-based Mendelian Randomization to confirm that *SCFD1* was the only gene that was genome-wide significant in mediating Amyotrophic Lateral Sclerosis risk via expression Quantitative Trait Loci (Summary-data-based Mendelian Randomization beta = 0.20, standard error = 0.04, *P*-value = 4.29×10^{-6}). Using *post-mortem* motor cortex, we tested whether expression Quantitative Trait Loci showed significant differences in expression between Amyotrophic Lateral Sclerosis ($n = 76$) and controls ($n = 25$), genome-wide. Of 20 757 genes analysed, the two most significant expression Quantitative Trait Loci to show differential in expression between Amyotrophic Lateral Sclerosis and controls involve two known Amyotrophic Lateral Sclerosis genes (*SCFD1* and *VCP*). *Cis*-acting *SCFD1* expression Quantitative Trait Loci downstream of the gene showed significant differences in expression between Amyotrophic Lateral Sclerosis and controls (top expression Quantitative Trait Loci beta = 0.34, standard error = 0.063, *P*-value = 4.54×10^{-7}). These *SCFD1* expression Quantitative Trait Loci also significantly modified Amyotrophic Lateral Sclerosis survival (number of samples = 4265, hazard ratio = 1.11, 95% confidence interval = 1.05–1.17, *P*-value = 2.06×10^{-4}) and act as an Amyotrophic Lateral Sclerosis trans-expression Quantitative Trait Loci hotspot for a wider network of genes enriched for *SCFD1* function and Amyotrophic Lateral Sclerosis pathways. Using gene-set analyses, we found the genes that correlate with this trans-expression Quantitative Trait Loci hotspot significantly increase risk of Amyotrophic Lateral Sclerosis (beta = 0.247, standard deviation = 0.017, $P = 0.001$) and schizophrenia (beta = 0.263, standard deviation = 0.008, *P*-value = 1.18×10^{-5}), a disease that genetically correlates with Amyotrophic Lateral Sclerosis. In summary, *SCFD1* expression Quantitative Trait Loci are a major factor in Amyotrophic Lateral Sclerosis, not only influencing disease risk but are differentially expressed in *post-mortem* Amyotrophic Lateral Sclerosis. *SCFD1* expression Quantitative Trait Loci show distinct expression profiles in Amyotrophic Lateral Sclerosis that correlate with a wider network of genes that also confer risk of the disease and modify the disease's duration.

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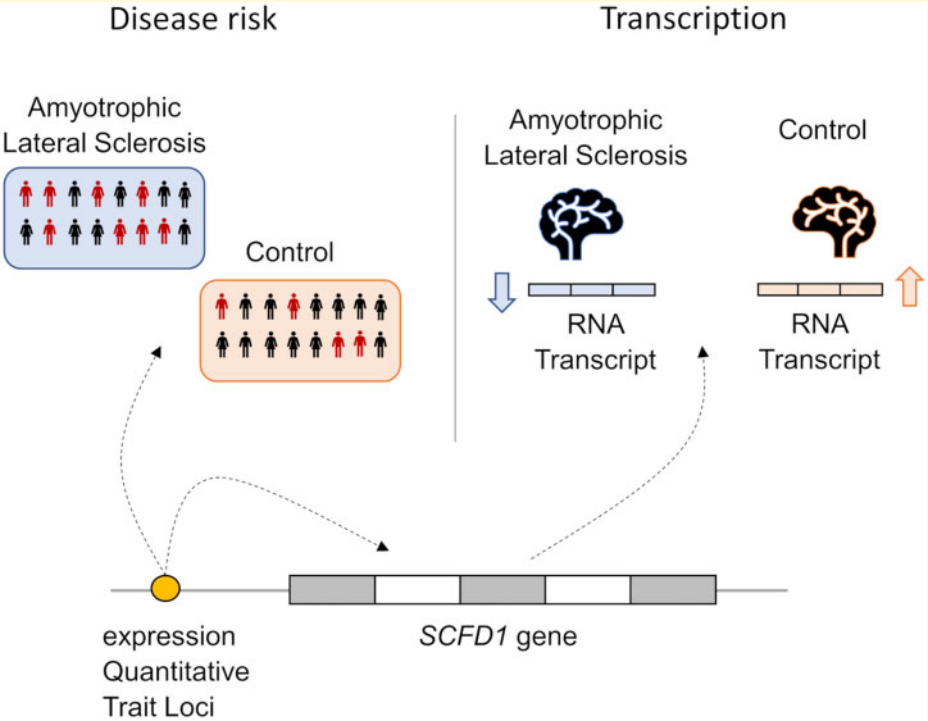
Keywords: amyotrophic lateral sclerosis; expression quantitative trait loci; GWAS; Mendelian randomization; vesicle-mediated transport

Abbreviations: ALS = amyotrophic lateral sclerosis; GWAS = genome-wide association study; CI = confidence Interval; D′ = D-prime; eQTL = expression Quantitative Trait Loci; ER = endoplasmic reticulum; FDR = false discovery rate; FTD = frontotemporal dementia; GO = gene ontology; GRCh37 = Genome Reference Consortium human build 37; GTEx = genotype-tissue expression project; HG19 = human genome build 19; hp-τ = hyperphosphorylated tau; KB = kilobase; NES = normalized effect size; r^2 = R-squared; RIN = RNA integrity number; rsID = reference SNP identifier; SD = standard deviation; SE = standard error; SMR = summary-data-based Mendelian Randomization; SNP = single nucleotide polymorphism; VST = variance stabilizing transformation

Graphical Abstract



Supplemental Online Content

Johnson JO, Chia R, Miller DE, et al; FALS Sequencing Consortium; American Genome Center; International ALS Genomics Consortium; ITALSGEN Consortium. Association of variants in the *SPTLC1* gene with juvenile amyotrophic lateral sclerosis. *JAMA Neurol*. Published online August 30, 2021. doi:10.1001/jamaneurol.2021.2598

This supplementary material has been provided by the authors to give readers additional information about their work.

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LETTER TO THE EDITOR

Unilateral freezing of gait or "magnetic feet phenomenon" caused by ischemic lesion involving fronto-striatal networks

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Keywords Freezing of the gait · Parkinsonism · Frontostriatal network

Dear Editor,

Freezing of gait (FOG) is defined as an episodic inability to generate effective stepping and is usually associated with parkinsonism. Very rarely, FOG is caused by focal lesions of the brain. We present a case of an 88-year-old woman affected by monolateral FOG due to an ischemic stroke of the periventricular portion of the corona radiata extending to the outer edge of the putamen. The clinical findings suggest a major role of cortico-striatal connections in the pathology of FOG.

We present a case of an 88-year-old woman who was admitted to our Department following a sudden onset of non-fluent aphasia associated with deterioration of gait. A rapid regression of the speech disorder was observed, but the gait did not improve: she was able to maintain an upright standing position, but her right foot remained glued to the floor. The leg showed no tremor in the attempt to lift the foot off the ground. After several attempts, she managed to detach her foot and take a few short, crawling steps. Difficulty in initiating the first step was associated with anxiety and fear of fall. Sensory cues did not make the initiation of the gait easier. Neurological examination showed no lower limb weakness or apraxia, bradykinesia, stiffness, tremor, or language disorders. Bicycling and rapid alternating flexion and extension movements of the feet were normal (Video 1). She could move from horizontal to sitting position without difficulty. We did not observe postural instability and or a tendency to fall. The age- and gender-normalized Mini-Mental State Examination score was within the normal range (MMSE 25/30). Our patient presented anxiety related to her motor disability in walking without evidence of apathy, disinhibition, and stereotype-repetitive behaviors. The result of the frontal assessment battery FAB test was 15.2 (adjusted for age and education). Since she did not show these clinical signs, we suggest ruling out a particular frontal behavioral impairment.

Brain MRI revealed an ischemic lesion of the portion of the corona radiata extending to the outer edge of the left putamen (Fig. 1). As the patient suffered from a dilated cardiomyopathy, a cardioembolic origin of the lesion was suspected. No significant change of motor symptoms was seen after treatment with L-Dopa\Benserazide 200\50 mg. Two years after the onset of unilateral FOG, and despite rehabilitation sessions, she still experienced extreme difficulty and latency to pick her right foot off the floor and start walking. Her walking started with the left leg, while the right leg appeared glued to the floor. She stood straight correctly, remained calm and aware of her disability, and denied fear of falling or anxiety during walking.

FOG refers to an episodic inability to start the gait. It is frequently associated with festination, postural instability, and other gait disorders such as reduction of step speed and amplitude, and changes in cadence [1]. FOG is frequently observed in advanced-stage Parkinson's disease (PD), atypical degenerative parkinsonism, vascular parkinsonism, and normal pressure hydrocephalus. Our patient showed a clear, selective unilateral FOG characterized by start hesitation with inability to start the gait with the right limb in the absence of paralysis, bradykinesia, tremor, lower limb apraxia, and

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ORIGINAL ARTICLE

SUMOylation Regulates TDP-43 Splicing Activity and Nucleocytoplasmic Distribution

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Abstract

The nuclear RNA-binding protein TDP-43 forms abnormal cytoplasmic aggregates in the brains of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) patients and several molecular mechanisms promoting TDP-43 cytoplasmic mislocalization and aggregation have been proposed, including defects in nucleocytoplasmic transport, stress granules (SG) disassembly and post-translational modifications (PTM). SUMOylation is a PTM which regulates a variety of cellular processes and, similarly to ubiquitination, targets lysine residues. To investigate the possible regulatory effects of SUMOylation on TDP-43 activity and trafficking, we first assessed that TDP-43 is SUMO-conjugated in the nuclear compartment both covalently and non-covalently in the RRM1 domain at the predicted lysine 136 and SUMO-interacting motif (SIM, 106–110 residues), respectively. By using the SUMO-mutant TDP-43 K136R protein, we demonstrated that SUMOylation modifies TDP-43 splicing activity, specifically exon skipping, and influences its sub-cellular localization and recruitment to SG after oxidative stress. When promoting deSUMOylation by SENP1 enzyme over-expression or by treatment with the cell-permeable SENP1 peptide TS-1, the cytoplasmic localization of TDP-43 increased, depending on its SUMOylation. Moreover, deSUMOylation by TS-1 peptide favoured the formation of small cytoplasmic aggregates of the C-terminal TDP-43 fragment p35, still containing the SUMO lysine target 136, but had no effect on the already formed p25 aggregates. Our data suggest that TDP-43 can be post-translationally modified by SUMOylation which may regulate its splicing function and trafficking, indicating a novel and druggable mechanism to explore as its dysregulation may lead to TDP-43 pathological aggregation in ALS and FTD.

Keywords TDP-43 · SUMOylation · Amyotrophic lateral sclerosis · Nucleocytoplasmic transport · Splicing

Introduction

TDP-43 is an ubiquitous RNA-binding protein (RBP) localized in the nucleus where it mainly regulates splicing but, by shuttling between the nucleus and the cytoplasm, it also controls RNA metabolism at different levels, including miRNA biogenesis, mRNA transport, stability and translation [1]. In the brain of patients suffering from the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), abnormal TDP-43 protein aggregates are found in the cytoplasm of both neuronal and glial cells [2–4]. In these pathological aggregates, TDP-43 is post-translationally modified by C-terminal cleavage, ubiquitination, phosphorylation and acetylation [5]. The occurring post-translational modifications (PTM) primarily seem to reduce TDP-43 solubility and to induce its aggregation [5], but acetylation was shown to decrease its RNA-binding and splicing activities [6]. However, how and whether all these PTM are interconnected in regulating TDP-43 function and in promoting its pathological aggregation is still unknown.

The PTM SUMOylation consists in the conjugation of different small ubiquitin-related modifiers (SUMO-1, 2/3 and 4) to specific lysines of the target protein through different steps which, similarly to ubiquitination, involve SUMO-E1 activating enzyme, SUMO-E2 conjugating enzyme (UBC9), and SUMO-E3 ligases. SUMOylation is reversible by means of the SENP proteases that cleave

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The Effect of *SMN* Gene Dosage on ALS Risk and Disease Severity

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Objective: The role of the survival of motor neuron (*SMN*) gene in amyotrophic lateral sclerosis (ALS) is unclear, with several conflicting reports. A decisive result on this topic is needed, given that treatment options are available now for *SMN* deficiency.

Methods: In this largest multicenter case control study to evaluate the effect of *SMN1* and *SMN2* copy numbers in ALS, we used whole genome sequencing data from Project MinE data freeze 2. *SMN* copy numbers of 6,375 patients with ALS and 2,412 controls were called from whole genome sequencing data, and the reliability of the calls was tested

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COVID-19

Testing olfactory dysfunction in acute and recovered COVID-19 patients: a single center study in Italy

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Abstract

Background Olfactory dysfunction in coronavirus disease 2019 (COVID-19) is common during acute illness and appears to last longer than other symptoms. The aim of this study was to objectively investigate olfactory dysfunction in two cohorts of patients at two different stages: during acute illness and after a median recovery of 4 months.

Methods Twenty-five acutely ill patients and 26 recovered subjects were investigated. Acute patients had a molecular diagnosis of COVID-19; recovered subjects had a positive antibody assay and a negative molecular test. A 33-item psychophysical olfactory identification test tailored for the Italian population was performed.

Results Median time from symptoms onset to olfactory test was 33 days in acute patients and 122 days in recovered subjects. The former scored a significantly higher number of errors at psychophysical testing (median [IQR]: 8 [13] vs 3 [2], $p < 0.001$) and were more frequently hyposmic (64% vs 19%, $p = 0.002$). Recovered subjects reported a variable time to subjective olfactory recovery, from days up to 4 months. Participants included in the study reported no significant nasal symptoms at olfactory testing. Among recovered subject who reported olfactory loss during acute COVID-19, four (27%) were still hyposmic. Demographic and clinical characteristics did not show significant associations with olfactory dysfunction.

Conclusion Moderate-to-severe hospitalized patients showed a high level and frequency of olfactory dysfunction compared to recovered subjects. In the latter group, subjects who reported persisting olfactory dysfunction showed abnormal scores on psychophysical testing, indicating that, at least in some subjects, persistent hyposmia may represent a long-term sequela of COVID-19.

Keywords Anosmia · Hyposmia · Olfactory testing · COVID-19 · SARS-CoV-2

Introduction

Olfactory dysfunction in coronavirus disease 2019 (COVID-19) is a common symptom appearing during the acute phase of the disease [1–5]. The frequency and degree of olfactory dysfunction during COVID-19 was assessed in some studies through heterogeneous psychophysical olfactory tests, mostly in mild disease [6–10]. These reports have shown high prevalence of olfactory dysfunction, from 40 to 98%. One study in hospitalized patients found that about 40 days after symptoms onset, olfactory dysfunction could be detected in 21% of patients [11]. Conversely, little is known about subjective and objective recovery of olfactory function in COVID-19, as only a few studies have been performed and their observation period was limited from 2 to 8 weeks after symptoms onset [12–15]. While these studies have shown relatively high recovery rates in the first weeks after symptoms onset, they still report around 40% of patients affected by olfactory dysfunction at the end of their follow-up. Overall, olfactory dysfunction in hospitalized patients with moderate-to-severe disease and long-term recovery of olfactory function after COVID-19 have not been well established.

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Genetic characterization of a cohort with familial parkinsonism and cognitive-behavioral syndrome: A Next Generation Sequencing study

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ABSTRACT

Objective: To perform the genetic characterization of a cohort with familial parkinsonism and cognitive-behavioral syndrome.

Methods: A Next Generation Sequencing – based targeted sequencing of 32 genes associated to various neurodegenerative phenotypes, plus a screening for *SNCA* Copy Number Variations and *C9orf72* repeat expansion, was applied in a cohort of 85 Italian patients presenting with parkinsonism and cognitive and/or behavioral syndrome and a positive familial history for any neurodegenerative disorder (i.e., dementia, movement disorders, amyotrophic lateral sclerosis).

Results: Through this combined genetic approach, we detected potentially relevant genetic variants in 25.8% of patients with familial parkinsonism and cognitive and/or behavioral syndrome. Peculiar phenotypes are described (Cortico-basal syndrome with *APP*, Posterior Cortical Atrophy with *GBA*, Progressive Supranuclear Palsy-like with *GRN,* Multiple System Atrophy with *TARDBP*).

The majority of patients presented a rigid-bradykinetic parkinsonian syndrome, while rest tremor was less common. Myoclonic jerks, pyramidal signs, dystonic postures and vertical gaze disturbances were more frequently associated with the presence of a pathogenic variant in one of the tested genes.

Conclusions: Given the syndromic approach adopted in our study, we were able to provide a detailed clinical description of patients beyond the boundaries of specific clinical diagnoses and describe peculiar phenotypes. This observation further supports the knowledge that genetic disorders present phenotypic overlaps across different neurodegenerative syndromes, highlighting the limitations of current clinical diagnostic criteria defining sharp boundaries between distinct conditions.

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RESEARCH ARTICLE

Association of Clinically Evident Eye Movement Abnormalities With Motor and Cognitive Features in Patients With Motor Neuron Disorders

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Abstract

Background and Objectives

Although oculomotor abnormalities (OMAs) are not usually considered prominent features of amyotrophic lateral sclerosis (ALS), they may represent potential clinical markers of neurodegeneration, especially when investigated together with cognitive and behavioral alterations. The aim of our study was to identify patterns of clinically evident OMAs in patients with ALS and to correlate such findings with cognitive-behavioral data.

Methods

Three consecutive inpatient cohorts of Italian patients with ALS and controls were retrospectively evaluated to assess the frequency of OMAs and cognitive-behavioral alterations. The ALS population was divided into a discovery cohort and a replication cohort. Controls included a cohort of cognitively impaired individuals and patients with Alzheimer disease (AD). Participants underwent bedside eye movement evaluation to determine the presence and pattern of OMAs. Cognitive assessment was performed with a standard neuropsychological battery (discovery ALS cohort and AD cohort) and the Italian Edinburgh Cognitive and Behavioural ALS Screen (ECAS) (replication ALS cohort).

Results

We recruited 864 individuals with ALS (635 discovery, 229 replication), 798 who were cognitively unimpaired and 171 with AD. OMAs were detected in 10.5% of our ALS cohort vs 1.6% of cognitively unimpaired controls ($p = 1.2 \times 10^{-14}$) and 11.4% of patients with AD (p = NS). The most frequent deficits were smooth pursuit and saccadic abnormalities. OMA frequency was higher in patients with bulbar onset, prominent upper motor neuron signs, and advanced disease stages. Cognitive dysfunction was significantly more frequent in patients with OMAs in both ALS cohorts ($p = 1.1 \times 10^{-25}$). Furthermore, OMAs significantly correlated with the severity of cognitive impairment and with pathologic scores at the ECAS ALS-specific domains. Last, OMAs could be observed in 35.0% of cognitively impaired patients with ALS vs 11.4% of patients with AD ($p = 6.4 \times 10^{-7}$), suggesting a possible involvement of frontal oculomotor areas in ALS.

Conclusion

Patients with ALS showed a range of clinically evident OMAs, and these alterations were significantly correlated with cognitive, but not behavioral, changes. OMAs may be a marker of neurodegeneration, and bedside assessment represents a rapid, highly specific tool for detecting cognitive impairment in ALS.

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biomolecules

MDPI

Brief Report

Exosome microRNAs in Amyotrophic Lateral Sclerosis: A Pilot Study

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Abstract: The pathogenesis of amyotrophic lateral sclerosis (ALS), a lethal neurodegenerative disease, remains undisclosed. Mutations in ALS related genes have been identified, albeit the majority of cases are unmutated. Clinical pathology of ALS suggests a prion-like cell-to-cell diffusion of the disease possibly mediated by exosomes, small endocytic vesicles involved in the propagation of RNA molecules and proteins. In this pilot study, we focused on exosomal microRNAs (miRNAs), key regulators of many signaling pathways. We analyzed serum-derived exosomes from ALS patients in comparison with healthy donors. Exosomes were obtained by a commercial kit. Purification of miRNAs was performed using spin column chromatography and RNA was reverse transcribed into cDNA. All samples were run on the miRCURY LNA™ Universal RT miRNA PCR Serum/Plasma Focus panel. An average of 29 miRNAs were detectable per sample. The supervised analysis did not identify any statistically significant difference among the groups indicating that none of the miRNA of our panel has a strong pathological role in ALS. However, selecting samples with the highest miRNA content, six biological processes shared across miRNAs through the intersection of the GO categories were identified. Our results, combined to those reported in the literature, indicated that further investigation is needed to elucidate the role of exosome-derived miRNA in ALS.

Keywords: exosome; miRNA; amyotrophic lateral sclerosis; biomarkers; serum

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1. Introduction

MicroRNAs (miRNAs) are single-stranded non-coding RNAs involved in the regulation of gene expression and in the control of many cellular and metabolic pathways, such as cell migration, proliferation, and differentiation [1,2]. Previous gene expression analyses have enlightened that a large amount of miRNAs within the central nervous system (CNS) can cross the blood brain barrier and consequently be secreted in the cerebrospinal fluid and peripheral blood [3]. Detectable and quantifiable changes of these miRNA levels can act as a disease sentinel in patients and, therefore, may be suitable as disease-related biomarkers [4]. These small RNAs are abundant in body fluids and may be transported bounded to proteins or carried by vesicles [5,6]. Extracellular vesicles represent a peculiar way for cell-to-cell communication. Among them, exosomes with a diameter of 30–100 nm, are released from the cell by exocytosis through fusion of multivesicular bodies with the plasmatic membrane [7] circulating in the extracellular space adjacent to the site where they

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RESEARCH ARTICLE

Genetic and epigenetic disease modifiers in an Italian *C9orf72* family expressing ALS, FTD or PD clinical phenotypes

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Abstract

Objective: The presence of the hexanucleotide repeat expansion (HRE) in *C9orf72* gene is associated to the ALS/FTD spectrum, but also to parkinsonisms. We here describe an Italian family with the father diagnosed with Parkinson disease (PD) at the age of 67 and the two daughters developing FTD and ALS at 45 years of age. We searched for *C9orf72* HRE with possible genetic and epigenetic modifiers to account for the intrafamilial phenotypic variability. *Methods*: *C9orf72* mutational analysis was performed by fragment length analysis, Repeat-primed PCR and Southern blot. Targeted next generation sequencing was used to analyze 48 genes associated to neurodegenerative diseases. Promoter methylation was analyzed by bisulfite sequencing. *Results:* Genetic analysis identified *C9orf72* HRE in all the affected members with a similar repeat expansion size. Both the father and the FTD daughter also carried the heterozygous p.Ile946Phe variant in *ATP13A2* gene, associated to PD. In addition, the father also showed a heterozygous *EIF4G1* variant (p.Ala13Pro), that might increase his susceptibility to develop PD. The DNA methylation analysis showed that all the 26 CpG sites within *C9orf72* promoter were unmethylated in all family members. *Conclusions:* Neither *C9orf72* HRE size nor promoter methylation act as disease modifiers within this family, at least in blood, not excluding HRE mosaicism and a different methylation pattern in the brain. However, the presence of rare genetic variants in PD genes suggests that they may influence the clinical manifestation in the father. Other genetic and/or epigenetic modifiers must be responsible for disease variability in this *C9orf72* family case.

Keywords: *C9orf72, genetic modifiers, DNA methylation*

Introduction

A hexanucleotide repeat expansion (HRE) in *C9orf72* gene is the most frequent cause of familial and sporadic amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1,2), ranging from 2–23 units in the normal population to >30–>4000 units in pathological conditions (3). In contrast to other repeat expansion disorders, no clear association between HRE size and phenotype severity or disease state (ALS/FTD) has been demonstrated so far. Genetic anticipation is not an evident phenomenon and, within the same pedigree, individuals with a similar HRE may manifest indifferently ALS, FTD, or mixed phenotypes (4–11). In addition, *C9orf72* HRE has been reported in a heterogeneous array of neurological disorders, other than ALS and FTD, including parkinsonism and psychosis (12,13). However, also within the ALS/FTD disease spectrum, the wide heterogeneity of clinical features and symptoms even intra-familiarly suggests that modifiers,

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Review

Association between renin-angiotensin-aldosterone system inhibitors and risk of dementia: A meta-analysis

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Keywords:
ACE-inhibitors
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Dementia, Alzheimer's disease, Vascular dementia

ABSTRACT

Objective: To evaluate the association of all RAAS inhibitors, ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) on dementia onset (any dementia, Alzheimer's disease and vascular dementia) using a meta-analytic approach.
Methods: A systematic MEDLINE search was carried out to identify all observational studies published up to the 30th September 2020 evaluating the association between RAAS inhibitors and risk of dementia. Studies were included if original investigations considering incident dementia cases, with ACEIs and/or ARBs as exposure and other antihypertensives (AHs) use as reference, and if reporting association estimates and relative variability measures. Random effect pooled relative risks (pRR) and the corresponding 95% confidence intervals (95%CI) were calculated according to DerSimonian and Laird's (DL) or to Hartung Knapp Sidik Jonkman (HKSJ) method depending on the number of studies and between-studies heterogeneity. A linear mixed meta-regression model (MM) was applied to take into account correlation among association estimates from the same study.
Results: 15 studies were included in the meta-analysis. ARBs but not ACEIs' use led to a significant reduction of the risk of any dementia (pRR 0.78, 95%CI_{MM} 0.70–0.87) and Alzheimer's disease (pRR 0.73, 95%CI_{MM} 0.60–0.90). Moreover, when compared to ACEIs, ARBs reduced of 14% the risk of any dementia (pRR 0.86, 95% CI_{DL} 0.79–0.94).
Conclusions: ARBs but not ACEIs led to a reduction in the risk of any dementia. The difference between ARBs and ACEIs in terms of preventive effectiveness could be due to distinct profiles of antagonism towards independent receptor pathways or to differential influences on amyloid metabolism.

1. Introduction

Dementia is an encompassing syndromic term for a decline in cognitive abilities of sufficient severity to interfere with the function of an individual during daily activities [1]. The term dementia does not imply an underlying etiology, although neurodegenerative diseases represent the most common causes. Among the different forms, Alzheimer's disease (AD) is the most common and vascular dementia (VaD) encompasses all forms of cognitive impairment related to vascular disease, also with mixed pathology. In 2017, the World Health Organization (WHO) estimated that, worldwide, around 50 million people are affected by dementia and there are nearly 10 million new cases every year. Although young-onset cases are increasingly recognized, dementia is a condition typically affecting older people. In fact, the main risk factors for the disease are ageing, diabetes, and hypertension [2]. Indeed, increasing evidence shows that raised blood pressure is an

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Original research

Value of systematic genetic screening of patients with amyotrophic lateral sclerosis

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ABSTRACT

Objective The clinical utility of routine genetic sequencing in amyotrophic lateral sclerosis (ALS) is uncertain. Our aim was to determine whether routine targeted sequencing of 44 ALS-relevant genes would have a significant impact on disease subclassification and clinical care.

Methods We performed targeted sequencing of a 44-gene panel in a prospective case series of 100 patients with ALS recruited consecutively from the Sheffield Motor Neuron Disorders Clinic, UK. All participants were diagnosed with ALS by a specialist Consultant Neurologist. 7/100 patients had familial ALS, but the majority were apparently sporadic cases.

Results 21% of patients with ALS carried a confirmed pathogenic or likely pathogenic mutation, of whom 93% had no family history of ALS. 15% met the inclusion criteria for a current ALS genetic-therapy trial. 5/21 patients with a pathogenic mutation had an additional variant of uncertain significance (VUS). An additional 21% of patients with ALS carried a VUS in an ALS-associated gene. Overall, 13% of patients carried more than one genetic variant (pathogenic or VUS). Patients with ALS carrying two variants developed disease at a significantly earlier age compared with patients with a single variant (median age of onset=56 vs 60 years, p=0.0074).

Conclusions Routine screening for ALS-associated pathogenic mutations in a specialised ALS referral clinic will impact clinical care in 21% of cases. An additional 21% of patients have variants in the ALS gene panel currently of unconfirmed significance after removing non-specific or predicted benign variants. Overall, variants within known ALS-linked genes are of potential clinical importance in 42% of patients.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterised by progressive injury and cell death of upper and lower motor neurons in the motor cortex, brainstem and spinal cord. This leads to progressive failure of the neuromuscular system with death, usually from respiratory failure, within 2–5 years of symptom onset in most cases. Up to 50% of cases also show mild cognitive impairment, with approximately 5% progressing to clinically recognised fronto-temporal dementia (FTD).[1] While the majority of ALS cases are considered sporadic (sALS), 5%–10% have been shown to be familial, usually with autosomal dominant inheritance, and the genetic cause of approximately 60%–70% of familial ALS (fALS) cases has now been identified.[2] The most common genetic cause of ALS is due to expansion of a GGGGCC (G4C2) hexanucleotide repeat in the first intron of the *C9orf72* gene. This expansion has a frequency of 43% in fALS and 7% in sALS cases in our UK cohort,[3] which is comparable with worldwide figures of 39.3% for fALS and 7.0% for sALS.[4] Mutations in *SOD1*,[5 6] *TARDBP*,[5 7 8] and *FUS*[5 9] genes, the next most common genetic causes of ALS, have also been reported in both patients with fALS and those with sALS. Therefore, it is clear that apparently sporadic cases can also carry potentially pathogenic variants in known ALS genes. In a recent study which screened 17 ALS-related genes, 27.8% of apparently sporadic cases carried a potentially pathogenic or rare variant in a known ALS gene.[10] In addition, it was noted that 3.8% of patients also carried multiple variants, with these cases having a significantly earlier age of onset. Another recent report from an Australian sporadic ALS cohort found that one-third of patients carried a variant of interest and 7% carried two or more variants, which again was correlated with an earlier age of onset.[11] It has previously been reported that ALS is a six-step process, with genes, environment and time (in the form of ageing) contributing to disease development.[12] It was proposed that individuals with a genetic variant would require fewer steps than those without such variants. Using data from an ALS registry in Italy, this proved to be the case, with individuals carrying *C9orf72*, *TARDBP* or *SOD1* mutations showing a three-step, four-step and two-step process.[13]

Currently, only cases with a familial history of ALS, dementia or with a young age of disease onset tend to be routinely offered genetic screening in a clinical setting, at least in the UK.[14] However, with the advent of therapies targeting specific genetic forms of the disease associated with *SOD1* or *C9orf72* mutations (Biogen sponsored clinical trials

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Contributors PJS and JK conceived and designed the study. JC-K, MDP, MRT, AM and PF contributed to the design, theoretical analysis and implementation of the analysis pipeline. SRS, MDP, JC-K, NSV, LT, CH, TMJ, CJM, PJS, NB, EP and ESAS were responsible for data acquisition. SRS, MDP, JC-K, NSV, JK and PJS were responsible for analysis of data. JK, PJS, MDP and JC-K were responsible for interpretation of data. PJS, JK and MDP supervised the project. The Project MinE ALS Sequencing Consortium (online supplementary table 4) was involved in data acquisition and analysis. All authors meet the four ICMJE authorship criteria, and were responsible for revising the manuscript, approving the final version for publication, and for accuracy and integrity of the work.

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REVIEW ARTICLE

Genetics of primary lateral sclerosis

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Abstract

With the exception of rare, juvenile-onset, autosomal recessive cases, primary lateral sclerosis (PLS) has long been considered an exclusively sporadic motor neuron disease. However, the identification of PLS cases within pedigrees with familial amyotrophic lateral sclerosis (ALS), together with the clinical and neuropathological overlap with other neurodegenerative disease with strong genetic component such as ALS and hereditary spastic paraparesis (HSP), suggest the existence of a genetic component in PLS as well. Here we will review the genetics of juvenile PLS-like syndromes and the contribution of mutations in ALS and HSP-associated genes to PLS pathogenesis.

Keywords: *Genetics, PLS, neuropathology*

Introduction

Primary lateral sclerosis (PLS) is an adult-onset, neurodegenerative disorder primarily affecting the upper motor neurons (UMN) that originate the corticospinal and corticobulbar tracts, thus leading to widespread spasticity and bulbar involvement. Although previous diagnostic criteria required the absence of family history (1), mainly to differentiate PLS from the partially overlapping hereditary spastic paraparesis (HSP), rare pedigrees with multiple individuals affected with PLS have been described (2–6). This observation, together with the shared clinical and pathological features with other motor neuron disorders with high genetic component, mainly amyotrophic lateral sclerosis (ALS), raises the question whether there are genetic factors associated to PLS susceptibility as well. We will first examine the contribution of ALS-associated genes in PLS, then we will evaluate the genetic overlap between PLS and other diseases characterized by corticospinal tract degeneration such as HSP, and lastly we will describe the genetics of juvenile PLS-like syndromes (JPLS) (Table 1). To account for the possibility that any observed genetic overlap between PLS, UMN-predominant ALS and HSP may in fact be due to misdiagnosis of these three conditions, whenever feasible we applied the novel consensus criteria for the diagnosis of PLS to critically review available literature (7).

ALS-associated genes in PLS

The occurrence of ALS and PLS phenotypes within the same pedigrees, although extremely rare, suggests the existence of a common genetic background between the two diseases; however, genetic studies have demonstrated only a minimal degree of genetic overlap so far. In fact, among major ALS-associated genes (*SOD1*, *TARDBP*, *FUS* and *c9orf72*), only the $(G_4C_2)_n$ hexanucleotide repeat expansion in *c9orf72* has been rarely observed in PLS patients (8,9,15). The screening a Dutch cohort of 110 individuals with PLS revealed a single mutated case, accounting for a mutational

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RESEARCH ARTICLE OPEN ACCESS

Structural MRI Signatures in Genetic Presentations of the Frontotemporal Dementia/Motor Neuron Disease Spectrum

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Abstract

Background and Objectives

To assess cortical, subcortical, and cerebellar gray matter (GM) atrophy using MRI in patients with disorders of the frontotemporal lobar degeneration (FTLD) spectrum with known genetic mutations.

Methods

Sixty-six patients carrying FTLD-related mutations were enrolled, including 44 with pure motor neuron disease (MND) and 22 with frontotemporal dementia (FTD). Sixty-one patients with sporadic FTLD (sFTLD) matched for age, sex, and disease severity with genetic FTLD (gFTLD) were also included, as well as 52 healthy controls. A whole-brain voxel-based morphometry (VBM) analysis was performed. GM volumes of subcortical and cerebellar structures were obtained.

Results

Compared with controls, GM atrophy on VBM was greater and more diffuse in genetic FTD, followed by sporadic FTD and genetic MND cases, whereas patients with sporadic MND (sMND) showed focal motor cortical atrophy. Patients carrying *C9orf72* and *GRN* mutations showed the most widespread cortical volume loss, in contrast with GM sparing in *SOD1* and *TARDBP*. Globally, patients with gFTLD showed greater atrophy of parietal cortices and thalami compared with sFTLD. In volumetric analysis, patients with gFTLD showed volume loss compared with sFTLD in the caudate nuclei and thalami, in particular comparing C9-MND with sMND cases. In the cerebellum, patients with gFTLD showed greater atrophy of the right lobule VIIb than sFTLD. Thalamic volumes of patients with gFTLD with a *C9orf72* mutation showed an inverse correlation with Frontal Behavioral Inventory scores.

Discussion

Measures of deep GM and cerebellar structural involvement may be useful markers of gFTLD, particularly *C9orf72*-related disorders, regardless of the clinical presentation within the FTLD spectrum.

From the Neuroimaging Research Unit (E.G.S., A.G., S.B., C.C., E.C., V.C., M.F., F.A.) and Experimental Neuropathology Unit (N.R., T.D.), Division of Neuroscience, Neurorehabilitation Unit (N.R., M.F.), Neurology Unit (E.G.S., G.M., F.C., M.F., F.A.), Laboratory of Clinical Molecular Biology (P. Carrera), and Neurophysiology Service (M.F.), IRCCS San Raffaele Scientific Institute; Vita-Salute San Raffaele University (E.G.S., A.G., C.C., V.C., M.F., F.A.); Unit of Neurology 5–Neuropathology (P. Caroppo, S.P., G.R.), Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan; Neurology Unit (L.T., I.A.), "San Gerardo" Hospital and University of Milano-Bicocca, Monza; Department of Neurology and Laboratory of Neuroscience (V.S.), IRCCS Istituto Auxologico Italiano; and "Dino Ferrari" Center, Department of Pathophysiology and Transplantation (V.S.), Università degli Studi di Milano, Milan, Italy.

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RESEARCH ARTICLE

Chitotriosidase as biomarker for early stage amyotrophic lateral sclerosis: a multicenter study

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Abstract

Objective: Levels of chitotriosidase (CHIT1) are increased in the cerebrospinal fluid (CSF) of amyotrophic lateral sclerosis (ALS) patients reflecting microglial activation. Here, we determine the diagnostic and prognostic potential of CHIT1 for early symptomatic ALS. *Methods*: Overall, 275 patients from 8 European neurological centers were examined. We included ALS with <6 and >6 months from symptom onset, other motoneuron diseases (oMND), ALS mimics (DCon) and non-neurodegenerative controls (Con). CSF CHIT1 levels were analyzed for diagnostic power and association with progression and survival in comparison to the benchmark neurofilament. The 24-bp duplication polymorphism of CHIT1 was analyzed in a subset of patients ($N = 65$). *Results:* Homozygous CHIT1 duplication mutation carriers (9%) invariably had undetectable CSF CHIT1 levels, while heterozygous carriers had similar levels as patients with wildtype CHIT1 ($p = 0.414$). In both early and late symptomatic ALS CHIT1 levels was increased, did not correlate with patients' progression rates, and was higher in patients diagnosed with higher diagnostic certainty. Neurofilament levels correlated with CHIT1 levels and prevailed over CHIT1 regarding diagnostic performance. Both CHIT1 and

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ORIGINAL ARTICLE

APPLIED PSYCHOLOGY: Health and Well-Being IAAP

It won't happen to me! Psychosocial factors influencing risk perception for respiratory infectious diseases: A scoping review

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Abstract

Understanding the determinants of risk perception for COVID-19 might help to promote self-preventive behaviours. This scoping review aimed to map the extent, variety and characteristics of the evidence on the possible determinants of risk perception for COVID-like diseases. PubMed, Scopus and Web of Science were searched for original, peer-reviewed English-written articles published up to March 2020 and investigating risk perception determinants for respiratory infectious diseases in adults. Titles and abstracts were screened, and full texts were analysed by the first author; when unsure, eligibility was discussed with the last author. Data were collected according to an extraction sheet developed by the first and last authors. The cross-sectional evidence covers a variety of diseases, countries and timings of testing. Mostly, questionnaires recorded socio-demographics, media exposure, trust in institutions, disease proximity and knowledge; psychological variables, including personality traits, distress and self-efficacy, were less investigated. A miscellaneous operationalization of risk perception emerged, including the likelihood of getting sick, perceived dangerousness, concerns or a combination of them. A comprehensive understanding of the

Gabriella Pravettoni and Barbara Poletti equally contributed to this study

frontiers in Psychology

ORIGINAL RESEARCH
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Attachment, Personality and Locus of Control: Psychological Determinants of Risk Perception and Preventive Behaviors for COVID-19

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Background: The understanding of factors that shape risk perception is crucial to modulate the perceived threat and, in turn, to promote optimal engagement in preventive actions.

Methods: An on-line, cross-sectional, survey was conducted in Italy between May and July 2020 to investigate risk perception for COVID-19 and the adoption of preventive measures. A total of 964 volunteers participated in the study. Possible predictors of risk perception were identified through a hierarchical multiple linear regression analysis, including sociodemographic, epidemiological and, most of all, psychological factors. A path analysis was adopted to probe the possible mediating role of risk perception on the relationship between the independent variables considered and the adoption of preventive measures.

Results: Focusing on the psychological predictors of risk perception, high levels of anxiety, an anxious attachment, and an external locus of control predicted higher perceived risk. Conversely, high levels of openness personality and of avoidant attachment predicted a lower perception of risk. In turn, the higher was the perceived risk the higher was the adoption of precautionary measures. Furthermore, psychological factors influenced the adoption of preventive behaviors both directly and indirectly through their effect on risk perception.

Conclusions: Our findings might be taken into high consideration by stakeholders, who are responsible for promoting a truthful perception of risk and proper compliance with precautionary measures.

Keywords: COVID-19, risk perception, preventive behaviors, psychological determinants, pandemic management

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COVID-19

The contribution of the Italian residents in neurology to the COVID-19 crisis: admirable generosity but neurological training remains their priority

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Abstract

Background The coronavirus disease 2019 (COVID-19) pandemic has severely impacted the Italian healthcare system, underscoring a dramatic shortage of specialized doctors in many disciplines. The situation affected the activity of the residents in neurology, who were also offered the possibility of being formally hired before their training completion.

Aims (1) To showcase examples of clinical and research activity of residents in neurology during the COVID-19 pandemic in Italy and (2) to illustrate the point of view of Italian residents in neurology about the possibility of being hired before the completion of their residency program.

Results Real-life reports from several areas in Lombardia—one of the Italian regions more affected by COVID-19—show that residents in neurology gave an outstanding demonstration of generosity, collaboration, reliability, and adaptation to the changing environment, while continuing their clinical training and research activities. A very small minority of the residents participated in the dedicated selections for being hired before completion of their training program. The large majority of them prioritized their training over the option of earlier employment.

Conclusions Italian residents in neurology generously contributed to the healthcare management of the COVID-19 pandemic in many ways, while remaining determined to pursue their training. Neurology is a rapidly evolving clinical field due to continuous diagnostic and therapeutic progress. Stakeholders need to listen to the strong message conveyed by our residents in neurology and endeavor to provide them with the most adequate training, to ensure high quality of care and excellence in research in the future.

Keywords COVID-19 · Residents in neurology · Training · Real-world experience · Early employment

Introduction

The pandemic due to the coronavirus disease 2019 (COVID-19) has severely impacted the healthcare system in the world and in Italy for a sustained period of time [1–3]. Since March 2020, the majority of Italian hospitals have rapidly shifted their target and mission from non-urgent, specialized, or highly specialized care to urgent and non-urgent COVID-19 care. During the first wave of the pandemic, the impact hit mostly the North Italian Regions, but then the entire Italian territory during the second wave, which affected also regions relatively spared by the first wave.

In this setting, and considering the pre-existent limited availability of specialists, Italian hospitals found themselves in a critical shortage of doctors. The scary gap was partially filled with the support of residents in those hospitals that delivered training for residents in the different medical programs. For most of the residents, the choice to help COVID-19-dedicated wards entailed the interruption of their discipline-specific training, which was anyway made inescapable by the generalized reconversion of wards dedicated to their discipline into COVID-dedicated structures.

The Italian government sought to facilitate and formalize the contribution of residents to the management of the COVID-19 crisis by releasing an urgent decree aimed at

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REVIEW
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Neurofilament Light Chain as Biomarker for Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two related currently incurable neurodegenerative diseases. ALS is characterized by degeneration of upper and lower motor neurons causing relentless paralysis of voluntary muscles, whereas in FTD, progressive atrophy of the frontal and temporal lobes of the brain results in deterioration of cognitive functions, language, personality, and behavior. In contrast to Alzheimer's disease (AD), ALS and FTD still lack a specific neurochemical biomarker reflecting neuropathology *ex vivo*. However, in the past 10 years, considerable progress has been made in the characterization of neurofilament light chain (NFL) as cerebrospinal fluid (CSF) and blood biomarker for both diseases. NFL is a structural component of the axonal cytoskeleton and is released into the CSF as a consequence of axonal damage or degeneration, thus behaving in general as a relatively non-specific marker of neuroaxonal pathology. However, in ALS, the elevation of its CSF levels exceeds that observed in most other neurological diseases, making it useful for the discrimination from mimic conditions and potentially worthy of consideration for introduction into diagnostic criteria. Moreover, NFL correlates with disease progression rate and is negatively associated with survival, thus providing prognostic information. In FTD patients, CSF NFL is elevated compared with healthy individuals and, to a lesser extent, patients with other forms of dementia, but the latter difference is not sufficient to enable a satisfying diagnostic performance at individual patient level. However, also in FTD, CSF NFL correlates with several measures of disease severity. Due to technological progress, NFL can now be quantified also in peripheral blood, where it is present at much lower concentrations compared with CSF, thus allowing less invasive sampling, scalability, and longitudinal measurements. The latter has promoted innovative studies demonstrating longitudinal kinetics of NFL in presymptomatic individuals harboring gene mutations causing ALS and FTD. Especially in ALS, NFL levels are generally stable over time, which, together with their correlation with progression rate, makes NFL an ideal pharmacodynamic biomarker for therapeutic trials. In this review, we illustrate the significance of NFL as biomarker for ALS and FTD and discuss unsolved issues and potential for future developments.

Keywords: amyotrophic lateral sclerosis, frontotemporal dementia, cerebrospinal fluid, biomarkers, neurofilament light chain

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genes

MDPI

Article

An Investigation of the Role of Common and Rare Variants in a Large Italian Multiplex Family of Multiple Sclerosis Patients

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Abstract: Known multiple sclerosis (MS) susceptibility variants can only explain half of the disease's estimated heritability, whereas low-frequency and rare variants may partly account for the missing heritability. Thus, here we sought to determine the occurrence of rare functional variants in a large Italian MS multiplex family with five affected members. For this purpose, we combined linkage analysis and next-generation sequencing (NGS)-based whole exome and whole genome sequencing (WES and WGS, respectively). The genetic burden attributable to known common MS variants was also assessed by weighted genetic risk score (wGRS). We found a significantly higher burden of common variants in the affected family members compared to that observed among sporadic MS patients and healthy controls (HCs). We also identified 34 genes containing at least one low-frequency functional variant shared among all affected family members, showing a significant enrichment in

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U-Fiber Leukoencephalopathy Due to a Novel Mutation in the *TACO1* Gene

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Translational activator of cytochrome c oxidase I (TACO1) is a mitochondrial translation factor involved in mitochondria-encoded cytochrome c oxidase subunit I (MT-CO1) synthesis.[1,2] Loss-of-function mutations in the *TACO1* gene cause respiratory chain complex IV deficiency. Clinically heterogeneous human diseases are due to cytochrome c oxidase (COX) deficiency, ranging from Leigh syndrome to myopathy, deafness, or ataxia. Recently, 2 different TACO1 mutations have been identified in 3 families with late-onset Leigh syndrome and a leukoencephalopathy involving predominantly basal ganglia and cystic changes.[3,4] Here, we report a subject carrying a novel homozygous truncating mutation in the *TACO1* gene and presenting an adult-onset slowly progressive spastic paraparesis with cognitive impairment and a subcortical U-fiber leukoencephalopathy.

Case Presentation

The proband is a 50-year-old Italian woman with a 20-year history of slowly progressive spastic gait and mild cognitive impairment. No family history and no clear consanguinity have been reported. She has a daughter, now 24-years-old, healthy. Neurologic examination showed diffuse signs of upper motor neuron involvement and an impairment of executive functions in a context of low cognitive ability setting. Neither involuntary movements nor cerebellar dysfunction signs were present. Optical coherence tomography highlighted a significant bilateral temporal retinal nerve fiber layer thickness reduction. Brain MRI showed a white matter disease with an extensive symmetrical involvement of U-fibers (figure 1A). Laboratory panels (including creatine kinase and lactic acid) and EMG were unremarkable. In a 10-year follow-up, we observed a spastic gait worsening leading to the necessary use of 2 sticks, and a cognitive impairment progression in terms of perseveration and reduced speed in information processing. MRI follow-up displayed a brain atrophy increase.

Genetics and Functional Studies

Whole-exome sequencing was performed on the proband, her healthy sister, and daughter after written informed consent. Two variants in *TACO1* and mitochondrial distribution and morphology regulator 1 (*MSTO1*) were identified in the proband: a novel homozygous truncating change c.676G>T (p.Glu226Ter) in *TACO1* and c.833A>G (p.Tyr278Cys) (rs143029385) in *MSTO1* (freq. 0.04% in ExAC). None of the variants segregated in the sister, whereas the daughter is a carrier of the *TACO1* change (figure 1B). Based on this, we speculated that *TACO1* variant might be the disease-causing mutation. Functional studies demonstrated that the newly identified *TACO1* mutation is highly pathogenic. Indeed, sodium dodecyl sulfate–

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