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

2022

"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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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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Juvenile-onset dystonia with spasticity in Leigh syndrome caused by a novel *NDUFA10* variant

ARTICLE INFO

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Leigh syndrome (LS) is a mitochondrial neurodegenerative disease with an incidence of ~1/40,000 live births [1].

LS usually manifests in the first two years of life. Disease onset and progression are related to metabolic triggers such as infections, vaccinations, fasting, dehydration, or surgery [1].

Patients with LS present intellectual and motor disability, including hypotonia, dystonia, spasticity, ataxia, and chorea-athetosis. They may also display epilepsy, feeding and respiratory difficulties, optic atrophy, oculomotor abnormalities, ptosis, and systemic involvement [1,2].

LS is characterized by bilateral T2-hyperintense lesions in basal ganglia and/or brainstem. Thalamus, cerebellum, optic nerve, and spinal cord may be involved as well. An increase in lactate levels and lactate/pyruvate ratio in serum and cerebrospinal fluid (CSF) is a common finding [2].

LS was etiologically linked to many genes, encoded by mitochondrial (mtDNA) or nuclear DNA (nDNA), and virtually involved in any mitochondrial function, such as oxidative phosphorylation (OXPHOS), mtDNA replication, and coenzyme Q metabolism. OXPHOS complex I deficiency is the most frequent cause of LS, and over 80% of these cases are due to mutations of nDNA-encoded genes [1,3].

Biallelic mutations of *NDUFA10*, encoding a subunit of OXPHOS complex I, are an extremely rare cause of LS. Three cases of LS with biallelic *NDUFA10* mutations were described so far [3–5]. Here we report the fourth case, distinguished for disease onset at 6 years with gait difficulties followed by severe dystonic-spastic tetraparesis. The IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) Ethics Committee approved this study.

The patient is a 43-year-old male born to non-consanguineous healthy parents from Southern Italy. He has a healthy sister and no familiarity for neurological disorders. Early psychomotor development was reportedly normal, gaining autonomous walking at the age of 15 months.

At the age of 6 years, walking impairment, frequent falls, and minimal dysarthria were reported. The patient underwent an initial diagnostic assessment. Electromyography and a wide spectrum laboratoristic screening performed on blood and CSF for neurometabolic disorders resulted normal. Brain MRI showed bilateral striatal T2-hyperintensities, with a greater involvement of the right putamen.

At 10 years, he was unable to stand and walk autonomously and displayed dystonic-spastic tetraparesis, bilateral ankle clonus, chorea-athetosis, dysarthria, and dysphagia for fluids. Treatment with clonazepam, trazodone, and L-DOPA/benserazide was started with benefit on rigidity and chorea-athetosis.

The disease progressed with a global deterioration of dysarthria, dysphagia, dystonia, and spasticity. The patient displayed a mild intellectual disability allowing him to write simple thoughts and perform simple arithmetic operations. Frequent depressive episodes were reported. The patient underwent further biochemical testing showing no abnormality but the elevation of p-OH-phenyllactic acid. A skeletal muscle biopsy showed several atrophic fibers and increased succinate-dehydrogenase activity, suggesting mitochondrial myopathy.

At the last evaluation, he presented a painful dystonic-spastic tetraparesis with a greater involvement of the left hemisoma, left-oriented laterocollis, bilateral Babinski sign and ankle clonus, bilateral striatal hand, hypotrophic lower limbs, dysarthria, fluid dysphagia, and eyes misalignment. The last brain MRI (Fig. 1A) confirmed bilateral striatal necrosis with a relative sparing of the anterior portion of the left putamen, in absence of any other alteration. Routine blood tests, electrocardiogram, electroencephalogram, and cardiac and abdominal ultrasonographies were normal. His electromyography showed axonal neuropathy for all four limbs. Enzymatic assays performed on his skeletal muscle biopsy demonstrated a deficiency of OXPHOS complexes I and I + III. The patient was treated with clonazepam, trazodone, L-DOPA/benserazide, baclofen, trihexyphenidyl, medical cannabis, botulinum toxin, venlafaxine, and clozapine.

Whole-exome sequencing (WES) was performed on genomic DNA of the patient. Bioinformatic filtering based on a virtual gene panel for LS looking for rare (allele frequency <0.001) nonsynonymous variants revealed two heterozygous *NDUFA10* (NM_004544.4) variants: c.233_235delCAG (p.Ala78del) and c.296G > A (p.Gly99Glu). Segregation analysis showed a biallelic status of these variants (Fig. 1B).

The p.Gly99Glu is a known pathogenic *NDUFA10* variant, already described in two Italian patients with LS [3,5]. The p.Ala78del is extremely rare (gnomAD allele frequency = 0.000004) and reported as a variant of unknown significance in genetic databases (i.e., ClinVar). It affects an evolutionary-conserved amino acid (Fig. 1C) and is predicted

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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

Mitochondrial DNA homeostasis impairment and dopaminergic dysfunction: A trembling balance

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ABSTRACT

Maintenance of mitochondrial DNA (mtDNA) homeostasis includes a variety of processes, such as mtDNA replication, repair, and nucleotides synthesis, aimed at preserving the structural and functional integrity of mtDNA molecules. Mutations in several nuclear genes (*i.e.*, *POLG*, *POLG2*, *TWNK*, *OPA1*, *DGUOK*, *MPV17*, *TYMP*) impair mtDNA maintenance, leading to clinical syndromes characterized by mtDNA depletion and/or deletions in affected tissues. In the past decades, studies have demonstrated a progressive accumulation of multiple mtDNA deletions in dopaminergic neurons of the substantia nigra in elderly population and, to a greater extent, in Parkinson's disease patients. Moreover, parkinsonism has been frequently described as a prominent clinical feature in mtDNA instability syndromes. Among Parkinson's disease-related genes with a significant role in mitochondrial biology, *PARK2* and *LRRK2* specifically take part in mtDNA maintenance. Moreover, a variety of murine models (*i.e.*, "Mutator", "MitoPark", "PD-mito*Pst*I", "Deletor", "Twinkle-dup" and "TwinkPark") provided *in vivo* evidence that mtDNA stability is required to preserve nigrostriatal integrity. Here, we review and discuss the clinical, genetic, and pathological background underlining the link between impaired mtDNA homeostasis and dopaminergic degeneration.

1. Introduction

Maintenance of mitochondrial DNA (mtDNA) homeostasis includes a variety of processes, such as mtDNA replication, repair, and nucleotides synthesis, aimed at preserving the structural and functional integrity of mtDNA molecules. Mutations in nuclear genes involved in mtDNA homeostasis (*i.e.*, *POLG*, *POLG2*, *TWNK*, *OPA1*, *DGUOK*, *MPV17*, *TYMP*) result in the loss (depletion) or altered integrity (deletions) of mtDNA molecules in post-mitotic tissues, leading to clinical presentations collectively termed "mtDNA maintenance disorders". The clinical landscape is dominated by muscle weakness, central nervous system (CNS) involvement and hepatic dysfunction, reflecting the high reliance of these tissues on oxidative metabolism.

While mtDNA depletion mainly gives rise to pediatric-onset syndromes (Moraes et al., 1991), the accumulation of partially deleted mitochondrial genomes (mtDNA multiple deletions) is more frequently observed in adult patients (Zeviani et al., 1989). Due to the involvement of nuclear genes, these disorders display autosomal dominant or recessive inheritance. Early-onset presentations were initially thought to have a genetic basis distinct from adult presentations. Nonetheless,

Abbreviations: mtDNA, mitochondrial DNA; SN, substantia nigra; PD, Parkinson's disease; ATP, adenosine triphosphate; ROS, reactive oxygen species; mtSSB, mitochondrial single-stranded binding protein; D-loop, displacement loop; MPTP, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; Nrf2, nuclear factor erythroid 2-related factor 2; Progressive external ophthalmoplegia, external ophthalmoplegia; mtDNA^{4977}, 5 kb common mtDNA deletion; MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; MERRF, myoclonic epilepsy with ragged red fibers; PCR, polymerase chain reaction; LR-PCR, long-range PCR; MSA, multiple system atrophy; DLB, dementia with Lewy bodies; AD, Alzheimer's disease; COX, cytochrome *c* oxidase; AHS, Alpers-Huttenlocher Syndrome; MCHS, myocerebrohepatopathy spectrum; ANS, ataxia neuropathy spectrum; SANDO, sensory ataxic neuropathy, dysarthria and ophthalmoparesis; [18F]β-CFT, fluorine-18-labeled 2β-carbomethoxy-3β-[4-fluorophenyl]tropane; PET, positron emission tomography; ^{123}I-β-CIT, iodine 123-labeled β-carboxymethyoxy-3-β-(4-iodophenyl) tropane; SPECT, single photon emission tomography; ^{123}I-FP-CIT, iodine 123-radiolabeled 2β-carboxymethyoxy-3-β-(4-iodophenyl)-N-(3-fluoropropyl)nortropane; Poly-Q, poly-glutamine; TP, thymidine phosphorylase; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; CS, citrate synthase; CNS, central nervous system.

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ORIGINAL ARTICLE

Insights into the identification of a molecular signature for amyotrophic lateral sclerosis exploiting integrated microRNA profiling of iPSC-derived motor neurons and exosomes

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Abstract

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

Keywords ALS · miRNA · Motor neurons · Exosomes · CSF

Abbreviations

ALS	Amyotrophic lateral sclerosis
CNS	Central nervous system
MNs	Motor neurons
sALS	Sporadic amyotrophic lateral sclerosis
fALS	Familial amyotrophic lateral sclerosis
miRNAs	MicroRNAs
ex-miRNAs	Exosomal microRNAs
CSF	Cerebrospinal fluid
EVs	Extracellular vesicles

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Author contributions MR and VM conceived and performed all the experiments. MR, ML and DR provided cell culture data. RS performed karyotype analysis. VM performed molecular biology experiments and data analysis, while LD carried out TLDA assays. DR and FB performed bioinformatics analysis. DG, MM, PM, KP and PVD collected CSF samples. MR, VM, PM and NH conducted qPCR experiments on CSF. DG analyzed CSF data and performed all the statistical analysis. MR, MN, DG and VM wrote the manuscript. MR produced Figures. SC and MN conceived the project, designed the research and reviewed the draft. NB, GPC, VB and PVD provided resources. All authors edited and gave critical input on the manuscript, providing data interpretation and contribution to the final version of the manuscript.

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Availability of data and material All data generated or analysed during this study are included in this published article [and its supplementary information files].

Declarations

Conflict of interest The authors report no competing interests.

Ethics approval The studies involving human samples were conducted in accordance with the ethical standards of the Declaration of Helsinki and with national legislation and institutional guidelines. Human fibroblast cell lines were obtained from Eurobiobank with informed consent approved by the ethical committee at Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan. All subjects provided written informed consent approved by the local ethical committee for the collection, storage and analysis of CSF samples (0,004,520, S50354, S55312, S59552). This experimental study was conducted in accordance with the international GLP and GCP guidelines.

Consent for publication Not applicable.

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Cellular and Molecular Life Sciences

REVIEW

Inhibition of myostatin and related signaling pathways for the treatment of muscle atrophy in motor neuron diseases

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Abstract

Myostatin is a negative regulator of skeletal muscle growth secreted by skeletal myocytes. In the past years, myostatin inhibition sparked interest among the scientific community for its potential to enhance muscle growth and to reduce, or even prevent, muscle atrophy. These characteristics make it a promising target for the treatment of muscle atrophy in motor neuron diseases, namely, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), which are rare neurological diseases, whereby the degeneration of motor neurons leads to progressive muscle loss and paralysis. These diseases carry a huge burden of morbidity and mortality but, despite this unfavorable scenario, several therapeutic advancements have been made in the past years. Indeed, a number of different curative therapies for SMA have been approved, leading to a revolution in the life expectancy and outcomes of SMA patients. Similarly, tofersen, an antisense oligonucleotide, is now undergoing clinical trial phase for use in ALS patients carrying the SOD1 mutation. However, these therapies are not able to completely halt or reverse progression of muscle damage. Recently, a trial evaluating apitegromab, a myostatin inhibitor, in SMA patients was started, following positive results from preclinical studies. In this context, myostatin inhibition could represent a useful strategy to tackle motor symptoms in these patients. The aim of this review is to describe the myostatin pathway and its role in motor neuron diseases, and to summarize and critically discuss preclinical and clinical studies of myostatin inhibitors in SMA and ALS. Then, we will highlight promises and pitfalls related to the use of myostatin inhibitors in the human setting, to aid the scientific community in the development of future clinical trials.

Keywords Myostatin · Motor neuron diseases · Muscle atrophy · Activin receptors, type II · Monoclonal antibodies

Introduction

Motor neuron diseases (MND) are a group of progressive neurodegenerative disorders which selectively affect the cellular population of motor neurons (MNs) [1, 2]. MN are localized either in the cortex (upper MNs) or in the brainstem and anterior horns of the spinal cord (lower MNs). The two most common and widely known MNDs are amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), which differ for pathogenic mechanisms, age at onset and presence of upper MN involvement [1, 2].

ALS is a fatal disorder that targets both upper and lower MNs, causing progressive weakness and atrophy of skeletal muscles, which usually leads to paralysis and death within 3–5 years [2]. ALS is divided in sporadic (sALS), when occurring in absence of family history, and familial (fALS), when at least two other family members are affected. sALS represents 85–90% of all cases and presents a later age of onset (58–63 years), while fALS accounts for the remaining 10–15% of cases and shows a slightly younger age of onset (47–53 years) [2, 3]. Potential causative mutations have been described in over 50 genes. Among them, *C9orf72, TARDBP, FUS* and *SOD1* account for almost 75% of fALS cases [2, 3]. As for now, the pathogenic mechanisms leading

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ORIGINAL COMMUNICATION

Genetic modifiers of upper limb function in Duchenne muscular dystrophy

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Abstract

Genetic modifiers of Duchenne muscular dystrophy (DMD) are variants located in genes different from the disease-causing gene *DMD*, but associated with differences in disease onset, progression, or response to treatment. Modifiers described so far have been tested mainly for associations with ambulatory function, while their effect on upper limb function, which is especially relevant for quality of life and independence in non-ambulatory patients, is unknown. We tested genotypes at several known modifier loci (*SPP1*, *LTBP4*, *CD40*, *ACTN3*) for association with Performance Upper Limb version 1.2 score in an Italian multicenter cohort, and with Brooke scale score in the Cooperative International Neuromuscular Group Duchenne Natural History Study (CINRG-DNHS), using generalized estimating equation (GEE) models of longitudinally collected data, with age and glucocorticoid treatment as covariates. *CD40* rs1883832, previously linked to earlier loss of ambulation, emerged as a modifier of upper limb function, negatively affecting shoulder and distal domains of PUL ($p = 0.023$ and 0.018, respectively) in the Italian cohort, as well as of Brooke score ($p = 0.018$) in the CINRG-DNHS. These findings will be useful for the design and interpretation of clinical trials in DMD, especially for non-ambulatory populations.

Keywords Duchenne muscular dystrophy · Genetic modifiers · Upper limb function · SPP1–osteopontin · CD40

Introduction

Duchenne muscular dystrophy (DMD) is a severe and progressive muscle disease caused by complete dystrophin deficiency in muscle fibers. It is an X-linked recessive disease, with an incidence of around 1 in 3800–4200 male births and prevalence between 19.9 and 95.5 in 1,000,000. Usually, symptoms are present in early childhood with delayed motor milestones and difficulties in rising from the floor, typically with a Gowers' manoeuver, and in climbing stairs. Progressive muscle degeneration causes loss of independent ambulation (LoA) typically around the age of 13. Respiratory and cardiac involvement develop later, and are major causes of death [1].

Even if all DMD patients carry out-of-frame mutations that disrupt protein expression completely, still it is possible to observe a spectrum of phenotype severity within DMD [2–5]. This is primarily measured by age at LoA, because of its impact on daily life and the overall health of patients, and its correlation with overall survival and other disease milestones, such as the onset of respiratory insufficiency and the need for scoliosis surgery [6]. All of these disease milestones may vary by several years, e.g. loss of ambulation may ensue from before 10 years to after 15 years of age.

Daniele Sabbatini, Aurora Fusto, Luca Bello and Elena Pegoraro authors contributed equally.

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Original research

Adults with spinal muscular atrophy: a large-scale natural history study shows gender effect on disease

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ABSTRACT

Background Natural history of spinal muscular atrophy (SMA) in adult age has not been fully elucidated yet, including factors predicting disease progression and response to treatments. Aim of this retrospective, cross-sectional study, is to investigate motor function across different ages, disease patterns and gender in adult SMA untreated patients.

Methods Inclusion criteria were as follows: (1) clinical and molecular diagnosis of SMA2, SMA3 or SMA4 and (2) clinical assessments performed in adult age (>18 years).

Results We included 64 (38.8%) females and 101 (61.2%) males (p=0.0025), among which 21 (12.7%) SMA2, 141 (85.5%) SMA3 and 3 (1.8%) SMA4. Ratio of sitters/walkers within the SMA3 subgroup was significantly (p=0.016) higher in males (46/38) than in females (19/38). Median age at onset was significantly (p=0.0071) earlier in females (3 years; range 0–16) than in males (4 years; range 0.3–28), especially in patients carrying 4 *SMN2* copies. Median Hammersmith Functional Rating Scale Expanded scores were significantly (p=0.0040) lower in males (16, range 0–64) than in females (40, range 0–62); median revised upper limb module scores were not significantly (p=0.059) different between males (24, 0-38) and females (33, range 0–38), although a trend towards worse performance in males was observed. In SMA3 patients carrying three or four SMN2 copies, an effect of female sex in prolonging ambulation was statistically significant (p=0.034).

Conclusions Our data showed a relevant gender effect on SMA motor function with higher disease severity in males especially in the young adult age and in SMA3 patients.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Natural history of spinal muscular atrophy (SMA) in adult age has not been fully clarified yet, including factors predicting disease progression and response to available treatments. The aim of this retrospective, cross-sectional study is to investigate motor function across different ages, disease patterns and gender in adult SMA untreated patients.

WHAT THIS STUDY ADDS

⇒ Our data showed a relevant gender effect on SMA motor function with higher disease severity in males especially in the young adult age and in SMA3 patients.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Gender should be considered as a factor predictive of disease severity and progression in SMA patients.

Spinal muscular atrophy (SMA) is a rare genetic disease of spinal and bulbar motor neurons leading to progressive weakness and atrophy of limb, axial, bulbar and respiratory muscles.[1] SMA is caused by mutations in the *SMN1* gene on chromosome 5q, with a homozygous deletion in exon 7 found in most cases.[2] *SMN2* is the paralogous of *SMN1*, from which it differs by a single C>T substitution in exon 7, determining a splicing defect with exclusion of exon 7 from *SMN2* mRNA and production of a truncated and unstable protein in around 90% of cases.[1] Only around 10% of full-length SMN protein is produced by *SMN2*. However, *SMN2* can be present in multiple copies, and acts as a genetic modifier of disease severity.[3] Based on age at onset and maximal motor function achieved, SMA is currently classified into four subtypes (SMA 1–4) associated with different prognosis.[1]

Adults represent a relevant portion of the overall SMA population. However, natural history of

markedly younger (mean age: 11.53 years) than those in our cohort, having excluded patients older than 30 years, and mean baseline HFMSE was much higher than in our study (44.0 vs 29.8).[21] Similarly, a gender effect on motor function was not observed in a longitudinal study including 79 SMA2/3 patients, even though mean age at baseline was again relatively low (11.3 years).[4] In a previous study including 268 SMA2 and SMA3 patients, mainly in the paediatric age (mean 10.65), females tended to have better HFMSE baseline values and smaller changes at 12-month assessment in both ambulant and non-ambulant patients, although differences were not significant.[5] These data suggest that the gender effect on motor function decline may be more easily observed since the end of the second decade of life and it is not fully expressed in younger patients.

To our knowledge, this is the first study showing the effect of gender on motor function in adult SMA patients through HFMSE, RULM and 6MWT scores. Previous studies suggested some discrepancies of the gender-related adult SMA natural history in terms of age at presentation and age of LoA, but our study reveals the magnitude of the gender effect on motor function tested with objective outcome measures.

Phenotypic differences by gender may be explained by multiple sex-specific variables, such as sex-related differences in mitochondrial biology, sex hormones and X chromosome-related modifiers. No significant impairment of the hypothalamic-pituitary-gonadal axis function was shown in male and female SMA patients, but male infertility was reported.[33 34] Interestingly, among the positive modifier genes of SMA, *PLS3*, *USP9X* and *UBA1* are linked to X-chromosome.[19 35 36] Genes in mitochondrial DNA or in the X chromosome are better fine-tuned in females than in males under the pressure of evolution. Deletion of exons 5 and 6 of the *NAIP* gene, located on chromosome 5, as *SMN1*, has been associated with a more severe phenotype in a large cohort of SMA1/2/3 patients and much more frequently found in female patients.[37] Furthermore, female mice showed greater endurance than males in the rotarod performance test in the mild SMA murine model.[38] Similarly, an impairment of the neuromuscular junction function in males was also showed in the same model.[38] Conversely, the gender-related impact of SMA on skeletal muscle involvement in mouse models is conflicting.[38 39] Finally, female SMA mice had better improvement than males when treated with specific antisense oligonucleotide restoring *SMN2* exon 7 inclusion.[40] Overall, these data from the literature suggest a possible higher disease severity in SMA males. The gender effect may be progressively stronger since puberty due to hormonal changes, as shown by our data in patients at the end of the second decade of life. In addition, the growth spurt during adolescence may potentially play a role in the motor decline of SMA patients, especially in males, with higher increase of weight and lean mass gain. Much less is known about the influence of sex-related factors in later stages of life in patients with myopathies and SMA.

This study has several limitations, mainly related to the retrospective cross-sectional design and the low SMA2 sample size, with the latter limiting the validity of observations in this SMA subgroup. However, retrospective studies are representative of the patient populations encountered in clinical practice, without the strict inclusion and exclusion criteria and sustained efforts required by longitudinal studies. We also believe that a cross-sectional design is a valid tool to investigate a possible gender effect on specific motor functional scores by age; of course, specific longitudinal studies in the future may provide more detailed data on possible different rates of disease progression based on gender.

In conclusion, our data suggest a relevant gender effect on motor function in paediatric and adult SMA patients. Further studies are needed to specifically address our findings and clarify the gender-related factors contributing to SMA disease progression.

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Correction notice Since this article was first published, figure 1 has been replaced to include a legend. This informs readers which colours relate to male and female results.

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Contributors LM planned the study, performed data analysis and their interpretation, drafted the manuscript and submitted the manuscript. LB performed data analysis and their interpretation and drafted the manuscript. SB collected data, contributed to data interpretation and revised the manuscript. AG collected data and revised the manuscript. CC collected data and revised the manuscript. LP collected data and revised the manuscript. MG collected data and revised the manuscript. FT collected data and revised the manuscript. FC collected data and revised the manuscript. AG collected data and revised the manuscript. MF collected data and revised the manuscript. GG collected data and revised the manuscript. VZ collected data and revised the manuscript. LC collected data and revised the manuscript. MM collected data and revised the manuscript. RT collected data and revised the manuscript. ES collected data and revised the manuscript. MM collected data and revised the manuscript. VV collected data and revised the manuscript. GR collected data and revised the manuscript. GS collected data and revised the manuscript. collected data and revised the manuscript. ED'E collected data and revised the

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RESEARCH

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Natural history of Type 1 spinal muscular atrophy: a retrospective, global, multicenter study

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Abstract

Background: ANCHOVY was a global, multicenter, chart-review study that aimed to describe the natural history of Type 1 spinal muscular atrophy (SMA) from a broad geographical area and provide further contextualization of results from the FIREFISH (NCT02913482) interventional study of risdiplam treatment in Type 1 SMA.

Methods: Data were extracted from medical records of patients with first symptoms attributable to Type 1 SMA between 28 days and 3 months of age, genetic confirmation of SMA, and confirmed survival of motor neuron 2 copy number of two or unknown. The study period started on 1 January 2008 for all sites; study end dates were site-specific due to local treatment availabilities. Primary endpoints were time to death and/or permanent ventilation and proportion of patients achieving motor milestones. Secondary endpoints included time to initiation of respiratory and feeding support.

Results: Data for 60 patients from nine countries across Asia, Europe and North and South America were analyzed. The median age (interquartile range [IQR]) for reaching death or permanent ventilation was ~ 7.3 (5.9–10.5) months. The median age (IQR) at permanent ventilation was ~ 12.7 (6.9–16.4) months and at death was ~ 41.2 (7.3–not applicable) months. No patients were able to sit without support or achieved any level of crawling, standing or walking.

Interpretation: Findings from ANCHOVY were consistent with published natural history data on Type 1 SMA demonstrating the disease's devastating course, which markedly differed from risdiplam-treated infants (FIREFISH Part 2). The results provide meaningful additions to the literature, including a broader geographical representation.

Keywords: ANCHOVY, FIREFISH, SMA natural history, Type 1 SMA, Spinal muscular atrophy

Background

Spinal muscular atrophy (SMA) is a severe, progressive, neuromuscular disease, and was the leading genetic cause of infant mortality prior to the availability of current disease-modifying treatments [1, 2]. It is caused by loss of functional survival of motor neuron (SMN) protein due to genetic mutations or deletions of the *SMN1* gene [1, 3–5]. *SMN2* is a paralogous SMN gene that also encodes SMN protein; however, during splicing, exon 7 is excluded from the transcript, resulting in low levels of functional SMN protein [4, 5]. Prior to the availability of disease-modifying treatments, SMA subtypes were classified as Type 0 through 4 (most to least severe), based on age at onset and the most advanced motor milestone

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included all patients who met the eligibility criteria of the study; this population was also used for the comparison with the FIREFISH Part 2 study data. Missing data were not imputed if not stated otherwise. The numbers of patients with missing data were reported for the HINE-2 assessments. Motor function and anthropometric data were summarized in 3-month age windows centered around the nominal age. For example, the Month 3 window was from 1.5 to 4.5 months of age.

For the comparison of time to death or permanent ventilation between ANCHOVY and FIREFISH, a sensitivity analysis was performed, herein referred to as the 'landmark analysis'. This analysis compensates for the differences in age at the start of the risk periods in each study. A time point was designated as the 'landmark age' and only patients who survived until the landmark age were analyzed. The landmark age was set at the youngest age that an infant had an event in FIREFISH Part 2. ANCHOVY data used in the landmark analysis included only patients who were event free at the landmark age.

Abbreviations

CI: Confidence interval; HINE-2: Hammersmith Infant Neurological Examination, Section 2; IQR: Interquartile range; PNCR: Pediatric Neuromuscular Clinical Research; SMA: Spinal muscular atrophy; SMN: Survival of motor neuron; SOC: Standard of care.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13023-022-02455-x.

Additional file 1: Secondary endpoints: Full list of ANCHOVY study secondary endpoints. **Fig. S1**. Patient flow diagram: ANCHOVY. **Fig. S2**. Time to permanent ventilation: ANCHOVY. Kaplan—Meier diagram illustrating probability of patients requiring permanent ventilation by up to 24 months of age. **Fig. S3**. Time to death: ANCHOVY. Kaplan—Meier diagram illustrating probability of patients dying by up to 24 months of age. **Fig. S4**. Time to abnormal swallowing: ANCHOVY. Kaplan—Meier diagram illustrating probability of onset of abnormal swallowing by up to 24 months of age. **Fig. S5**. Height and weight: ANCHOVY. Graphs illustrating height and weight measurements up to 48 months of age. **Table S1**. Other HINE-2 motor milestones: ANCHOVY. Table listing the numbers of patients achieving the following HINE-2 motor milestones at 3-monthly windows up to 24 months of age: voluntary grasp, kicking, rolling crawling, standing and walking.

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Author contributions

All authors contributed to the study conception and design. Analysis and interpretation were performed by all authors. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to their sensitive medical nature and the risk or reidentification. Reasonable requests for aggregate data may be made to global.scientific-communications@roche.com.

Declarations

Ethics approval and consent to participate

The study protocol was approved by an institutional review board/ethics committee at each study site and the study was conducted in accordance with Good Clinical Practice guidelines and the laws and regulations of each country in which the research was conducted. When required by local regulations, written informed consent was provided by parents or caregivers of the patients.

Consent for publication

Not applicable.

Competing interests

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Molecular Neurodegeneration

RESEARCH ARTICLE Open Access

Transcriptome deregulation of peripheral monocytes and whole blood in *GBA*-related Parkinson's disease

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Abstract

Background: Genetic mutations in beta-glucocerebrosidase (*GBA*) represent the major genetic risk factor for Parkinson's disease (PD). *GBA* participates in both the endo-lysosomal pathway and the immune response, two important mechanisms involved in the pathogenesis of PD. However, modifiers of *GBA* penetrance have not yet been fully elucidated.

Methods: We characterized the transcriptomic profiles of circulating monocytes in a population of patients with PD and healthy controls (CTRL) with and without *GBA* variants ($n = 23$ PD/GBA, 13 CTRL/GBA, 56 PD, 66 CTRL) and whole blood ($n = 616$ PD, 362 CTRL, 127 PD/GBA, 165 CTRL/GBA). Differential expression analysis, pathway enrichment analysis, and outlier detection were performed. Ultrastructural characterization of isolated CD14+ monocytes in the four groups was also performed through electron microscopy.

Results: We observed hundreds of differentially expressed genes and dysregulated pathways when comparing manifesting and non-manifesting *GBA* mutation carriers. Specifically, when compared to idiopathic PD, PD/GBA showed dysregulation in genes involved in alpha-synuclein degradation, aging and amyloid processing. Gene-based outlier analysis confirmed the involvement of lysosomal, membrane trafficking, and mitochondrial processing in manifesting compared to non-manifesting *GBA*-carriers, as also observed at the ultrastructural levels. Transcriptomic results were only partially replicated in an independent cohort of whole blood samples, suggesting cell-type specific changes.

Conclusions: Overall, our transcriptomic analysis of primary monocytes identified gene targets and biological processes that can help in understanding the pathogenic mechanisms associated with *GBA* mutations in the context of PD.

Keywords: Parkinson's disease, Monocytes, *GBA*, beta-glucocerebrosidase, Transcriptomic analysis

Background

Mutations of the *GBA* gene, encoding beta-glucocerebrosidase (GCase), have long been recognized as the major genetic risk factor for Parkinson's disease (PD) [1–4]. Mono- and biallelic mutations of *GBA* can increase the risk of developing PD up to 10 times compared to the

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CD14+ monocytes and whole blood. We compared the directionality of differentially expression genes between a) GBA/PD vs GBA/CTRL and PD vs CTRL in CD14+ monocytes ($n = 197$); b) GBA/PD vs GBA/CTRL and PD vs CTRL in whole blood ($n = 207$); c) GBA/PD vs GBA/CTRL in CD14+ monocytes and whole blood ($n = 16$); d) PD vs CTRL in CD14+ monocytes and whole blood ($n = 103$). **Supplementary Fig. 11.** Correlation between gene expression levels in isolated CD14+ monocytes and whole blood. Genes with expression with more than 1 CPM in 30% of the samples were considered from both cohorts (discovery cohort: isolated CD14+ monocytes (total number of genes: 13711), validation cohort: whole blood - PPMI cohort (total number of genes: 18111)). Spearman correlation between levels of normalized mean gene expression across subjects within each cohort per sub-group of subjects was calculated ($R = 0.78$ $p < 0.001$). Genes were normalized with TMM and voom, as detailed in the main text. **Supplementary Fig. 12.** Validation in whole blood of differentially expressed genes in monocytes. a) Differential levels of expression of the targeted genes (*ATP13A2*, *LRRK2*, *NOTCH1*, between manifesting and non-manifesting carriers in whole blood from manifesting and non-manifesting GBA-mutation carriers. b) Differential normalized expression count of *SNCA*, *LMNA,* and *GBA* between PD/GBA and PD, compared to CTRL/GBA and CTRL subjects in whole blood. In a) and b) each dot represents a subject. Dots are colored based on *GBA* mutations (as reported in the legend: *GBA* mild mutations (N370S, E326K, R496H), *GBA* severe mutations (L444P/A456P/RecNciI, V394L, 84GG, 84GG/T369M, N370S/RecNciI)). *p*-value of different expression levels is reported on top (statistics: Mann-Whitney U test). c) Pathway enrichment analysis of differentially expressed genes in whole blood between PD/GBA vs PD subjects with *p*-value < 0.01 for GO terms are reported. Dark blue: pathways related to cell transport; Green: pathways related to immune response; Light blue: other pathways.

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Authors' contributions
Conceptualization: TR, GMR; Methodology: GMR, TR, RAV, EN, EU, KPL, ONJ, SDK, WJ, AS; Formal analysis and investigation: GMR, RAV, EN, EU, KPL, AA, MP, BH, KA, CA, MZ, ST, ONJ, SDK, WJ, AS; Writing - original draft preparation: GMR, TR; Writing - review and editing: GMR, TR, RAV, EN, EU, KPL, AA, MP, BH, KA, CA, MZ, ST, ONJ, SDK, WJ, AA, JFC, ADF, GPC, SJF; Funding acquisition: TR, GMR; Resources: TR, SJF; Supervision: TR. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets supporting the conclusions of this article (Raw RNA-seq data) are available as part of the Myeloid cells in Neurodegenerative Disease (MyND) study via dbGAP (study accession ID: phs002400.v1.p1) at https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs002400.v1.p1. RNA-seq data for Parkinson's Progression Markers Initiative (PPMI) cohort were obtained from the Accelerating Medicines Partnership program for Parkinson's disease (AMP-PD) Knowledge Platform. For up-to-date information on the study, https://www.amp-pd.org.

Declarations

Ethics approval and consent to participate
All the procedures involving human subjects were performed upon written informed consent, approval from the institutional review board and in accord with the Helsinki Declaration of 1975. Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare no competing interests.

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Skeletal Muscle

CASE REPORT Open Access

Megaconial congenital muscular dystrophy due to novel CHKB variants: a case report and literature review

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Abstract

Background: Choline kinase beta (CHKB) catalyzes the first step in the de novo biosynthesis of phosphatidyl choline and phosphatidylethanolamine via the Kennedy pathway. Derangement of this pathway might also influence the homeostasis of mitochondrial membranes.

Autosomal recessive *CHKB* mutations cause a rare form of congenital muscular dystrophy known as megaconial congenital muscular dystrophy (MCMD).

Case presentation: We describe a novel proband presenting MCMD due to unpublished *CHKB* mutations. The patient is a 6-year-old boy who came to our attention for cognitive impairment and slowly progressive muscular weakness. He was the first son of non-consanguineous healthy parents from Sri Lanka. Neurological examination showed proximal weakness at four limbs, weak osteotendinous reflexes, Gowers' maneuver, and waddling gate. Creatine kinase levels were mildly increased. EMG and brain MRI were normal. Left quadriceps skeletal muscle biopsy showed a myopathic pattern with nuclear centralizations and connective tissue increase. Histological and histochemical staining suggested subsarcolemmal localization and dimensional increase of mitochondria. Ultrastructural analysis confirmed the presence of enlarged ("megaconial") mitochondria. Direct sequencing of *CHKB* identified two novel defects: the c.1060G > C (p.Gly354Arg) substitution and the c.448-56_29del intronic deletion, segregating from father and mother, respectively. Subcloning of RT-PCR amplicons from patient's muscle RNA showed that c.448-56_29del results in the partial retention (14 nucleotides) of intron 3, altering physiological splicing and transcript stability. Biochemical studies showed reduced levels of the mitochondrial fission factor DRP1 and the severe impairment of mitochondrial respiratory chain activity in patient's muscle compared to controls.

Conclusions: This report expands the molecular findings associated with MCMD and confirms the importance of considering *CHKB* variants in the differential diagnosis of patients presenting with muscular dystrophy and mental retardation. The clinical outcome of MCMD patients seems to be influenced by *CHKB* molecular defects. Histological

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function in the rmd mouse model, confirming the cell-autonomous nature of the disease. More importantly, AAV6-based intramuscular gene therapy improved dystrophy phenotype even after disease onset in preclinical models [16]. Altered lipid metabolism was also demonstrated to result in the increase of the arrhythmogenic lipid acylcarnitine predisposing to arrhythmia in hypertrophic cardiac muscles [17]. Tavasoli and colleagues have recently demonstrated that a temporal change in lipid metabolism occurs in Chkb−/− affected muscles. They observed that impaired β-oxidation of fatty acids in mitochondria results in triacylglycerol accumulation as the disease progresses. Interestingly, the decrease in peroxisome proliferator-activated receptors (PPAR) and downstream target gene expression can be reversed by pharmacological PPAR agonism [18].

Irregular mitochondrial morphology is linked to hampered mitochondrial fission consequent to decreased levels of the fission protein DRP1, compromising OXPHOS activity [19]. Aksu-Menges and colleagues have recently observed altered mitochondrial morphology, reduced levels of mitochondrial fission proteins and derangement in several mitochondrial pathways in human primary skeletal muscle cells from a MCMD patient [20]. Our study confirms these findings in the muscle of our patient: engaged autophagy was indirectly suggested by increased levels of p62 and LC3 in some muscle fibers while decreased levels of DRP1 were associated with a severe multi-complex defect in presence of normal levels of respiratory chain protein subunits. The rarefication of mitochondria in the center of muscle fibers, observed in our case as well as in previous reports [1, 20], might be a consequence of sustained mitophagy.

Nowadays, modern diagnostic approach based on NGS sequencing bypass the need of invasive procedures to achieve a molecular diagnosis in a relevant number of patients with neuromuscular disorders. Nevertheless, we highlight the appropriateness of muscle biopsy for the validation of genetic findings and, as in the case of MCMD, for the identification of pathognomonic features which unequivocally direct the molecular analysis.

Conclusions

Our findings expand the genetic repertoire of MCMD and support the role of altered mitochondrial morphology and dynamics in the establishment of the severe respiratory chain defect which underline muscle pathology in this form of congenital myopathy. Additional cases and prolonged follow up of *CHKB*-mutated patients are required to challenge the genotype–phenotype correlation advanced in this report.

Abbreviations

MCMD: Megaconial congenital muscular dystrophy; EMG: Electromyography; MRI: Magnetic resonance imaging; ENT: Ear, nose, and throat evaluation; CK: Creatine kinase; COX: Cytochrome c oxidase; OXPHOS: Oxidative phosphorylation; PPAR: Peroxisome proliferator-activated receptors.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13395-022-00306-8.

Additional file1: Supplementary Table 1. Clinical, instrumental, histological and molecular features of CHKB-mutated MCMD patients reported so far (y: years; m: months; d: days; NA: not assessed; DCM: dilated cardiomyopathy; LVFS: left ventricular systolic function; PDA: Patent ductus arteriosus).

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Authors' contributions

FM and DR designed the study and edited the manuscript. DR and SA performed molecular studies. MR, SZ, LN, and PC performed histological and ultrastructural analysis of skeletal muscle. FF and MG performed biochemical studies. FM, GS, VN, AG, and DM contributed to the clinical examination of the patient. DV and MS contributed to muscle biopsy and data interpretation. MS, SC, and GPC revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The "Comitato Etico Milano Area 2 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico" (Milan, Italy) approved the study. Informed consent was obtained from all subjects involved in the study.

Consent for publication

Written informed consent was obtained from the patients for publication of this Case Report and any accompanying images. A copy of the written consent is available to Editors of this journal on request.

Competing interests

The authors declare that they have no competing interests.

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RESEARCH Open Access

Muscle histological changes in a large cohort of patients affected with Becker muscular dystrophy

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Abstract

Becker muscular dystrophy (BMD) is a severe X-linked muscle disease. Age of onset, clinical variability, speed of progression and affected tissues display wide variability, making a clinical trial design for drug development very complex. The histopathological changes in skeletal muscle tissue are central to the pathogenesis, but they have not been thoroughly elucidated yet. Here we analysed muscle biopsies from a large cohort of BMD patients, focusing our attention on the histopathological muscle parameters, as fibrosis, fatty replacement, fibre cross sectional area, necrosis, regenerating fibres, splitting fibres, internalized nuclei and dystrophy evaluation. We correlated histological parameters with both demographic features and clinical functional evaluations. The most interesting results of our study are the accurate quantification of fibroadipose tissue replacement and the identification of some histopathological aspects that well correlate with clinical performances. Through correlation analysis, we divided our patients into three clusters with well-defined histological and clinical features. In conclusion, this is the first study that analyses in detail the histological characteristics of muscle biopsies in a large cohort of BMD patients, correlating them to a functional impairment. The collection of these data help to better understand the histopathological progression of the disease and can be useful to validate any pharmacological trial in which the modification of muscle biopsy is utilized as outcome measure.

Keywords: Histology, Becker muscular distrophy, Muscle biopsies, Fibrosis

Introduction

Skeletal muscle dystrophies are a large and heterogeneous group of inherited disorders characterized by progressive muscle weakness. X-linked Duchenne muscular dystrophy (DMD, OMIM 310,200) and Becker muscular dystrophy (BMD, OMIM 300,376) are among the most severe.

In both disorders, most of the identified mutations are large deletions, spanning one or more exons, the remaining patients harbour exon duplications or less frequently point mutations and small rearrangements [1, 2].

Muscle histopathological changes are central to DMD/BMD pathogenesis. The lack of dystrophin results in sarcolemma instability and increased vulnerability to mechanical stress, causing inflammation, fibre necrosis and fibre regeneration. These changes lead to constant cycles of degeneration and regeneration, but, with age, the repair phase becomes less and less successful as a consequence of exhaustion of satellite cell pools [3]. Muscle fibres are replaced with fat and connective

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Data obtained from our statistical analysis suggested the importance of selected suitable clinical tests to be applied during pharmacological treatment or clinical trials, to be able to efficiently monitor patients' clinical progress.

It is important to underline that this study recruited patients participating to a clinical trial and therefore meeting specific inclusion criteria. In details, ambulant BMD patients aged ≥ 18 to ≤ 65 years and able to perform 6MWT at screening with a minimum distance of 200 m and maximum distance of 450 m, were recruited. These inclusion criteria prevented us from examining muscle biopsies from younger and older patients, therefore, individuals with a very mild or otherwise very severe disease were excluded.

Conclusion

At present, this work has collected one of the largest cohorts of ambulant BMD patients, providing relevant information about histological picture and showing extremely significant correlations between histological traits and some functional data making this information useful for any pharmacological trial in which the modification of muscle biopsy is utilized as outcome measures.

Supplementary Information

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Additional file 1 Western Blot Analysis

Additional file 2 Bidimensional score plot of the principal component analysis (PCA) applied to the data set of the 45 BMD patients (each dot represents a patient)

Additional file 3 Three-dimensional score plot of the PLS-DA model showing the three clusters in which BMD patients are classified according to different histological and clinical traits. Each dot represents a patient. Green dots identified patients of cluster 1, blue dots of cluster 2 and red dots of cluster 3.The axis score t[1] represents the latent variable of the model. The latent variable is a mathematical construct that 'summarizes' the variables registered in the study. PLS-DA: partial least squaresdiscriminant analysis.

Additional file 4 Loading plot of the PLS-DA model. The loading plot is complementary to the score plot and summarizes how the X-variables relate to each other as well as to group belonging (Y-variable symbolized by a group dot). X-variables located near a group dot are positively associated with that group. PLS-DA: partial least squares-discriminant analysis

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Author contributions

MR, DV, SC; MM, PB, GPC contributed to the design the study; MR, SZ, FF, PC conducted experiments; DV, FM, EM collected clinical data; MR, DV, SM, FM analysed the data; SM performed the statistical analysis; FT performed muscle biopsies; MS, MM, PB, GPC supervised the study; MR, SM, SZ wrote the manuscript; all authors read and approved the final manuscript.

Declarations

Competing interests

MR, DV, SM, SZ, FM, EM, FF, PC, FT, MS, MM—Disclosures: None. SC is employee of Italfarmaco SpA, sponsor of the clinical study. PB is employee of Italfarmaco SpA, sponsor of the clinical study. GPC participated to Advisory boards of Italfarmaco SpA.

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RESEARCH

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Expanding the clinical-pathological and genetic spectrum of *RYR1*-related congenital myopathies with cores and minicores: an Italian population study

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Abstract

Mutations in the *RYR1* gene, encoding ryanodine receptor 1 (RyR1), are a well-known cause of Central Core Disease (CCD) and Multi-minicore Disease (MmD). We screened a cohort of 153 patients carrying an histopathological diagnosis of core myopathy (cores and minicores) for *RYR1* mutation. At least one *RYR1* mutation was identified in 69 of them and these patients were further studied. Clinical and histopathological features were collected. Clinical phenotype was highly heterogeneous ranging from asymptomatic or paucisymptomatic hyperCKemia to severe muscle weakness and skeletal deformity with loss of ambulation. Sixty-eight *RYR1* mutations, generally missense, were identified, of which 16 were novel. The combined analysis of the clinical presentation, disease progression and the structural bioinformatic analyses of *RYR1* allowed to associate some phenotypes to mutations in specific domains. In addition, this study highlighted the structural bioinformatics potential in the prediction of the pathogenicity of *RYR1* mutations. Further improvement in the comprehension of genotype–phenotype relationship of core myopathies can be expected in the next future: the actual lack of the human RyR1 crystal structure paired with the presence of large intrinsically disordered regions in RyR1, and the frequent presence of more than one *RYR1* mutation in core myopathy patients, require designing novel investigation strategies to completely address RyR1 mutation effect.

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Supplementary Information

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Additional file 1: Bioinformatics pipeline used for mutations effect prediction.

Additional file 2: Affected RyR1 domains and clinical description.

Additional file 3: Histological description of core myopathies patients.

Additional file 4: Patients *RYR1* mutations and clinical description.

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Author contributions
Conceptualization: E.P., C. B.; Data Curation: A. F., D. C.; Supervision: E. P., C. B.; Writing—Original Draft Preparation: A. F, E.P. C. B. G. M.; Formal Analysis: D. C., G.M., D. S., L.B., S. T., F. M. S.; Funding Acquisition, E. P., C. B. Writing—review & editing, A. F., D. C., C. F., V. C., G. A., A. D'A., L. M., F. M., M. P., G. T., D. S., L. B., R. B., P. B., F. F., E. S. B., G. C., S. M., T. M., I. M., C. P., A. B., A. D., V. N., A. P., M. G., C. D., E. R., E. M., G. M., S. T., F. S., C. B. and E. P.

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Availability of data and materials
All data generated or analysed during this study are included in this published article [Table 1, Additional file 2: T1, Additional file 3: T2, Additional file 4: T3].

Declarations

Ethics approval and consent to participate
The study was conducted according to the guidelines of the Declaration of Helsinki, and ap-proved by the Institutional Ethic Committee at each participating center. We report here the Ethics Committee approvals of the two senior authors: the study was approved by the Ethical Committee of the Istituto Giannina Gaslini Genova on March 10, 2009 (number 567 DSc/fg) and by the Ethical Committee of the University of Padova—Hospital on May 11, 2009 (number 1879P/0025745).

Consent for publication
Written informed consent has been obtained from the patients to publish this paper.

Competing interests
The authors declare that they have no competing interests.

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Clinical and genetic features of a cohort of patients with *MFN2*-related neuropathy

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Charcot–Marie–Tooth disease type 2A (CMT2A) is a rare inherited axonal neuropathy caused by mutations in *MFN2* gene, which encodes Mitofusin 2, a transmembrane protein of the outer mitochondrial membrane. We performed a cross-sectional analysis on thirteen patients carrying mutations in *MFN2*, from ten families, describing their clinical and genetic characteristics. Evaluated patients presented a variable age of onset and a wide phenotypic spectrum, with most patients presenting a severe phenotype. A novel heterozygous missense variant was detected, p.K357E. It is located at a highly conserved position and predicted as pathogenic by in silico tools. At a clinical level, the p.K357E carrier shows a severe sensorimotor axonal neuropathy. In conclusion, our work expands the genetic spectrum of CMT2A, disclosing a novel mutation and its related clinical effect, and provides a detailed description of the clinical features of a cohort of patients with *MFN2* mutations. Obtaining a precise genetic diagnosis in affected families is crucial both for family planning and prenatal diagnosis, and in a therapeutic perspective, as we are entering the era of personalized therapy for genetic diseases.

Abbreviations

CMT	Charcot–Marie–Tooth
CMTESv2	Charcot–Marie–Tooth Examination Score version 2
CMTESv2-R	Rasch analysis-weighted CMTESv2
CMTNSv2	Charcot–Marie–Tooth Neuropathy Score version 2
CMTPeds	CMT pediatric scale
MFN1/2	Mitofusin1/2
MNCV	Motor nerve conduction velocity
NIV	Non-invasive ventilatory support

Charcot–Marie–Tooth disease (CMT) includes a wide spectrum of primary inherited sensory-motor neuropathies associated with more than 100 different genetic culprits[1]. With an overall prevalence of 1/1200-2500, it represents the most common genetically inherited neuromuscular disorder[2]. CMTs are classified according to their neurophysiological properties and inheritance pattern. Demyelinating CMT type 1 is characterized by reduced motor nerve conduction velocity (MNCV), while axonal CMT type 2 shows preserved MNCV[1]. Among CMT2, CMT2A is the most frequent form, accounting for approximately 10–40% of axonal CMT cases and 4–7% of all CMTs with a genetic diagnosis[2–6].

CMT2A is associated with mutations in the nuclear-encoded mitochondrial gene *mitofusin 2 (MFN2)*, which is translated into the 757-amino acid long protein Mitofusin2 (MFN2). MFN2 is a highly conserved GTPase,

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BRIEF COMMUNICATION

Analysis of *HTT* CAG repeat expansion in Italian patients with amyotrophic lateral sclerosis

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[a]These authors equally contributed to the work.

Abstract

HTT full-penetrance pathogenic repeat expansions, the genetic cause of Huntington's disease (HD), have been recently reported in a minority of frontotemporal dementia/amyotrophic lateral sclerosis (ALS) patients (0.13%). We analyzed *HTT* CAG repeats in an Italian cohort of ALS patients (n = 467) by repeat-primed polymerase chain reaction. One patient harbored two expanded alleles in the *HTT* gene (42 and 37 CAG repeats). The absence of HD typical symptoms and the clinical picture consistent with ALS, corroborated by the diagnostic assessment, apparently excluded a misdiagnosis of HD.

Introduction

Dewan and colleagues have recently reported *HTT* full-penetrance pathogenic repeat expansions in three probands (0.12%) out of 2442 frontotemporal dementia (FTD)/amyotrophic lateral sclerosis (ALS), patients.[1] After expanding the analysis to an independent cohort of 3674 FTD/ALS patients, five additional carriers of *HTT* pathogenic expansions were identified (0.14%). Comparing these data to the prevalence of pathogenic *HTT* repeat expansions in the general population (0.03%),[2,3] the authors concluded that the carrier rate was significantly higher in FTD/ALS patients.

Thomas and colleagues have recently challenged this finding, highlighting several points which argue against the role of *HTT* pathogenic expansions in FTD/ALS.[4] Among them, the authors cited a previously published work which reported a 0.18% carrier rate of *HTT* repeat expansions in the general population. Accordingly, they suggested that the occurrence of *HTT* pathogenic expansions in FTD/ALS might merely reflect their prevalence among the general population.[5] Furthermore, Thomas and colleagues questioned the lack of clinical description of the cases. Indeed, they could have been misdiagnosed due to the clinical heterogeneity of HD, especially in juvenile forms, and to the age-dependent penetrance of *HTT* pathogenic expansions.[6,7] Regarding neuropathology, Thion and coauthors stated that the absence of neostriatal atrophy was coherent with the small repeat expansions of

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Review

Targeting PTB for Glia-to-Neuron Reprogramming In Vitro and In Vivo for Therapeutic Development in Neurological Diseases

Matilde Contardo [1,†], Roberta De Gioia [2,†], Delia Gagliardi [1], Giacomo Pietro Comi [1,2], Linda Ottoboni [2], Monica Nizzardo [2,‡] and Stefania Corti [1,2,*,‡]

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Abstract: In vivo cell reprogramming of glial cells offers a promising way to generate new neurons in the adult mammalian nervous system. This approach might compensate for neuronal loss occurring in neurological disorders, but clinically viable tools are needed to advance this strategy from bench to bedside. Recently published work has described the successful neuronal conversion of glial cells through the repression of a single gene, polypyrimidine tract-binding protein 1 (*Ptbp1*), which encodes a key RNA-binding protein. Newly converted neurons not only express correct markers but they also functionally integrate into endogenous brain circuits and modify disease symptoms in in vivo models of neurodegenerative diseases. However, doubts about the nature of "converted" neurons, in particular in vivo, have been raised, based on concerns about tracking reporter genes in converted cells. More robust lineage tracing is needed to draw definitive conclusions about the reliability of this strategy. In vivo reprogramming and the possibility of implementing it with approaches that could be translated into the clinic with antisense oligonucleotides targeting a single gene like *Ptbp1* are hot topics. They warrant further investigation with stringent methods and criteria of evaluation for the ultimate treatment of neurological diseases.

Keywords: PTB; reprogramming; neuron; neurodegenerative diseases

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1. Introduction

Neurodegenerative diseases are disabling and often fatal disorders characterized by the progressive loss of specific neuronal subpopulations in various parts of the nervous system and thus specific profiles of neurological dysfunction.

Neurons in the human central nervous system (CNS) are not normally replaced through adult neurogenesis once they are lost, aside from a negligible fraction [1–3]. Thus, methods to promote the generation of new neural cells in the adult mammalian brain have been intensively investigated during the past decades [4]. Three main approaches to produce new neurons in the adult brain have been explored: (1) cell transplantation of exogenous neuronal cells/precursors [3,5,6], (2) activation of the endogenous neurogenic capacity of neuronal progenitors in specific zones [7], and (3) reprogramming (or direct conversion or transdifferentiation) of non-neuronal cells, conventionally of abundant glial cells into neurons [8–11].

The strategy of direct neuronal conversion is based on the combinatorial expression of lineage-specific neural transcription factors (TFs) that can turn fibroblasts or glial cells into neurons In Vitro, and likely also in vivo, without passage through a stem cell state [12].

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Review

Stathmins and Motor Neuron Diseases: Pathophysiology and Therapeutic Targets

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Abstract: Motor neuron diseases (MNDs) are a group of fatal, neurodegenerative disorders with different etiology, clinical course and presentation, caused by the loss of upper and lower motor neurons (MNs). MNs are highly specialized cells equipped with long, axonal processes; axonal defects are some of the main players underlying the pathogenesis of these disorders. Microtubules are key components of the neuronal cytoskeleton characterized by dynamic instability, switching between rapid polymerization and shrinkage. Proteins of the stathmin family affect microtubule dynamics regulating the assembly and the dismantling of tubulin. Stathmin-2 (STMN2) is one of the most abundantly expressed genes in MNs. Following axonal injury, STMN2 expression is upregulated, and the protein is transported toward the growth cones of regenerating axons. STMN2 has a critical role in axonal maintenance, and its dysregulation plays an important role in neurodegenerative processes. Stathmin-1 (STMN1) is a ubiquitous protein that is highly expressed during the development of the nervous system, and its phosphorylation controls microtubule dynamics. In the present review, we summarize what is currently known about the involvement of stathmin alterations in MNDs and the potential therapeutic effect of their modulation, with a specific focus on the most common forms of MND, amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA).

Keywords: stathmin; motor neuron diseases; ALS; SMA; STMN2; STMN1; axonal defects; cytoskeleton; microtubules

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1. Stathmins Are Relevant for Axonal Stability

Motor neurons (MNs) are highly specialized cells equipped with long, axonal processes. Proper cytoskeletal structure is fundamental for maintaining shape, axonal stability, anterograde and retrograde transport and inter-neuronal signaling. Microtubules are essential for axonal outgrowth and regeneration and in maintaining the integrity of axonal signal transduction and cellular transport systems [1]. Axonal defects are some of the main players of the pathogenesis of motor neuron disorders (MNDs), and understanding the biology underlying these processes may increase the comprehension and the development of therapeutic targets in these diseases.

Microtubules are characterized by dynamic instability: they undergo periods of polymerization, shrinkage and rest, depending on the continuous balance between assembly and disassembly which is largely mediated by microtubule-associated proteins such as stathmins.

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Review

New Insights into Cerebral Vessel Disease Landscapes at Single-Cell Resolution: Pathogenetic and Therapeutic Perspectives

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Abstract: Cerebrovascular diseases are a leading cause of death and disability globally. The development of new therapeutic targets for cerebrovascular diseases (e.g., ischemic, and hemorrhagic stroke, vascular dementia) is limited by a lack of knowledge of the cellular and molecular biology of health and disease conditions and the factors that cause injury to cerebrovascular structures. Here, we describe the role of advances in omics technology, particularly RNA sequencing, in studying high-dimensional, multifaceted profiles of thousands of individual blood and vessel cells at single-cell resolution. This analysis enables the dissection of the heterogeneity of diseased cerebral vessels and their atherosclerotic plaques, including the microenvironment, cell evolutionary trajectory, and immune response pathway. In animal models, RNA sequencing permits the tracking of individual cells (including immunological, endothelial, and vascular smooth muscle cells) that compose atherosclerotic plaques and their alteration under experimental settings such as phenotypic transition. We describe how single-cell RNA transcriptomics in humans allows mapping to the molecular and cellular levels of atherosclerotic plaques in cerebral arteries, tracking individual lymphocytes and macrophages, and how these data can aid in identifying novel immune mechanisms that could be exploited as therapeutic targets for cerebrovascular diseases. Single-cell multi-omics approaches will likely provide the unprecedented resolution and depth of data needed to generate clinically relevant cellular and molecular signatures for the precise treatment of cerebrovascular diseases.

Keywords: cerebral vessel; atherosclerosis; cerebrovascular disease; transcriptomics; stroke; single-cell sequencing; single-cell omics; RNA

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1. Introduction

Neurovascular diseases are a top cause of disability, morbidity, and death globally [1], with an estimated 143 million disability-adjusted life-years in 2019 expected to grow with an expanding and increasingly older population [2].

Therapeutic approaches in acute stroke are targeted at rapid recanalizing of the blocked artery, either with intravenous thrombolysis or with thrombus lysis by endovascular thrombectomy [3,4]. Often, this recanalization is not successful or is even inefficient in removing the occlusion. Although therapeutic strategies have significantly improved, this disease still poses an enormous burden on human health, and translational research in the cerebrovascular field is an urgent unmet need to promote healthy living worldwide.

Atherosclerosis is important in the onset and progression of cerebral vascular disease. Cells and fibrous and lipid-rich material can accumulate and form arterial plaques in

cells

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Article

Biallelic Variants in *ENDOG* Associated with Mitochondrial Myopathy and Multiple mtDNA Deletions

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Abstract: Endonuclease G (ENDOG) is a nuclear-encoded mitochondrial-localized nuclease. Although its precise biological function remains unclear, its proximity to mitochondrial DNA (mtDNA) makes it an excellent candidate to participate in mtDNA replication, metabolism and maintenance. Indeed, several roles for ENDOG have been hypothesized, including maturation of RNA primers during mtDNA replication, splicing of polycistronic transcripts and mtDNA repair. To date, *ENDOG* has been deemed as a determinant of cardiac hypertrophy, but no pathogenic variants or genetically defined patients linked to this gene have been described. Here, we report biallelic *ENDOG* variants identified by NGS in a patient with progressive external ophthalmoplegia, mitochondrial myopathy and multiple mtDNA deletions in muscle. The absence of the ENDOG protein in the patient's muscle and fibroblasts indicates that the identified variants are pathogenic. The presence of multiple mtDNA deletions supports the role of ENDOG in mtDNA maintenance; moreover, the patient's clinical presentation is very similar to mitochondrial diseases caused by mutations in other genes involved in mtDNA homeostasis. Although the patient's fibroblasts did not present multiple mtDNA deletions or delay in the replication process, interestingly, we detected an accumulation of low-level heteroplasmy mtDNA point mutations compared with age-matched controls. This may indicate a possible role of ENDOG in mtDNA replication or repair. Our report provides evidence of the association of *ENDOG* variants with mitochondrial myopathy.

Keywords: endonuclease G; ENDOG; mitochondrial DNA; mitochondrial myopathy; multiple mtDNA deletions

check for updates

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1. Introduction

Endonuclease G (ENDOG) is a nuclear-encoded nuclease, member of the conserved DNA/RNA non-specific ββα-Me-finger nuclease family [1]. The ENDOG protein is synthesized in the cytoplasm as an inactive 33 kDa propeptide, which is activated by proteolytic cleavage of the mitochondrial targeting sequence, thus producing a mature 28 kDa enzyme which acts as a homodimer [1–3].

Initial experiments suggested an exclusive localization of ENDOG within the mitochondrial intermembrane space; later on, it was found to be mainly bound to the mitochondrial inner membrane, facing the matrix [4]. Given its spatial closeness to mtDNA, as well as

absence of ENDOG casts doubt on the assertion that loss-of-function mutations in *ENDOG* are associated with impaired cardiac function. Although the functional results obtained are preliminary, we provide novel evidence about a possible role of ENDOG linked to mtDNA maintenance. More studies are needed to further test involvement of ENDOG in mtDNA metabolism, not limited to replication but also to the complex repair systems for mtDNA, which are still poorly understood.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cells11060974/s1. Table S1: biochemical analysis of OXPHOS complexes; Table S2: Additional candidate variants from NGS analyses; Figure S1: Analysis of the mtDNA by NGS; Figure S2: Structural analysis of residues affected by the missense variants; Figure S3: Immunofluorescence studies in fibroblasts; Figure S4: Western blot analysis of C1QBP; Figure S5: *ENDOG* transcript analysis in patient's fibroblasts.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. The patient provided his written informed consent to participate in this study, approved by the Ethics Committee of the Neurological Institute Besta (CI43, 24 February 2021).

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Immunofluorescence signal intensity measurements as a semi-quantitative tool to assess sarcoglycan complex expression in muscle biopsy

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ABSTRACT

Sarcoglycanopathies are highly heterogeneous in terms of disease progression, muscular weakness, loss of ambulation and cardiac/respiratory involvement. Their clinical severity usually correlates with the residual protein amount, which makes protein quantification extremely relevant. Sarcoglycanopathy diagnosis is genetic, but skeletal muscle analysis - by both immunohistochemistry and Western blot (WB) - is still mandatory to establish the correct diagnostic process. Unfortunately, however, WB analysis cannot be performed if the bioptic specimen is scarce. This study provides a sensitive tool for semi-quantification of residual amount of sarcoglycans in patients affected by sarcoglycanopathies, based on immunofluorescence staining on skeletal muscle sections, image acquisition and software elaboration. We applied this method to eleven sarcoglycanopathies, seven Becker muscular dystrophies, as pathological control group, and four age-matched controls. Fluorescence data showed a significantly reduced expression of the mutated sarcoglycan in all patients when compared to their respective age-matched healthy controls, and a variable reduction of the other sarcoglycans. The reduction is due to the effect of gene mutation and not to the increasing age of controls. Fluorescence normalized data analyzed in relation to the age of onset of the disease, showed a negative correlation of α-sarcoglycan fluorescence signal *vs* fibrosis in patients with an early age of onset and a negative correlation between δ-sarcoglycan signal and fibrosis in both intermediate and late age of onset groups. The availability of a method that allows objective quantification of the sarcolemmal proteins, faster and less consuming than WB analysis and able to detect low residual sarcoglycan expression with great sensitivity, proves useful also in view of possible inferences on disease prognosis. The proposed method could be employed also to monitor the efficacy of therapeutic interventions and during clinical trials.

Key words: sarcoglycans; immunofluorescence; protein quantification; histology; fibrosis.

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Contributions: SZ, FM, FP, MS, conceived the idea, interpreted the results, revised the literature, and wrote the manuscript; MS, MM, GC, SC, DV, MR performed a critical revision of the manuscript for important intellectual content; FF, PC, participated in the acquisition of data. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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CASE REPORT
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Case Report: Rare Homozygous *RNASEH1* Mutations Associated With Adult-Onset Mitochondrial Encephalomyopathy and Multiple Mitochondrial DNA Deletions

Arianna Manini[1], Leonardo Caporali[2], Megi Meneri[1,3], Simona Zanotti[4], Daniela Piga[3], Ignazio Giuseppe Arena[5], Stefania Corti[1,3], Antonio Toscano[5], Giacomo Pietro Comi[1,4], Olimpia Musumeci[5], Valerio Carelli[2,6]* and Dario Ronchi[1,3]**

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Mitochondrial DNA (mtDNA) maintenance disorders embrace a broad range of clinical syndromes distinguished by the evidence of mtDNA depletion and/or deletions in affected tissues. Among the nuclear genes associated with mtDNA maintenance disorders, *RNASEH1* mutations produce a homogeneous phenotype, with progressive external ophthalmoplegia (PEO), ptosis, limb weakness, cerebellar ataxia, and dysphagia. The encoded enzyme, ribonuclease H1, is involved in mtDNA replication, whose impairment leads to an increase in replication intermediates resulting from mtDNA replication slowdown. Here, we describe two unrelated Italian probands (Patient 1 and Patient 2) affected by chronic PEO, ptosis, and muscle weakness. Cerebellar features and severe dysphagia requiring enteral feeding were observed in one patient. In both cases, muscle biopsy revealed diffuse mitochondrial abnormalities and multiple mtDNA deletions. A targeted next-generation sequencing analysis revealed the homozygous *RNASEH1* mutations c.129-3C>G and c.424G>A in patients 1 and 2, respectively. The c.129-3C>G substitution has never been described as disease-related and resulted in the loss of exon 2 in Patient 1 muscle *RNASEH1* transcript. Overall, we recommend implementing the use of high-throughput sequencing approaches in the clinical setting to reach genetic diagnosis in case of suspected presentations with impaired mtDNA homeostasis.

Keywords: *RNASEH1*, ribonuclease H1, mitochondrial DNA, mtDNA maintenance disorders, myopathy, CPEO

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INTRODUCTION

Mitochondrial DNA (mtDNA) maintenance disorders, which produce a variety of clinical presentations, including myopathy, progressive external ophthalmoparesis (PEO), ptosis, parkinsonism, bulbar dysfunction, and cerebellar features, originate from mutations in more than twenty-five nuclear genes involved in mtDNA homeostasis (Ahmed et al., 2015; Viscomi

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REVIEW
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Adeno-Associated Virus (AAV)-Mediated Gene Therapy for Duchenne Muscular Dystrophy: The Issue of Transgene Persistence

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Duchenne muscular dystrophy (DMD) is an X-linked recessive, infancy-onset neuromuscular disorder characterized by progressive muscle weakness and atrophy, leading to delay of motor milestones, loss of autonomous ambulation, respiratory failure, cardiomyopathy, and premature death. DMD originates from mutations in the *DMD* gene that result in a complete absence of dystrophin. Dystrophin is a cytoskeletal protein which belongs to the dystrophin-associated protein complex, involved in cellular signaling and myofiber membrane stabilization. To date, the few available therapeutic options are aimed at lessening disease progression, but persistent loss of muscle tissue and function and premature death are unavoidable. In this scenario, one of the most promising therapeutic strategies for DMD is represented by adeno-associated virus (AAV)-mediated gene therapy. DMD gene therapy relies on the administration of exogenous micro-dystrophin, a miniature version of the dystrophin gene lacking unnecessary domains and encoding a truncated, but functional, dystrophin protein. Limited transgene persistence represents one of the most significant issues that jeopardize the translatability of DMD gene replacement strategies from the bench to the bedside. Here, we critically review preclinical and clinical studies of AAV-mediated gene therapy in DMD, focusing on long-term transgene persistence in transduced tissues, which can deeply affect effectiveness and sustainability of gene replacement in DMD. We also discuss the role played by the overactivation of the immune host system in limiting long-term expression of genetic material. In this perspective, further studies aimed at better elucidating the need for immune suppression in AAV-treated subjects are warranted in order to allow for life-long therapy in DMD patients.

Keywords: Duchenne muscular dystrophy, adeno-associated virus, gene therapy, persistence, dystrophin, microdystrophin

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Case report: Sodium and chloride muscle channelopathy coexistence: A complicated phenotype and a challenging diagnosis

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Non-dystrophic myotonias (NDM) encompass chloride and sodium channelopathy. Mutations in *CLCN1* lead to either the autosomal dominant form or the recessive form of myotonia congenita (MC). The main symptom is stiffness worsening after rest and improving by physical exercise. Patients with recessive mutations often show muscle hypertrophy, and transient weakness mostly in their lower limbs. Mutations in *SCN4A* can lead to Hyper-, Hypo- or Normo-kalemic Periodic Paralysis or to different forms of myotonia (Paramyotonia Congenita-PMC and Sodium Channel Myotonia-SCM and severe neonatal episodic laryngospasm-SNEL). SCM often presents facial muscle stiffness, cold sensitivity, and muscle pain, whereas myotonia worsens in PMC patients with the repetition of the muscle activity and cold. Patients affected by chloride or sodium channelopathies may show similar phenotypes and symptoms, making the diagnosis more difficult to reach. Herein we present a woman in whom sodium and chloride channelopathies coexist yielding a complex phenotype with features typical of both MC and PMC. Disease onset was in the second decade with asthenia, weakness, warm up and limb stiffness, and her symptoms had been worsening through the years leading to frequent heavy retrosternal compression, tachycardia, stiffness, and symmetrical pain in her lower limbs. She presented severe lid lag myotonia, a hypertrophic appearance at four limbs and myotonic discharges at EMG. Her symptoms have been triggered by exposure to cold and her daily life was impaired. All together, clinical signs and instrumental data led to the hypothesis of PMC and to the administration of mexiletine, then replaced by acetazolamide because of gastrointestinal side effects. Analysis of *SCN4A* revealed a new variant, p.Glu1607del. Nonetheless the severity of myotonia in the lower limbs and her general stiffness led to hypothesize that the

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CASE REPORT
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Case Report: Thymidine Kinase 2 (TK2) Deficiency: A Novel Mutation Associated With Childhood-Onset Mitochondrial Myopathy and Atypical Progression

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The nuclear gene *TK2* encodes the mitochondrial thymidine kinase, an enzyme involved in the phosphorylation of deoxycytidine and deoxythymidine nucleosides. Biallelic *TK2* mutations are associated with a spectrum of clinical presentations mainly affecting skeletal muscle and featuring muscle mitochondrial DNA (mtDNA) instability. Current classification includes infantile- (≤1 year), childhood- (1–12 years), and late-onset (≥12 years) forms. In addition to age at onset, these forms differ for progression, life expectancy, and signs of mtDNA instability (mtDNA depletion vs. accumulation of multiple mtDNA deletions). Childhood-onset TK2 deficiency typically causes a rapidly progressive proximal myopathy, which leads to wheelchair-bound status within 10 years of disease onset, and severe respiratory impairment. Muscle biopsy usually reveals a combination of mitochondrial myopathy and dystrophic features with reduced mtDNA content. Here we report the case of an Italian patient presenting childhood-onset, slowly progressive mitochondrial myopathy, ptosis, hypoacusis, dysphonia, and dysphagia, harboring the *TK2* variants c.278A>G and c.543del, the latter unreported so far. Compared to other childhood-onset *TK2*-patients, our case displays atypical features, including slowly progressive muscle weakness and absence of respiratory failure, which are usually observed in late-onset forms. This report extends the genetic background of TK2-related myopathy, highlighting the clinical overlap among different forms.

Keywords: thymidine kinase 2, TK2, mitochondrial DNA, mtDNA maintenance defects, myopathy, deoxynucleosides

INTRODUCTION

Mitochondrial DNA (mtDNA) maintenance defects are a heterogeneous group of clinical syndromes characterized by mtDNA deletions and/or depletion and derived from mutations in nuclear genes variably involved in mtDNA homeostasis (i.e., *POLG1*, *POLG2*, *TWNK*, *DGUOK*, *TYMP*) (1–5).

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CASE REPORT
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Newly Diagnosed Hepatic Encephalopathy Presenting as Non-convulsive Status Epilepticus: A Case Report and Literature Review

Marco Olivero[1], *Delia Gagliardi*[1,2], *Gianluca Costamagna*[1], *Daniele Velardo*[2], *Francesca Magri*[3], *Fabio Triulzi*[4], *Giorgio Conte*[4], *Giacomo P. Comi*[1,3], *Stefania Corti*[1,2]* *and Megi Meneri*[1,2]

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Background: Hepatic encephalopathy is characterized by psychiatric and neurological abnormalities, including epileptic seizure and non-convulsive and convulsive status epilepticus. Conventional brain magnetic resonance imaging is useful in supporting diagnosis since it can reveal specific radiological findings. In the literature, there is no description of hepatic encephalopathy onset as non-convulsive status epilepticus; we provide the first report.

Case Summary: We report a case of a 67-year-old woman, without history of cirrhosis, presenting altered mental state, normal brain computed tomography imaging, and electroencephalography suggestive of epileptic activity. We suspected non-convulsive status epilepticus, and we administered diazepam and levetiracetam with clinical improvement. Thus, we made a diagnosis of non-convulsive status epilepticus. A radiological study with brain magnetic resonance imaging showed bilateral hyperintensity on T1-weighted sequences of globus pallidus and hyperintensity of both corticospinal tracts on T2-weighted fluid-attenuated inversion recovery sequences. Blood tests revealed hyperammonemia, mild abnormality of liver function indices, and chronic Hepatitis B and D virus coinfection. Hepatic elastosonography suggested liver cirrhosis. The patient started antiviral therapy with entecavir and prevention of hepatic encephalopathy with rifaximin and lactulose; she was discharged with a normal mental state.

Conclusions: Hepatic encephalopathy can present as an initial manifestation with non-convulsive status epilepticus. Electroencephalography is useful for differentiating non-convulsive status epilepticus from an episode of hepatic encephalopathy, and neuroimaging aids the diagnostic process.

Keywords: hepatic encephalopathy, non-convulsive status epilepticus, brain magnetic resonance imaging, case report, corticospinal tract, globus pallidus

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ORIGINAL RESEARCH
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Cognitive and Autonomic Dysfunction in Multiple System Atrophy Type P and C: A Comparative Study

Giulia Lazzeri[1,2], *Giulia Franco*[1,2], *Teresa Difonzo*[1], *Angelica Carandina*[3,4], *Chiara Gramegna*[5], *Maurizio Vergari*[6], *Federica Arienti*[1,2], *Anisa Naci*[6], *Costanza Scatà*[3,7], *Edoardo Monfrini*[1,2], *Gabriel Dias Rodrigues*[4], *Nicola Montano*[4], *Giacomo P. Comi*[1,2], *Maria Cristina Saetti*[1,2], *Eleonora Tobaldini*[4] *and Alessio Di Fonzo*[1,2]*

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Multiple System Atrophy (MSA) is a rare neurodegenerative disease, clinically defined by a combination of autonomic dysfunction and motor involvement, that may be predominantly extrapyramidal (MSA-P) or cerebellar (MSA-C). Although dementia is generally considered a red flag against the clinical diagnosis of MSA, in the last decade the evidence of cognitive impairment in MSA patients has been growing. Cognitive dysfunction appears to involve mainly, but not exclusively, executive functions, and may have different characteristics and progression in the two subtypes of the disease (i.e., MSA-P and MSA-C). Despite continued efforts, combining *in-vivo* imaging studies as well as pathological studies, the physiopathological bases of cognitive involvement in MSA are still unclear. In this view, the possible link between cardiovascular autonomic impairment and decreased cognitive performance, extensively investigated in PD, needs to be clarified as well. In the present study, we evaluated a cohort of 20 MSA patients (9 MSA-P, 11 MSA-C) by means of a neuropsychological battery, hemodynamic assessment (heart rate and arterial blood pressure) during rest and active standing and bedside autonomic function tests assessed by heart rate variability (HRV) parameters and sympathetic skin response (SSR) in the same experimental session. Overall, global cognitive functioning, as indicated by the MoCA score, was preserved in most patients. However, short- and long-term memory and attentional and frontal-executive functions were moderately impaired. When comparing MSA-P and MSA-C, the latter obtained lower scores in tests of executive functions and verbal memory. Conversely, no statistically significant difference in cardiovascular autonomic parameters was identified between MSA-P and

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Case Reports: Novel Missense Variants in the Filamin C Actin Binding Domain Cause Variable Phenotypes

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Filamin C is a large dimeric actin-binding protein, most prevalent in skeletal and cardiac muscle Z-discs, where it participates in sarcomere mechanical stabilization and intracellular signaling, interacting with numerous binding partners. Dominant heterozygous mutations of Filamin C gene cause several forms of myopathy and structural or arrhythmogenic cardiomyopathy. In this report we describe clinical and molecular findings of two Italian patients, in whom we identified two novel missense variants located within the Filamin C actin binding domain. Muscle imaging, histological and ultrastructural findings are also reported. Our results underline the extreme inter- and intrafamilial variability of clinical manifestations, hence the need to extend the investigation also to asymptomatic relatives, and the relevance of a broad diagnostic approach involving muscle electron microscopy, skeletal muscle magnetic resonance imaging and next generation sequencing techniques.

Keywords: Filamin C, actin binding domain, distal myopathy, muscle electron microscopy, muscle magnetic resonance imaging, next generation sequencing

INTRODUCTION

Heterozygous defects in the human Filamin C gene (*FLNC*) located on chromosome 7q32.1 result in clinical forms of myopathy and cardiomyopathy with marked phenotypic variability (1, 2). FLNC-related myopathies comprise three main presentations, according to type and location of the molecular defect: (i) missense or splice site changes affecting the rod domain result in late onset, progressive, proximal muscular weakness with large sarcoplasmic inclusions; (ii) frameshift mutations in the rod domain cause distal myopathy without sarcoplasmic inclusions; (iii) missense variants in the actin-binding domain (ABD) result in proximal or distal myopathy with non-specific myopathic changes (3–5). More recently, patients displaying restrictive, hypertrophic, dilated and arrhythmogenic cardiomyopathies have been found harboring truncating and missense *FLNC* mutations (6). Here we describe two novel *FLNC* variants located in the actin-binding domain associated with different phenotypes in two distinct Italian families.

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Article

Antisense Morpholino-Based In Vitro Correction of a Pseudoexon-Generating Variant in the *SGCB* Gene

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Abstract: Limb-girdle muscular dystrophies (LGMD) are clinically and genetically heterogenous presentations displaying predominantly proximal muscle weakness due to the loss of skeletal muscle fibers. Beta-sarcoglycanopathy (LGMDR4) results from biallelic molecular defects in *SGCB* and features pediatric onset with limb-girdle involvement, often complicated by respiratory and heart dysfunction. Here we describe a patient who presented at the age of 12 years reporting high creatine kinase levels and onset of cramps after strenuous exercise. Instrumental investigations, including a muscle biopsy, pointed towards a diagnosis of beta-sarcoglycanopathy. NGS panel sequencing identified two variants in the *SGCB* gene, one of which (c.243+1548T>C) was found to promote the inclusion of a pseudoexon between exons 2 and 3 in the *SGCB* transcript. Interestingly, we detected the same genotype in a previously reported LGMDR4 patient, deceased more than twenty years ago, who had escaped molecular diagnosis so far. After the delivery of morpholino oligomers targeting the pseudoexon in patient-specific induced pluripotent stem cells, we observed the correction of the physiological splicing and partial restoration of protein levels. Our findings prompt the analysis of the c.243+1548T>C variant in suspected LGMDR4 patients, especially those harbouring monoallelic *SGCB* variants, and provide a further example of the efficacy of antisense technology for the correction of molecular defects resulting in splicing abnormalities.

Keywords: LGMD; *SGCB*; beta-sarcoglycan; morpholino

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1. Introduction

Limb-girdle muscular dystrophies (LGMD) are hereditary disorders characterized by the loss of skeletal muscle fibers, resulting in predominantly proximal muscle weakness at onset. The impressive genetic heterogeneity is acknowledged by the report of mutations in more than 30 genes, displaying dominant and recessive patterns of inheritance, associated with LGMD presentation [1].

Biallelic variants in *SGCA*, *SGCB*, *SGCG*, and *SGCD* encoding for alpha-, beta-, gamma-, and delta-sarcoglycan proteins [2], respectively, are the molecular determinants of rare recessive LGMD forms, collectively termed sarcoglycanopathies (LGMDR3-6, according to the updated nomenclature) [3]. Sarcoglycans interact with the dystroglycan complex, which links the subsarcolemmal protein dystrophin to the basement membrane. In this way, sarcoglycans participate in the maintenance of muscle membrane integrity during muscle fibers' contraction and relaxation process [4]. Indeed, molecular defects in any of

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ORIGINAL ARTICLE

WILEY

MicroRNAs as serum biomarkers in Becker muscular dystrophy

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Abstract

Becker muscular dystrophy (BMD) is an X-linked neuromuscular disorder due to mutation in the *DMD* gene, encoding dystrophin. Despite a wide clinical variability, BMD is characterized by progressive muscle degeneration and proximal muscle weakness. Interestingly, a dysregulated expression of muscle-specific microRNAs (miRNAs), called myomirs, has been found in patients affected with muscular dystrophies, although few studies have been conducted in BMD. We analysed the serum expression levels of a subset of myomirs in a cohort of 29 ambulant individuals affected by BMD and further classified according to the degree of alterations at muscle biopsy and in 11 age-matched healthy controls. We found a significant upregulation of serum miR-1, miR-133a, miR-133b and miR-206 in our cohort of BMD patients, supporting the role of these miRNAs in the pathophysiology of the disease, and we identified serum cut-off levels discriminating patients from healthy controls, confiming the potential of circulating miRNAs as promising noninvasive biomarkers. Moreover, serum levels of miR-133b were found to be associated with fibrosis at muscle biopsy and with patients' motor performances, suggesting that miR-133b might be a useful prognostic marker for BMD patients. Taken together, our data showed that these serum myomirs may represent an effective tool that may support stratification of BMD patients, providing the opportunity of both monitoring disease progression and assessing the treatment efficacy in the context of clinical trials.

KEYWORDS
Becker muscular dystrophy, biomarkers, BMD, miR-133b, miRNA, serum, skeletal muscle

1 | INTRODUCTION

Becker muscular dystrophy (BMD) is a neuromuscular disorder due to in-frame mutations in the *DMD* gene, located on the X chromosome.[1] This gene encodes for dystrophin, whose lack leads to structural damages and disruption of the membrane of skeletal muscles with consequent activation of inflammation and regeneration in the early phases of the disease and increase of connective and

Delia Gagliardi, Mafalda Rizzuti, Francesca Magri and Daniele Velardo equally contributed to this work.

Letter

Molecular analysis of SMARD1 patient-derived cells demonstrates that nonsense-mediated mRNA decay is impaired

INTRODUCTION

Biallelic mutations in the immunoglobulin μ-binding protein 2 (IGHMBP2) gene lead to motor neuron (MN) degeneration in the brain stem and anterior horns of the spinal cord, causing fatal spinal muscular atrophy with respiratory distress type I (SMARD1). Patients exhibit a certain degree of phenotypic variability that has not been explained.[1] No effective therapy is currently available, and understanding the function of IGHMBP2 is crucial for identifying specific disease targets.

IGHMBP2 is a DNA/RNA helicase protein involved in different cellular processes, but its precise function is unknown. IGHMBP2 exhibits similarities to the human regulator of nonsense transcripts homolog UPF1,[2] which is part of the core complex required for nonsense-mediated mRNA decay (NMD), a translation-dependent RNA degradation pathway implicated in different subtypes of amyotrophic lateral sclerosis (ALS).[3]

We analysed fibroblasts, induced pluripotent stem cells (iPSCs), and their derived MNs from eight patients with SMARD1 carrying different IGHMBP2 mutations. All cell types exhibited a marked deficiency in IGHMBP2 protein but not mRNA. We further demonstrated that the IGHMBP2 transcript is regulated by the NMD pathway, which resulted inhibited in SMARD1 condition.

RESULTS

Our eight SMARD1 patients are summarised in online supplemental table 1. The identified mutations included four missense and four nonsense mutations, three point deletions, one inversion and one insertion mutation (figure 1A). We collected peripheral blood mononuclear cells and/or fibroblasts from the patients and three unaffected subjects (online supplemental table 2). We successfully reprogrammed iPSCs from four patients (online supplemental figure 1A) and differentiated them into MNs (online supplemental figure 1B) that exhibited pathological features of increased apoptosis and decreased axon length (online supplemental figure 1C,D).

Western blot analysis of MNs, fibroblasts and iPSCs from patients and controls (online supplemental figure 2) showed three migration bands specific for IGHMBP2 (~110 kDa,~75 kDa and ~55 kDa). Online supplemental figure 3 summarises the data regarding protein isoforms. Only the ~110 kDa band corresponded to the full-length and functioning IGHMPB2 protein[4]; it was significantly reduced in all SMARD1 samples (figure 1B; online supplemental figure 2) and nearly absent in cell lines with nonsense mutations. Immunofluorescence confirmed the western blot data in MNs (figure 1C) and iPSCs (online supplemental figure 4), with no difference in localisation. Interestingly, our analysis suggested that the reduction in IGHMBP2 was not the result of decreased mRNA (figure 1C; online supplemental figure 5A).

To determine whether the upregulation of IGHMBP2 mRNA in SMARD1 was attributable to impaired mRNA turnover, we evaluated the efficacy of IGHMBP2 mRNA decay after transcriptional inhibition in iPSCs. The ratio of mRNA before and after actinomycin D treatment was increased in SMARD1 iPSCs (online supplemental figure 5B), suggesting an impairment of IGHMPB2 transcript degradation. The treatment of SHSY-5Y neuroblastoma cells and control iPSCs with cycloheximide (CHX), which indirectly inhibits NMD by blocking translation, induced an increase of IGHMBP2 mRNA levels suggesting NMD regulation of IGHMBP2 transcript (online supplemental figure 5C).

We observed an increase in the abundance of a set of NMD target genes in SMARD1 MNs (figure 1E) and iPSCs (online supplemental figure 6A) compared with controls. Remarkably, the NMD-activating compound tranilast significatively decreased IGHMBP2 expression in SMARD1 MNs (figure 1G), and iPSCs (online supplemental figure 5D) and rescued the mRNA accumulation of some NMD targets both in MNs (figure 1F) and in iPSCs (online supplemental figure 6B). Importantly, NMD reactivation was also able to significantly rescue pathological MN hallmarks (figure 1H1). Moreover, in control iPSCs, NMD inhibition by CHX induced a variable increase in NMD-sensitive transcript isoforms (hnRNPL and TRA2B), which was less steep in SMARD1 iPSCs (online supplemental figure 7).

DISCUSSION

SMARD1 is a rare but fatal disease with onset in early childhood. It affects the lower MNs, causing distal limb paralysis and respiratory distress. In the present study, we described eight new SMARD1 cases and reported updated data for two previously described cases. Given the rarity of this disease, this represents a substantial cohort of SMARD1 patients. Our results confirmed reduced expression of full-length IGHMBP2 protein (to<5%) in all cell types. In cases involving nonsense mutations, IGHMBP2 was absent, whereas the protein was mildly reduced in the presence of a missense mutation.

We also demonstrated that very low IGHMBP2 protein generally predicts a severe phenotype. However, SMARD1 patients did not have significantly reduced IGHMBP2 mRNA levels, confirming previous findings.[1] We demonstrated that IGHMPB2 mRNA is regulated by NMD, a mechanism that eliminates mRNAs containing premature translation–termination codons, but also regulates the expression of a large number of genes and that NMD is impaired in SMARD1. Several mRNAs that are normally target of NMD were upregulated in SMARD1 iPSCs and MNs, and these changes were rescued by NMD pathway reactivation. Thus, NMD is emerging as a critical regulator of neuronal development, MN viability and axon growth. Insufficient NMD may indeed underlie neurodegeneration, such as in ALS.[3 5] Therefore, it is conceivable that NMD deficiency represents a pathogenic mechanism for SMARD1, causing accumulation of aberrant defective mRNA to which MNs are particularly sensitive. NMD rescue can reestablish the mRNA balance in MNs improving their pathological phenotype. Importantly, reactivation of the NMD pathway was able to rescue axon length and apoptosis in affected MNs, supporting the NMD pathway as a potential target, as previously suggested for other MN disorders.[3] Therefore, further investigations of drugs that can rescue NMD activity for potential therapeutic use in inherited motor neuropathies that share the same pathological molecular mechanism are warranted.

METHODS

Cell culture

Human samples were reprogrammed into iPSCs using the CytoTune-iPS V.2.0 Sendai Reprogramming Kit (Life Technologies). Spinal MNs were then obtained following a rapid multistage protocol.

The iPSCs and/or MNs were used in standard western blot analysis, and underwent immunohistochemistry for anti-human IGHMBP2 (Millipore) and

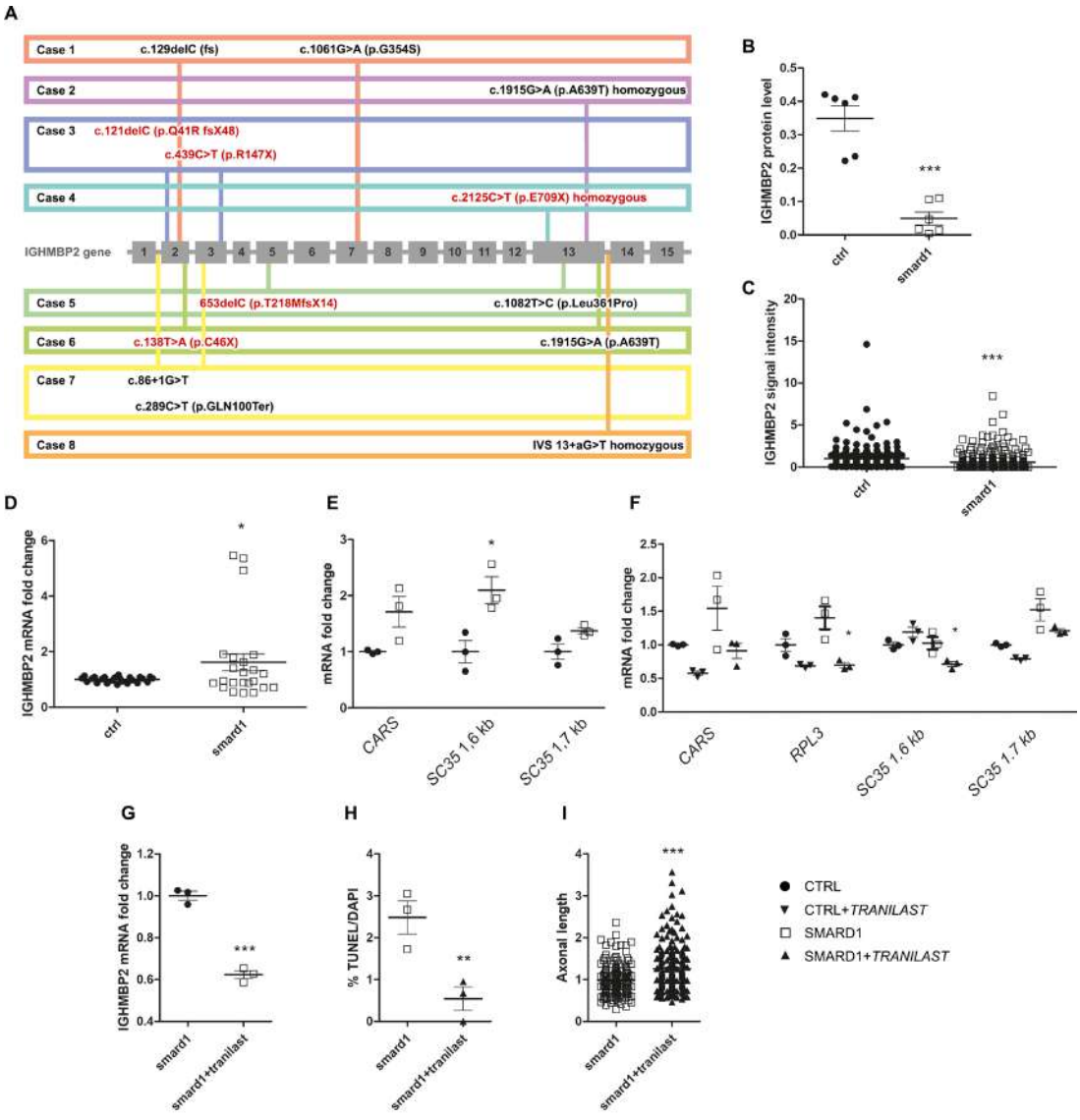

Figure 1 IGHMBP2 levels and nonsense-mediated mRNA decay (NMD) decay in hiPSC-derived motor neurons (MNs). (A) Schematic representation of the distribution along the immunoglobulin μ-binding protein 2 (IGHMBP2) gene of mutations found in the patient cohort. STOP codon mutations are indicated in red. (B) 110 kDa IGHMBP2 protein, assessed by western blot, decrease in spinal muscular atrophy with respiratory distress type I (SMARD1) MNs versus ctrl (***p<0.001, Student's t-test). (C) Immunocytochemistry quantification confirmed lower levels of IGHMBP2 in affected MNs ***p<0.001, *p<0.05, Student's t-test, ctrl versus patients (smard1, case 2, 3 and 6). (D) qPCR analyses of IGHMBP2 mRNA levels in affected MNs showed no correlation with protein level reduction, increasing in SMARD1 lines,*p<0.05, Student's t-test, ctrl versus patients. (E) mRNA levels of NMD target genes were increased in SMARD1 MNs versus ctrl (**p<0.01, Student's t-test). (F) RNA levels of NMD target genes were decreased after tranilast treatment in SMARD1 MNs (smard1, case 2, 3 and 6) versus ctrl (*p<0.05, Student's t-test). (G) mRNA levels of IGHMBP2 were rescued after treatment with tranilast (5 μM) in SMARD1 MNs, ***p<0.001, Student's t-test. (H,I) Treatment with the activator of NMD tranilast (5 μM) rescued pathological hallmarks of SMARD1 MNs (smard1, case 2, 3 and 6), namely apoptosis evaluated by tunel assay (E, **p<0.01, Student's t-test) and axon length reduction (F, ***p<0.001, Student's t-test). In B, E–H, each data point represents the mean obtained from three technical replicates for each biological replicate (n=3, smard1: case 2, 3 and 6). In D, each data point represents a technical replicate (biological replicates n=3 for ctrl, n=4 for smard1, case 2, 3, 6 and 7). In C and I, each point represents data from a single cell. Values are presented±SEM. All the images are original and made by the authors.

SMI-32 (Millipore) and the terminal deoxynucleotidyl transferase dUTP nick end labelling system protocol (Promega). IGHMBP2 expression was evaluated by standard TaqMan qPCR assay. For transcripts known to be regulated by NMD, SYBR Green Real Time PCR was used.

The iPSCs and/or MNs were treated with transcription inhibitor actinomycin D at 2.5 μg/mL, with 100 μg/mL CHX for 6 hour and 5 μM tranilast (T0318-10MG) for 24 hours.

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TWNK in Parkinson's Disease: A Movement Disorder and Mitochondrial Disease Center Perspective Study

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ABSTRACT: Background: Parkinsonian features have been described in patients harboring variants in nuclear genes encoding for proteins involved in mitochondrial DNA maintenance, such as *TWNK*.
Objectives: The aim was to screen for *TWNK* variants in an Italian cohort of Parkinson's disease (PD) patients and to assess the occurrence of parkinsonism in patients presenting with *TWNK*-related autosomal dominant progressive external ophthalmoplegia (*TWNK*-adPEO).
Methods: Genomic DNA of 263 consecutively collected PD patients who underwent diagnostic genetic testing was analyzed with a targeted custom gene panel including *TWNK*, as well as genes causative of monogenic PD. Genetic and clinical data of 18 *TWNK*-adPEO patients with parkinsonism were retrospectively analyzed.
Results: Six of 263 PD patients (2%), presenting either with isolated PD (n = 4) or in combination with bilateral ptosis (n = 2), carried *TWNK* likely pathogenic variants. Among 18 *TWNK*-adPEO patients, 5 (28%) had parkinsonism.
Conclusions: We show candidate *TWNK* variants occurring in PD without PEO. This finding will require further confirmatory studies. © 2022 Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson Movement Disorder Society.

Key Words: *TWNK*; twinkle; Parkinson's disease; parkinsonism; mitochondrial DNA

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Pathogenesis of Parkinson's disease (PD) has long been associated with mitochondrial dysfunction.[1] Dopaminergic neurons of the substantia nigra pars compacta seem to be particularly vulnerable to mitochondrial damage.[2] Although sequencing of mitochondrial DNA (mtDNA) failed to reveal pathogenic mutations associated with PD, population-specific common variants defining mtDNA haplogroups have been implicated as possible risk factors.[3] In addition, age-related accumulation of somatic mtDNA deletions in the substantia nigra has been reported to occur more significantly in PD patients than in age-matched controls.[4,5] Moreover, the regulation of mtDNA copy number seems to be affected in PD, leading to a relative mtDNA depletion.[6,7]

CLINICAL/SCIENTIFIC NOTE OPEN ACCESS

Expanding the Phenotypic Spectrum of Vocal Cord and Pharyngeal Weakness With Distal Myopathy due to the p.S85C MATR3 Mutation

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Abstract

Objectives

The c.254C>G (p.S85C) *MATR3* variant causes vocal cord and pharyngeal weakness with distal myopathy (VCPDM), which is characterized by progressive, asymmetric, predominantly distal muscle weakness, dysphonia, dysphagia, and respiratory impairment. Herein, we describe an Italian patient who harbored the p.S85C MATR3 variant and showed a composite phenotype of VCPDM and sensorimotor polyneuropathy.

Methods

The proband underwent neurologic evaluation, muscular MRI of the lower limbs, neurophysiologic assessment, muscle biopsy, and spirometry. After excluding common acquired and genetic causes of sensorimotor polyneuropathy, a larger group of genes involved in inherited forms of neuropathy, distal myopathy, and motor neuron disorders were analyzed by next-generation sequencing targeted panels.

Results

The patient, affected by progressive distal muscle weakness and hypotrophy, myalgias, dysphonia, dysphagia, respiratory impairment, and sensory abnormalities, harbored the heterozygous c.254C>G (p.S85C) *MATR3* substitution. Neurophysiologic assessment revealed a severe sensorimotor polyneuropathy. Variation of fiber size, central nuclei, and nonrimmed vacuoles were evident at muscle biopsy.

Discussion

This finding extends the *MATR3*-associated VCPDM phenotypic spectrum and suggests considering *MATR3* analysis in suspected congenital polyneuropathies with odd features, including dysphonia, dysphagia, and respiratory insufficiency.

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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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Prevalence of Spinal Muscular Atrophy in the Era of Disease-Modifying Therapies: An Italian Nationwide Survey

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ANNALS of Clinical and Translational Neurology
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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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Peculiar histological and ultrastructural skeletal muscle alterations in a patient with CMV infection and autoimmune myositis: case evaluation and brief literature review

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We report the case of a young woman with CMV infection, high level of creatine kinase and myopathy. Electromyography showed a myopathic pattern. Muscle biopsy showed a marked increase of NADH enzymatic activity in the central area of almost all type I fibres, few degenerative and necrotic fibres and scattered mononuclear cell infiltrates. Ultrastructural analysis showed a marked disarrangement of sarcomeric structure and large inclusions of thin filaments in some fibres, while immunohistochemistry evidenced alteration in desmin, actin and αB-crystallin protein signals. PCR for CMV detection on muscle sections was negative. Histological, immunological and ultrastructural evaluations were compatible with a necrotic inflammatory myopathy. The correlations between CMV liver infection and the myopathic pattern are discussed. This case underscores the need to consider CMV infection in the differential diagnosis of myopathy with undetermined aetiology, quickly providing directions for a targeted muscle pharmacological intervention.

Key words: CMV, muscle biopsy, myofibrillar disorganization, Z-band streaming

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Introduction

Viral infections have been frequently reported in association with development of secondary myopathies characterized by different forms of muscle involvement that can vary from mild to severe inflammatory myopathy. Literature reported evidences of nemaline myopathy and myositis after human immunodeficiency infection (HIV) [1], myositis after infection by hepatitis B and C [2], Epstein-Barr virus [3], herpes simplex virus [4] and, less frequently, cytomegalovirus (CMV) [5]. Few cases of severe rhabdomyolysis in association with CMV infection [6,7], and a case of polymyositis associated with primary CMV infection were reported [5].

Herein, we describe the case of a young woman with hepatitis by primary CMV infection, muscle weakness, myalgia, oedema and increased serum creatine kinase (CK) levels associated with severe and marked structural alterations in skeletal muscle, whose symptoms improved after

ralysis probably associated with CMV infection was reported [7].

Although the presence of viral particles has not been confirmed in skeletal muscle by real-time PCR or immunohistochemistry, and the mechanism through which the virus could affect skeletal muscle is still unknown, we can hypothesize that the CMV infection has caused the observed alterations in skeletal muscle as an indirect host-derived effect. Indeed, besides the direct viral liver infection, indirect effects probably mediated by the immunological response can cause detrimental consequences including skeletal muscle alterations [9]. However, a possible direct viral muscle infection cannot be completely excluded. Indeed, viral count could have remained below threshold detection level due to methodological limits and/or very low (latent) viral activity when PCR was performed. Ultrastructural changes have been reported in different types of CMV-infected cells as direct effects: in human bone marrow fibroblasts, mitochondrial enlargement, production of dense bodies and cytoplasmic accumulation were observed [10]. During in vitro CMV infection, a rapid and progressive alteration of actin, microfilaments and cytoskeleton was observed in both human embryo and lung fibroblasts [11], however what happens in cells and tissues not directly invaded by the virus is still poorly understood.

The clear improvement of the electromyographic pattern following the acute phase, confirms the non-primary nature of the myopathy.

To summarize, several issues – clinical presentation, serological and neurophysiological evidence, skeletal muscle findings and progressive improvement of clinical and instrumental parameters after therapy – favour the hypothesis of an autoimmune/inflammatory myopathy. We could not demonstrate the presence of viral particles in skeletal muscle, but, as explained, an indirect host-derived effect is likely implicated, not to mention concomitance between liver infection and onset of myopathic symptoms.

The study of this case has an important implication for the medical internist approach towards primary CMV viral infection; indeed, the presence of symptoms induced by viral hepatitis could cause underestimation of severe effects on other tissues/organs including skeletal muscle.

Also, our report underscores the need to consider CMV infection in the differential diagnosis of myopathy with undetermined aetiology, providing directions for a targeted muscle pharmacological intervention.

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Conflict of interest statement

The Authors declare no conflict of interest

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Authors' contributions

MR and SZ wrote the manuscript, MR, LN, MS, SZ interpreted the results, revised the literature and revised the manuscript. VM performed clinical evaluation.

Ethical consideration

All procedures were in accordance with the standards of the bioethical committee and the Declaration of Helsinki.

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7 Hirohama D, Shimizu T, Hashimura K, et al. Reversibile respiratory failure due to rhabdomyolysis associated with cytomegalo-

Immunofluorescence signal intensity measurements as a semi-quantitative tool to assess sarcoglycan complex expression in muscle biopsy

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ABSTRACT

Sarcoglycanopathies are highly heterogeneous in terms of disease progression, muscular weakness, loss of ambulation and cardiac/respiratory involvement. Their clinical severity usually correlates with the residual protein amount, which makes protein quantification extremely relevant. Sarcoglycanopathy diagnosis is genetic, but skeletal muscle analysis - by both immunohistochemistry and Western blot (WB) - is still mandatory to establish the correct diagnostic process. Unfortunately, however, WB analysis cannot be performed if the bioptic specimen is scarce. This study provides a sensitive tool for semi-quantification of residual amount of sarcoglycans in patients affected by sarcoglycanopathies, based on immunofluorescence staining on skeletal muscle sections, image acquisition and software elaboration. We applied this method to eleven sarcoglycanopathies, seven Becker muscular dystrophies, as pathological control group, and four age-matched controls. Fluorescence data showed a significantly reduced expression of the mutated sarcoglycan in all patients when compared to their respective age-matched healthy controls, and a variable reduction of the other sarcoglycans. The reduction is due to the effect of gene mutation and not to the increasing age of controls. Fluorescence normalized data analyzed in relation to the age of onset of the disease, showed a negative correlation of α-sarcoglycan fluorescence signal *vs* fibrosis in patients with an early age of onset and a negative correlation between δ-sarcoglycan signal and fibrosis in both intermediate and late age of onset groups. The availability of a method that allows objective quantification of the sarcolemmal proteins, faster and less consuming than WB analysis and able to detect low residual sarcoglycan expression with great sensitivity, proves useful also in view of possible inferences on disease prognosis. The proposed method could be employed also to monitor the efficacy of therapeutic interventions and during clinical trials.

Key words: sarcoglycans; immunofluorescence; protein quantification; histology; fibrosis.

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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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Analysis of miRNA rare variants in amyotrophic lateral sclerosis and *in silico* prediction of their biological effects

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Background: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and/or lower motor neurons and characterized by complex etiology. Familial cases show high genetic heterogeneity and sporadic cases (90%) are associated with several genetic and environmental risk factors. Among the genetic risk factors, the contribution of non-coding elements, such as microRNAs (miRNAs), to ALS disease susceptibility remains largely unexplored.

Aim: This work aims to identify rare variants in miRNA genes in sporadic ALS (sALS) patients which may cause a defective miRNA maturation or altered target gene recognition by changing miRNA secondary structure or seed sequence, respectively.

Methods: Rare variants located in miRNA loci with a minor allele frequency (MAF) < 0.01 were extracted from whole genome sequencing (WGS) data of 100 sALS patients. The secondary pre-miRNA structures were predicted using MiRVas to evaluate the impact of the variants on RNA folding process. Human TargetScan was used to retrieve all the potential target genes of miRNAs with variants in the seed region. Over Representation Analysis (ORA) was conducted to compare the lists of target genes for the reference and mutated miRNAs in the seed sequence.

Results: Our analysis identified 86 rare variants in 77 distinct miRNAs and distributed in different parts of the miRNA precursors. The presence of these variants changed miRNA secondary structures in ~70% of MiRVas predictions. By focusing on the 6 rare variants mapping within the seed sequence, the predicted target genes increased in number compared to the reference miRNA and included novel targets in a proportion ranging from 30 to 82%. Interestingly,

Bridging the Gap: Gene Therapy in a Patient With Spinal Muscular Atrophy Type 1

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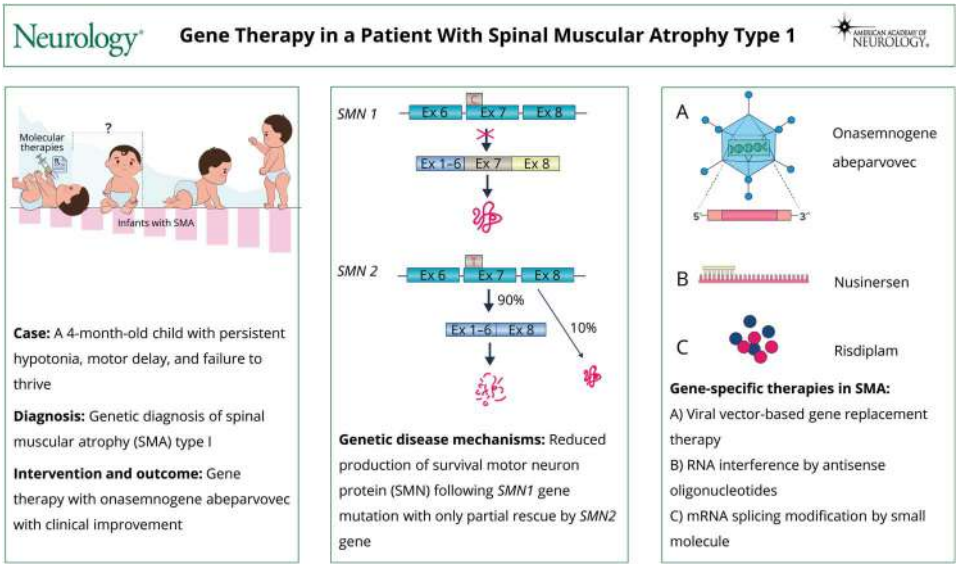

Abstract

Molecular therapies exploit the understanding of pathogenic mechanisms to reconstitute impaired gene function or manipulate flawed RNA expression. These therapies include (1) RNA interference by antisense oligonucleotides, (2) mRNA modification using small molecules, and (3) gene replacement therapy, the viral-mediated intracellular delivery of exogenous nucleic acids to reverse a genetic defect. Several molecular therapies are approved for treating spinal muscular atrophy (SMA), a recessive genetic disorder caused by survival motor neuron (*SMN*)*1* gene alterations. SMA involves degeneration of lower motor neurons, which leads to progressive muscle weakness, hypotonia, and hypotrophy. Onasemnogene abeparvovec is a gene replacement therapy for SMA that uses adeno-associated virus delivery of functional *SMN1* cDNA to motor neurons. Two other molecular therapies modulate *SMN2* transcription: nusinersen, an antisense oligonucleotide, and risdiplam, a small molecule designed to modify faulty mRNA expression. The most suitable individualized treatment for SMA is not established. Here, we describe remarkable clinical improvement in a 4-month-old patient with SMA type 1 who received onasemnogene abeparvovec therapy. This case represents an explanatory bridge from bench to bedside with regard to therapeutic approaches for genetic disorders in neurology. Knowledge of the detailed mechanisms underlying genetic neurologic disorders, particularly monogenic conditions, is paramount for developing tailored therapies. When multiple disease-modifying therapies are available, early genetic diagnosis is crucial for appropriate therapy selection, highlighting the importance of early identification and intervention. A combination of drugs, each targeting unique genetic pathomechanisms, may provide additional clinical benefits.

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ORIGINAL ARTICLE

WILEY

MicroRNAs as serum biomarkers in Becker muscular dystrophy

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Abstract

Becker muscular dystrophy (BMD) is an X-linked neuromuscular disorder due to mutation in the *DMD* gene, encoding dystrophin. Despite a wide clinical variability, BMD is characterized by progressive muscle degeneration and proximal muscle weakness. Interestingly, a dysregulated expression of muscle-specific microRNAs (miRNAs), called myomirs, has been found in patients affected with muscular dystrophies, although few studies have been conducted in BMD. We analysed the serum expression levels of a subset of myomirs in a cohort of 29 ambulant individuals affected by BMD and further classified according to the degree of alterations at muscle biopsy and in 11 age-matched healthy controls. We found a significant upregulation of serum miR-1, miR-133a, miR-133b and miR-206 in our cohort of BMD patients, supporting the role of these miRNAs in the pathophysiology of the disease, and we identified serum cut-off levels discriminating patients from healthy controls, confiming the potential of circulating miRNAs as promising noninvasive biomarkers. Moreover, serum levels of miR-133b were found to be associated with fibrosis at muscle biopsy and with patients' motor performances, suggesting that miR-133b might be a useful prognostic marker for BMD patients. Taken together, our data showed that these serum myomirs may represent an effective tool that may support stratification of BMD patients, providing the opportunity of both monitoring disease progression and assessing the treatment efficacy in the context of clinical trials.

KEYWORDS
Becker muscular dystrophy, biomarkers, BMD, miR-133b, miRNA, serum, skeletal muscle

1 | INTRODUCTION

Becker muscular dystrophy (BMD) is a neuromuscular disorder due to in-frame mutations in the *DMD* gene, located on the X chromosome.[1] This gene encodes for dystrophin, whose lack leads to structural damages and disruption of the membrane of skeletal muscles with consequent activation of inflammation and regeneration in the early phases of the disease and increase of connective and

Delia Gagliardi, Mafalda Rizzuti, Francesca Magri and Daniele Velardo equally contributed to this work.

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Lab Resource: Multiple Cell Lines

Generation of two hiPSC lines (UMILi027-A and UMILi028-A) from early and late-onset Congenital Central hypoventilation Syndrome (CCHS) patients carrying a polyalanine expansion mutation in the *PHOX2B* gene

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A B S T R A C T

Congenital Central Hypoventilation Syndrome (CCHS) is a rare disorder of the autonomic nervous system (ANS), characterized by inadequate control of autonomic ventilation and global autonomic dysfunction. Heterozygous polyalanine repeat expansion mutations in exon 3 of the transcription factor Paired-like homeobox 2B (*PHOX2B*) gene occur in 90% of CCHS cases. In this study, we describe the generation and characterization of two human induced pluripotent stem cell (hiPSC) lines from female CCHS patients carrying a heterozygous + 5 alanine expansion mutation. The generated iPSC lines show a normal karyotype, express pluripotency markers and are able to differentiate into the three germ layers.

Resource Table

Unique stem cell lines identifier	1. UMILi027-A 2. UMILi028-A
Alternative name(s) of stem cell lines	N/A
Institution	Department of Medical Biotechnology and Translational Medicine (BIOMETRA), Università degli Studi di Milano, Milan, Italy.
Contact information of distributor	Diego Fornasari diego.fornasari@unimi.it
Type of cell lines	iPSC
Origin	Human
Additional origin info required	UMILi027-A Age: 40 years Sex: F Ethnicity: Central European UMILi028-A Age: 24 years Sex: F Ethnicity: Central European
Cell Source	Skin fibroblasts
Clonality	Mixed
Associated disease	Congenital Central Hypoventilation Syndrome (CCHS)

(*continued on next column*)

(*continued*)

(*continued on next page*)

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Short communication

A Bayesian approach to Essential Tremor plus: A preliminary analysis of the TITAN cohort

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ABSTRACT

Background: The construct of Essential Tremor plus (ET-plus) refers to patients who also have rest tremor and/or mild neurologic signs of unknown significance. It is unclear whether soft signs represent confounding factors or are useful in suspecting an alternative condition.

Methods: Using a Bayesian approach to ET-plus patients recruited in The ITAlian tremor Network (TITAN), we analyzed the probability that these patients do not have ET.

Results: The data of 274 ET-plus patients were extracted from the TITAN database. The majority of patients (240/274; 87.5%) had a single soft sign. The post-test probability of not having ET was different according to the specific soft sign: namely, 0.64 (rest tremor); 0.46 (questionable dystonia); 0.85 (questionable bradykinesia); 0.19 (soft gait impairment); and 0.09 (questionable cognitive issues). In patients with multiple soft signs, the post-test probability of not having ET was higher than 50% for 7 out of 11 combinations, accounting for 44.1% of subjects. Overall, the post-test probability of not having ET was higher than 50% in up to 71.5% of ET-plus patients.

Discussion: We have here shown that: 1) the soft signs differently contribute in modulating the probability that a patient does not have ET; and 2) the effect of multiple soft signs are not always addictive. Future studies are needed to collect prevalence figures of soft signs in different neurological disorders as well as in the elderly and to calculate their value in predicting the development of an alternative tremor syndrome.

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Review

A Practical Approach to Early-Onset Parkinsonism

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Abstract. Early-onset parkinsonism (EO parkinsonism), defined as subjects with disease onset before the age of 40 or 50 years, can be the main clinical presentation of a variety of conditions that are important to differentiate. Although rarer than classical late-onset Parkinson's disease (PD) and not infrequently overlapping with forms of juvenile onset PD, a correct diagnosis of the specific cause of EO parkinsonism is critical for offering appropriate counseling to patients, for family and work planning, and to select the most appropriate symptomatic or etiopathogenic treatments. Clinical features, radiological and laboratory findings are crucial for guiding the differential diagnosis. Here we summarize the most important conditions associated with primary and secondary EO parkinsonism. We also proposed a practical approach based on the current literature and expert opinion to help movement disorders specialists and neurologists navigate this complex and challenging landscape.

Keywords: Parkinsonian disorders, Parkinson's disease, autosomal recessive early-onset, secondary Parkinson's disease, dopa-responsive dystonia, adult-onset dystonia-parkinsonism, genetic counseling

INTRODUCTION

The term "early-onset Parkinson's disease" (EO PD, or young-onset PD - YOPD) refers to cases of PD with onset between the age of 21 and 40 years, as reported by Quinn et al. in their seminal paper from 1987, or between 21 and 50 years, according to other authors [1–4]. Compared with idiopathic cases of PD (iPD), patients with EOPD usually present a slower progression of the motor symptoms, a prevalence of bradykinesia over tremor, focal dystonia at onset or during off-state, satisfactory response even to low doses of levodopa, earlier motor complications (such as motor fluctuations and dyskinesias), a lower incidence of cognitive impairment and non-motor symptoms, while anxiety and depression are frequent [1, 5–16]. A positive family history can be often identified in these patients, suggesting an important role of genetics in the pathogenesis of several of these cases [15, 16].

Based on retrospective observational studies, classical EOPD accounts for about 3–7% of all cases of PD in the Western world and up to 10–14% in Japan [1, 3, 17–19], with an incidence between 0.29 and 3.3 per 100,000 persons-years in the current literature [8, 17, 20, 21].

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CASE REPORT
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Case Report: Effect of Targeted Therapy With Carbamazepine in KCNQ2 Neonatal Epilepsy

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We present a family case of neonatal-onset *KCNQ2*-related epilepsy due to a novel intronic mutation. Three members of an Italian family (father and offspring) presented with neonatal-onset asymmetric tonic and clonic seizures with peculiar video-electroencephalography and aEEG features referring to sequential seizures. The father and the first son underwent standard of care treatments in line with current neonatal intensive care unit protocols, with a prolonged hospitalization before reaching full seizure control with carbamazepine. After the experience acquired with her family and the latest advances in the literature, the younger daughter was directly treated with carbamazepine, obtaining rapid seizure control and short hospitalization. They all had normal development. Carbamazepine is rarely administered as a first-line option in neonatal seizures. Recent evidence suggests that neonatal intensive care unit protocols should implement a trial with sodium channel blockers such as carbamazepine as first-option anti-seizure medication and a fast access to genetic testing in neonates with sequential seizures without structural brain injury or acute causes. Moreover, we report and discuss the laboratory studies performed on a novel causative intronic mutation in *KCNQ2* (c.1525+5 G>A in IVS13), since pathogenicity may be difficult to prove for intronic variants.

Keywords: *KCNQ2, SCN2A*, self-limited neonatal epilepsy, carbamazepine (CBZ), developmental and epileptic encephalopathy (DEE), EEG, sodium channel blocker, intronic mutation

INTRODUCTION

Seizures are the most frequent neurological sign observed in the neonatal intensive care unit (NICU) (1), and according to etiology, seizures can be classified into structural, metabolic, toxic, infectious, and genetic (2). Early recognition of the specific etiology has a significant impact on therapeutic management of neonatal seizures and neonatal epilepsies.

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RESEARCH

Open Access

Clinical uses of Bupropion in patients with Parkinson's disease and comorbid depressive or neuropsychiatric symptoms: a scoping review

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Abstract

Objective: Bupropion, an antidepressant inhibiting the reuptake of dopamine and noradrenaline, should be useful to treat depressive symptoms in patients with Parkinson's disease (PD). Limited and conflicting literature data questioned its effectiveness and safety in depressed PD patients and extended its use to other neuropsychiatric symptoms associated with this disorder.

Design: The databases PubMed, Embase, Web of Sciences, Cochrane Library, and the grey literature were searched. Following a scoping review methodology, articles focusing on Bupropion uses in PD patients who manifested depressive or other neuropsychiatric alterations were reviewed.

Results: Twenty-three articles were selected, including 7 original articles, 3 systematic reviews or meta-analyses, 11 case reports, 1 clinical guideline, and 1 expert opinion. Bupropion showed considerable effectiveness in reducing depressive symptoms, particularly in relation to apathy. Solitary findings showed a restorative effect on compulsive behaviour secondary to treatment with dopamine as well as on anxiety symptoms. The effect on motor symptoms remains controversial. The safety profile of this medication seems positive, but additional precautions should be used in subjects with psychotic symptoms.

Conclusion: The available literature lacks good evidence to support the use of Bupropion in PD patients presenting depressive symptoms. Further investigations are needed to extend and confirm reported findings and to produce accurate clinical guidelines.

Keywords: Bupropion, Parkinson's disease, Depression, Neuropsychiatric symptoms, Pharmacological treatment

Background

Motor symptoms are the cardinal manifestation of Parkinson's disease (PD), however, the clinical picture typically also manifests with non-motor symptoms like neuropsychiatric alterations, autonomic dysfunctions, sleep disturbances, sensory deficits, and cognitive impairment [1, 2]. Non-motor symptoms often anticipate the diagnosis of PD and their underrecognition might lead to delay in the correct diagnosis and treatment [3]. Additionally, the frequent overlap between neurological and psychiatric symptoms complicates the course of the illness and remains a real challenge in terms

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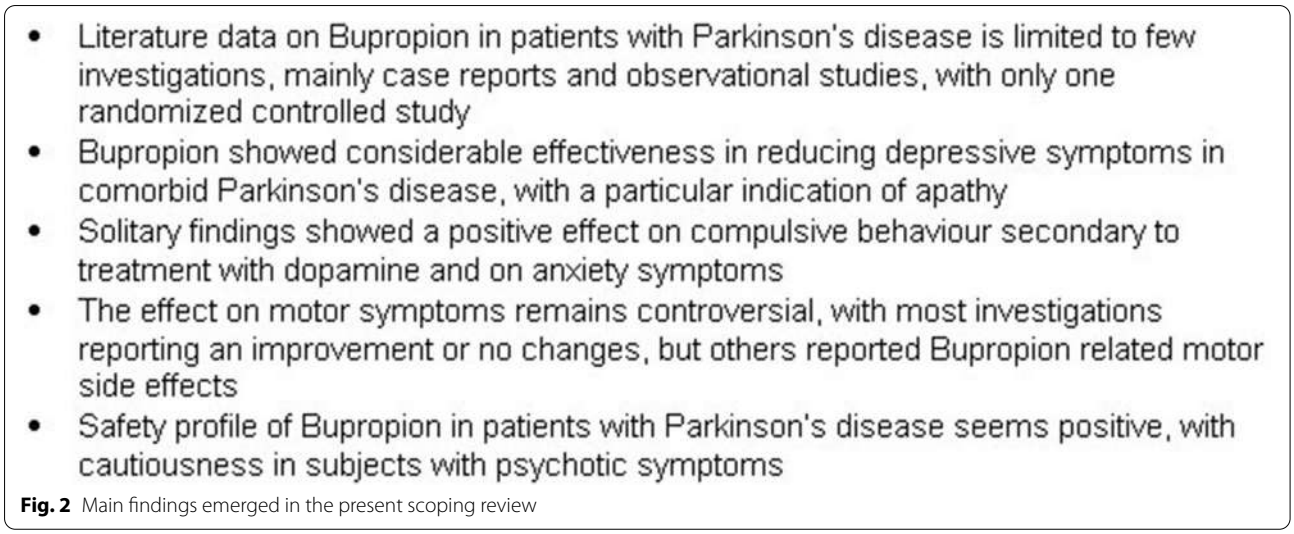

Fig. 2 Main findings emerged in the present scoping review

Conclusion

The present scoping review sought to provide a comprehensive and updated overview of Bupropion clinical uses in patients with PD who manifested depression or other neuropsychiatric symptoms. Figure 2 describes the main findings and related recommendations that emerged from the present work.

Considering the current literature limitations and the scarce number of patients with non-motor symptoms treated with Bupropion, it was not possible to stratify them according to specific disease variables, like severity, duration, or pharmacotherapy. However, we tentatively delineated a patient's profile more suitable for treatment with Bupropion. Patients with PD and depressive symptoms in particular apathy seem to favor the use of this medication, which should preferably not be used in subjects who present a history of psychosis and in ones with a long history of PD or unstable response to treatment with dopamine.

Considering the unique mechanism of action of the medication and the encouraging results emerged in the present scoping review, further investigations in this area, in particular RCTs with larger sample sizes, are encouraged and needed to overcome current literature limitations and to better understand the efficacy and safety profile of the compound in this specific population.

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Authors' contributions

All authors were involved in drafting the manuscript and agreed to its publication. All authors read and approved the final version of the manuscript.

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Availability of data and materials

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Declarations

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Not applicable.

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Competing interests

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CORRECTION

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Originally, the article was published with an error. The affiliation of the author Giulia Paparella should only be " Neuromed Institute IRCCS, Pozzilli, IS, Italy".

The original article has been corrected.

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ORIGINAL RESEARCH
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Dysautonomia in Parkinson's Disease: Impact of Glucocerebrosidase Gene Mutations on Cardiovascular Autonomic Control

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Evidence from clinical practice suggests that PD patients with the Glucocerebrosidase gene mutations (GBA-PD) are characterized by more severe dysautonomic symptoms than patients with idiopathic PD (iPD). Therefore, an accurate assessment of cardiovascular autonomic control (CAC) is necessary to clarify the role of GBA mutations in the pathophysiology of PD. We evaluated the CAC at rest and during orthostatic challenge of 15 iPD, 15 GBA-PD and 15 healthy controls (CTR). ECG and respiration were recorded in supine position and during active standing. The analysis of Heart Rate Variability (HRV) was performed on ECG recordings using two different approaches, linear spectral analysis and non-linear symbolic analysis. GBA-PD patients presented more frequently an akinetic-rigid phenotype and cognitive dysfunction than iPD patients. Both iPD and GBA-PD group were characterized by a lower spectral HRV than CTR group. At rest, the GBA-PD group was characterized by a lower parasympathetic modulation and a shift of the sympathovagal balance toward a sympathetic predominance compared to the CTR group. Moreover, the GBA-PD patients presented a lower HR increment and a lower or absent reduction of the vagal modulation in response to the active standing than iPD patients. Lastly, the cardiovascular autonomic dysfunction in PD patients was associated with longer disease duration, and with the occurrence of REM sleep behavior disorder and constipation. Our findings suggest a more severe impairment of the CAC in PD patients with GBA mutations. These results and further studies on the role of GBA mutations could allow a stratification based on cardiovascular risk in PD patients and the implementation of specific prevention programs.

Keywords: Parkinson's Disease, glucocerebrosidase gene mutations, cardiovascular autonomic control, dysautonomia, heart rate variability (HRV)

Case report

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Genetic evaluation in phenotypically discordant monozygotic twins with Coats Disease

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Abstract

Purpose: To report the unique case of a pair of phenotypically discordant monozygotic twins, with one of them affected by unilateral Coats disease.

Case report: Both patients underwent a complete ophthalmologic evaluation and were genetically tested with whole-exome sequencing (WES). Any known or unknown potential genetic determinant of Coats disease wasn't found.

Conclusion: It may suggest a non-genetic etiology for this disorder. This represents, to the best of our knowledge, the first case of genetic analysis of monozygotic twins, one of whom is affected by Coats disease. Further studies are warranted, including performing genetic analysis directly on retinal biopsy tissue.

Keywords

Coats disease, Coats, genetic, monozygotic twins, genetic analysis, genetic evaluation, phenotypically discordant monozygotic twins, retinal telangiectasia, idiopatic retinal vasculopathy

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Introduction

Coats disease is an idiopathic retinal vasculopathy characterized by retinal telangiectasia, intraretinal or subretinal exudation, micro and macro-aneurysm, and exudative retinal detachment.[1] Vascular abnormalities are more common in the peripheral retina, and exudation occurs mostly in the macular area.[2] Coats disease can manifest at any age, but the majority of patients are children with a diagnosis in their first or second decades of life.[3] It's a rare disease, with an incidence estimated at 0.09 per 100,000 population in the UK.[4] It occurs predominantly in males without any ethnic differences. This disease is usually unilateral, with a bilateral manifestation in less than 10% of cases.[2] In the last decades more sophisticated diagnostic techniques[2,5] and treatments of Coats disease have been proposed. Vitreoretinal or subretinal/external drainage surgery, laser photocoagulation,[6] and periocular and/or intravitreal medications have led to a reduction in the need for enucleation, especially in advanced-stage Coats disease.[1] Coats disease is usually not associated with systemic disease and its genetic etiology is still debated. Several candidate gene mutations have been described, including the Norrie Disease Protein (*NDP*),[7] *CRB1*,[8] *PANK2*,[9] *TERC*,[10] *ABCD4*.[11] In addition, the hypothesis of a somatic mutation has been proposed in the years given the congenital, nonfamilial, and unilateral features of the disease[7]

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ARTICLE OPEN

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LRRK2 kinase activity regulates GCase level and enzymatic activity differently depending on cell type in Parkinson's disease

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Leucine-rich repeat kinase 2 (LRRK2) is a kinase involved in different cellular functions, including autophagy, endolysosomal pathways, and immune function. Mutations in LRRK2 cause autosomal-dominant forms of Parkinson's disease (PD). Heterozygous mutations in GBA1, the gene encoding the lysosomal enzyme glucocerebrosidase (GCase), are the most common genetic risk factors for PD. Moreover, GCase function is altered in idiopathic PD and in other genetic forms of the disease. Recent work suggests that LRRK2 kinase activity can regulate GCase function. However, both a positive and a negative correlation have been described. To gain insights into the impact of LRRK2 on GCase, we performed a comprehensive analysis of GCase levels and activity in complementary LRRK2 models, including (i) LRRK2 G2019S knock in (GSKI) mice, (ii) peripheral blood mononuclear cell (PBMCs), plasma, and fibroblasts from PD patients carrying LRRK2 G2019S mutation, (iii) patient iPSCs-derived neurons; (iv) endogenous and overexpressed cell models. In some of these models we found a positive correlation between the activities of LRRK2 and GCase, which was further confirmed in cell lines with genetic and pharmacological manipulation of LRRK2 kinase activity. GCase protein level is reduced in GSKI brain tissues and in G2019S iPSCs-derived neurons, but increased in fibroblasts and PBMCs from patients, suggesting cell-type-specific effects. Overall, our study indicates that LRRK2 kinase activity affects both the levels and the catalytic activity of GCase in a cell-type-specific manner, with important implications in the context of therapeutic application of LRRK2 inhibitors in GBA1-linked and idiopathic PD.

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INTRODUCTION

Mutations in *LRRK2* cause autosomal dominant Parkinson's disease (PD) with age- and mutation-dependent penetrance[1–3], whereas heterozygous mutations in *GBA1* are the most common genetic risk factors for PD and the cause of the lysosomal storage disorder Gaucher disease when present in homozygosis[4,5]. Leucine-rich repeat kinase 2 (LRRK2) is a large, multi-domain protein with two enzymatic domains, a Ser/Thr kinase domain and a small GTPase domain (ROC), where the bulk of the pathogenic PD-linked mutations are located. While its full range of cellular functions has yet to be characterized, it has been robustly associated with endo-lysosomal pathways and vesicular trafficking (reviewed in Bonet-Ponce and Cookson, 2021[6]). These activities are likely mediated by its phosphorylation of multiple members of the Rab GTPase family, which is increased in the context of the disease-linked mutations[7], and potentially also in cases of PD not linked to mutations in *LRRK2*[8].

The main function of the lysosomal enzyme glucocerebrosidase (GCase) is to hydrolyze glucosylceramide and glucosylsphingosine to glucose and either ceramide or sphingosine, respectively; and most of the mutations in *GBA1* associated with PD risk reduce the activity of GCase[4,9]. High levels of α-synuclein, another protein mutated in PD and the major component of Lewy bodies, inhibit autophagic flux and the lysosomal activity of GCase[10]. GCase activity has been shown to be reduced also in peripheral monocyte extracts from PD patients without mutations in *GBA1*[11,12] and in PD brains[13], overall suggesting that alterations of GCase activity may be a common underlying feature of PD, similar to what has been proposed for changes in LRRK2 kinase activity.

Using a novel method of assessing GCase activity in dried blood spots, in 2015 Alcalay and colleagues reported a significant increase in GCase activity in carriers of the *LRRK2* G2019S mutation[14], suggesting that carriers of the gain of function mutation have higher GCase activity and therefore the activities of the two enzymes are positively correlated in blood cells. Shortly after, using brain lysates from LRRK2 knock-out (KO) mice, we found that loss of LRRK2 results in decreased GCase levels, which corresponded to an increase in GCase-specific activity[15]. Because of the role played by LRRK2 in the vesicular and endo-lysosomal systems, several studies have followed to assess the link between mutant LRRK2 and GCase activities.

Recently, GCase activity of induced pluripotent stem cell (iPSC)-derived dopamine neurons from LRRK2-PD patients, carrying

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Oligomeric α-synuclein and tau aggregates in NDEVs differentiate Parkinson's disease from atypical parkinsonisms

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Exosomes

ABSTRACT

The early differential diagnosis of Parkinson's disease (PD) and atypical Parkinsonian syndromes (APS), including corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), is challenging because of an overlap of clinical features and the lack of reliable biomarkers. Neural-derived extracellular vesicles (NDEVs) isolated from blood provide a window into the brain's biochemistry and may assist in distinguishing between PD and APS. We verified in a case-control study whether oligomeric α-Synuclein and Tau aggregates isolated from NDEVs could allow the differential diagnosis of these conditions.

Blood sampling and clinical data, including disease duration, motor severity, global cognition, and levodopa equivalent daily dose (LEDD), were collected from patients with a diagnosis of either PD ($n = 70$), PSP ($n = 21$), or CBD ($n = 19$). NDEVs were isolated from serum by immunocapture using an antibody against the neuronal surface marker L1CAM; oligomeric α-Synuclein and aggregated Tau were measured by ELISA.

NDEVs analyses showed that oligomeric α-Synuclein is significantly augmented in PD compared to APS, whereas Tau aggregates are significantly increased in APS compared to PD ($p < 0.0001$). ROC analyses showed that these two biomarkers have a "good" power of classification ($p < 0.0001$ for both proteins), with high sensitivity and specificity, with NDEVs concentration of Tau aggregates and oligomeric α-Synuclein being respectively the best biomarker for PD/PSP and PD/CBD diagnostic differentiation.

Logistic and multiple regression analysis confirmed that NDEVs-derived oligomeric α-Synuclein and Tau aggregates differentiate PD from CBD and PSP ($p < 0.001$). Notably, a positive correlation between NDEVs oligomeric α-Synuclein and disease severity (disease duration, $p = 0.023$; Modified H&Y, $p = 0.015$; UPDRS motor scores, $p = 0.004$) was found in PD patients and, in these same patients, NDEVs Tau aggregates concentration inversely correlated with global cognitive scores ($p = 0.043$).

A minimally invasive blood test measuring the concentration of α-synuclein and Tau aggregates in NDEVs can represent a promising tool to distinguish with high sensitivity and specificity PD from CBD or PSP patients. Optimization and validation of these data will be needed to confirm the diagnostic value of these biomarkers in distinguishing synucleinopathies from taupathies.

Abbreviations: APS, Atypical Parkinsonian Syndromes; CD81, Cluster of Differentiation 81; CI, confidence interval; L1CAM, L1 Cell Adhesion Molecule; LEDD, Levodopa Equivalent Daily Dose; MISEV, Minimal Information for Studies of Extracellular Vesicles; NDEVs, Neural Derived Extracellular Vesicles.

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2021) L1CAM -based immunoprecipitation extracellular vesicles is nevertheless expected to enrich NDEVs rather than to yield a pure population. Moreover, in-depth characterization of L1CAM-isolated NDEV's clearly showed that these particles carry specific exosomal and neural markers (Agliardi et al., 2021; Dutta et al., 2021). Reinforcing the idea that NDEVs isolated in this way are indeed an extremely useful tool to allow a glimpse into the CNS.

It has become evident that the clinicopathological heterogeneity of PSP and CBD impedes the development of specific clinical diagnostic criteria. Many studies have attempted to identify clinical features from clinicopathologic series in order to predict the underlying pathology. The overlapping clinical spectrum of PD and APS can make the differential diagnosis of these conditions very challenging. The difficulty in discriminate between these forms is particularly evident in the early stages, when neurological sings and neuroimaging features can be indistinguishable. In this scenario, the need for precise, reliable and easily measurable biomarkers is warranted.

The results presented here will need to be validated in larger independent cohorts and will need to be confirmed using next generation ELISA methods, that reach sub-picogram concentration sensitivity. It also has to be noted that the final diagnosis of patients, which was used to determine diagnostic accuracy, was based on clinical evaluation alone and has not yet been confirmed by neuropathologic examination. Although a team of movement disorders specialists has identified clinical diagnoses according to international diagnostic criteria, we cannot rule out that some patients may have received an erroneous diagnosis. These limitations notwithstanding, these results strongly suggest that NDEVs-associated oligomeric α-Synuclein and Tau aggregates concentration may serve as minimally invasive biomarkers for the early differential diagnosis of PD and APS, and could have a prognostic value in PD patients.

5. Conclusions

Data herein not only confirm very recent studies showing that increased α-synuclein in NDEVs can predict and differentiates PD from APS (Jiang et al., 2020), but also expand the knowledge by showing that the evaluation of α-synuclein and aggregated Tau in NDEVs allows to distinguish between PD and APS. This new observation suggests that these proteins have a promising potential to become disease-specific biomarkers in the clinical settings.

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Relevant conflicts of interest

Nothing to declare.

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

CRediT authorship contribution statement

Mario Meloni: Conceptualization, Project administration, Funding acquisition, Resources, Writing – original draft, Writing – review & editing. **Cristina Agliardi:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Franca Rosa Guerini:** Formal analysis, Visualization, Writing – review & editing. **Milena Zanzottera:** Investigation, Methodology. **Elisabetta Bolognesi:** Investigation, Formal analysis, Writing – review & editing. **Silvia Picciolini:** Investigation, Writing – review & editing. **Massimo Marano:** Resources, Writing – review & editing. **Alessandro Magliozzi:** Resources, Writing – review & editing. **Alessio Di Fonzo:** Resources, Writing – review & editing. **Andrea Arighi:** Resources, Writing – review & editing. **Chiara Fenoglio:** Resources, Writing – review & editing. **Giulia Franco:** Resources, Writing – review & editing. **Federica Arienti:** Resources, Writing – review & editing. **Francesca Lea Saibene:** Resources, Writing – review & editing. **Jorge Navarro:** Resources, Writing – review & editing. **Mario Clerici:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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Review article

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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Progressive myoclonus without epilepsy due to a *NUS1* frameshift insertion: Dyssynergia cerebellaris myoclonica revisited

ARTICLE INFO

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1. Main text

Within neurogenetic disorders, myoclonus usually occurs as part of a more complex phenotype, such as epileptic encephalopathy or cerebellar ataxia [1]. Heterozygous pathogenic variants of the *NUS1* gene have been linked to infantile-onset epilepsy, intellectual disability, cerebellar ataxia, neuropsychiatric features, and movement disorders, including dystonia, tremor, and myoclonus (Supplementary Table 1) [2–6]. The *NUS1* gene encodes a transmembrane receptor for the neural and cardiovascular regulator Nogo-B (NUS1 or NgBR) [7]. In addition, NUS1 is essential for dolichol synthesis and protein glycosylation in the endoplasmic reticulum (ER) [7]. Here we present a non-epileptic *NUS1* patient presenting with a progressive myoclonic syndrome and mild cerebellar signs.

The proband was a 14-years-old right-handed male, the only child of non-consanguineous parents (Fig. 1A), without family history of neurological disorders. He was born at term after an uncomplicated pregnancy. His mother reported infantile-onset motor clumsiness but no additional psychomotor development delay. At the age of 11, he started to develop involuntary twitching movements of his face, shortly followed by bilateral jerky distal movements of the arms. His handwriting deteriorated, although it was already poor. Social interaction and academic performance were impacted by a suspected mild intellectual disability.

He was initially evaluated at the age of 11. Brain MRI and laboratory analyses (including amino acids in serum, urinary copper and serum ceruloplasmin levels, CRP, ESR, CPK, thyroid function, RA Latex, Ab anti-ANA, urate, liver function test, creatinine, and electrolytes) had non-contributory findings. The jerks progressively increased in frequency and amplitude, significantly impacting his quality of life. At the age of 13, clonazepam was begun, initially at a dose of 2 mg twice daily that was soon reduced to 1 mg twice daily because of marked daytime sleepiness and limited benefit.

He was first assessed at our center at the age of 14. Neurological examination showed almost continuous, multifocal myoclonus affecting his face, tongue, and upper limbs (distal more than proximal), both at rest and with action. There was mild dysdiadochokinesis. Gait was narrow-based, but standing on one leg and tandem walking were impaired. The remainder of the neurologic and general examination were unremarkable (**Video 1**). Brain MRI at that time showed mild atrophy of the rostral part of the cerebellar vermis (Fig. 1B). EEG revealed diffuse excessive fast activity (likely due to clonazepam) without epileptiform discharges.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.parkreldis.2022.03.016

Whole-exome sequencing (WES) of genomic DNA of the proband was performed as part of a research protocol approved by the Institutional Review Board of NYU Langone Health. Written informed consent and assent for study participation and video publication were obtained from the patient and his mother. Variant prioritization looking for rare (AF<0.001) nonsynonymous variants in genes associated with neurological disorders revealed a frameshift insertion in exon 4 of *NUS1*, causing a premature stop codon (NM_138459.5: c.754_755insGTTTTCTTCCCTGGCACATCAG, p.Thr261Serfs*9). The pathogenicity of the variant was supported by the following ACMG criteria: PVS1, PM2, and PP3 [8]. PCR amplification and subsequent Sanger sequencing of *NUS1* exon 4 confirmed the presence of the frameshift insertion in the proband while indicating wild-type status of the mother (Fig. 1C, D, and 1E). The father of the patient was not available for testing since he was no longer in contact with the family but was reportedly healthy.

The causative role of the identified nonsense mutation is consistent with the mechanism of haploinsufficiency previously described for this gene. Although it was not possible to test the proband's father, we suspect that the identified mutation occurred *de novo* since he was reportedly healthy, while *NUS1* pathogenic variants are considered fully penetrant at young age [4].

Previously reported pathogenic variants of *NUS1* are mostly severe deleterious mutations (frameshift, stop, splice-disruptive, exon deletions, and chromosomal deletions) with an expected complete protein loss (Table 1) [4]. The missense variant (p.Gly102Asp) was identified in a unique patient presenting with a milder phenotype (dystonia, myoclonic jerks, and mild intellectual disability) [9]. The frameshift variant found in this subject affects the C-terminus of the protein,

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cerebellar features, myoclonus, and epilepsy. The cerebellar symptoms ("dyssynergia cerebellaris") mostly presented with mild cerebellar dysarthria and upper limb ataxia, with less involvement of the gait and station, as described in the manuscript "the evidences of dyssynergia are appendicular rather than trunkal in distribution and that higher types of movement are chiefly affected" [10]. There has been some debate and overlaps in the literature, regarding these terms being used to define cases of progressive myoclonic epilepsy and progressive myoclonic ataxia, characterized by different degrees of rate of progression, severity of the intellectual impairment, and seizures [11]. Nonetheless, we suggest that the initial description proposed by Ramsay Hunt may be still relevant indicating a syndrome characterized by cortical myoclonus, ataxia, intellectual disability, and seizures with a spectrum of degrees of severity of these traits [12]. New genetics and biochemical diagnosis are helping define these conditions and better counseling patients about disease progression, as in the case we propose here. Interestingly, in his paper, Ramsay Hunt noticed that "there was no history of the familial occurrence of either myoclonus-epilepsy or cerebellar disease" [10]. This is consistent with the following identification of mostly *de novo* autosomal dominant genetic mutations causing these conditions, such as in our patient. Therefore, the present report helps further characterizing "dyssynergia cerebellaris myoclonica" genetics.

Author contribution

(1) Conception and design of the study and data acquisition: EM, CM, GR; analysis and interpretation of data: EM, GR, CM, ADF, SF, (2) Drafting the article: EM, GR, CM; critical revising of the article for important intellectual content: ADF, SF, (3) Final approval of the version to be submitted: ED, CM, ADF, SF, GR.

Article respects all the ethical requirements expressed in the Author declaration.

Authorization for videotaping for publication for scientific purposes was signed by the patient.

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Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2022.03.016.

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Konstantin Senkevich, MD, PhD,[1,2] and Ziv Gan-Or, MD, PhD[1,2,3*]

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Data Availability Statement

Data used in the preparation of this article were obtained from the AMP PD Knowledge Platform and from UK biobank. For up-to-date information on the AMP PD study, visit https://www.amp-pd.org. UK Biobank data is available for qualified researchers upon request.

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Reply to: "No Association between Rare *TWNK* Variants and Parkinson's Disease in European Cohorts"

We thank Drs. Senkevich and Gan-Or for their interest in our work[1] and for their attempt to further explore the association between rare variants of *TWNK* and Parkinson's disease (PD). To this aim, they performed a burden analysis of rare deleterious variants by mining existing whole-exome sequencing datasets from two cohorts of PD patients and controls of European ancestry. Next, they evaluated the frequency of *TWNK* variants previously identified in our PD cohort in their patients and controls. They conclude against an association of rare *TWNK* variants with PD.[2] Although we acknowledge the importance of further exploring the link between *TWNK* variants in PD, we raise some perplexities regarding their conclusions.

First, none of the variants identified by us in the Italian PD cohort were detected, suggesting that these variants are very rare, possibly private. This observation does not argue against their possible pathogenic role in PD, as rare variants might contribute to the risk of PD along with more frequent variants in other mitochondrial genes.[3] In line with this hypothesis, a large screening of multiple PD cohorts from Norway did identify an enrichment of variants in *TWNK*, as well as in other genes implicated in mitochondrial DNA (mtDNA) replication and maintenance.[4]

Second, we believe that a burden analysis for rare variants may not comprehensively explore the contribution of *TWNK* variants to PD. *TWNK* variants are a well-known cause of autosomal dominant progressive external ophthalmoplegia (adPEO), a syndrome that could remain underdiagnosed especially in late-onset patients or in those presenting very subtle

Key Words: Parkinson's disease; TWNK; parkinsonism; twinkle; mtDNA

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mitochondrial features.[5] Therefore, it would not be surprising to detect *TWNK* pathogenic variants also in the control cohort.

Finally, they identified two known adPEO-causative *TWNK* variants, p.R303W in two controls and p.Y537H in several PD and control subjects, in line with the known frequencies of both variants (0.0009% and 0.03% in Caucasian non-Finnish individuals from GnomAD). It would be interesting to explore whether any of the subjects carrying such variants already have or will develop any signs of PEO in the future. It is worth mentioning that these variants were not found in our PD cohort, but only in adPEO patients also displaying parkinsonian signs. Therefore, this finding does not provide any evidence against the possible contribution of other *TWNK* variants toward PD risk.

In conclusion, we agree that caution is needed when assessing the contribution to PD etiology of genes whose pathogenic variants can lead to syndromes other than PD (eg, *TWNK* and *POLG*). Genetic association studies in this direction should take into account in both PD and control cohorts the possible presence of even subtle signs of PEO, a clinical history of ptosis or blepharoplasty surgery, and/or a positive family history for adPEO. This approach would reduce the chance to detect rare *TWNK* variants, no matter whether in controls or patients, related to an undiagnosed PEO. We encourage pursuing more genetic and functional studies to determine the pathogenic impact of distinct *TWNK* rare and common variants on mitochondrial function, which may help establishing their individual contribution on PD risk.

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Data Availability Statement

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

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Neurodevelopmental Gene-Related Dystonia: A Pediatric Case with *NAA15* Variant

We read with great interest the case report by Straka et al.[1] on the adult male patient with dystonia-parkinsonism and a variant in the *NAA15* (OMIM #617787) gene. N-terminal acetylation is one of the most frequent cotranslational and posttranslational protein modifications.[2] The canonical human N-terminal acetyltransferase has three subunits: a catalytic subunit (NAA10), an auxiliary subunit (NAA15), and a regulatory subunit (HYPK).[3] Both *NAA10* and *NAA15* are associated with neurodevelopmental disorders. Because dystonia is a rare feature of *NAA15*-related disorders and has been documented in only 1 of 38 patients (2.6%) in a large cohort,[4] we would like to report an additional case.

A 13-year-old girl was referred for gait disturbance and developmental delay evaluation. The legal guardians gave their consent for publication, and the study received ethical approval by the Ethics Committee (Institutional Review Board #7659).

Pregnancy, delivery, and the neonatal period were uneventful, except for neonatal hypoglycemia. As a result of the maternal cognitive and mental issues, the patient lives in a foster home. She was suffering from astigmatism and hyperopia,

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RESEARCH ARTICLE

Role of Lysosomal Gene Variants in Modulating *GBA*-Associated Parkinson's Disease Risk

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ABSTRACT: Background: To date, variants in the *GBA* gene represent the most frequent large-effect genetic factor associated with Parkinson's disease (PD). However, the reason why individuals with the same *GBA* variant may or may not develop neurodegeneration and PD is still unclear.

Objectives: Therefore, we evaluated the contribution of rare variants in genes responsible for lysosomal storage disorders (LSDs) to *GBA*-PD risk, comparing the burden of deleterious variants in LSD genes in PD patients versus asymptomatic subjects, all carriers of deleterious variants in *GBA*.

Methods: We used a custom next-generation sequencing panel, including 50 LSD genes, to screen 305 patients and 207 controls (discovery cohort). Replication and meta-analysis were performed in two replication cohorts of *GBA*-variant carriers, of 250 patients and 287 controls, for whom exome or genome data were available.

Results: Statistical analysis in the discovery cohort revealed a significantly increased burden of deleterious variants in LSD genes in patients ($P = 0.0029$). Moreover, our analyses evidenced that the two strongest modifiers of *GBA* penetrance are a second variation in *GBA* (5.6% vs. 1.4%, $P = 0.023$) and variants in genes causing mucopolysaccharidoses (6.9% vs. 1%, $P = 0.0020$). These results were confirmed in the meta-analysis, where we observed pooled odds ratios of 1.42 (95% confidence interval [CI] = 1.10–1.83, $P = 0.0063$), 4.36 (95% CI = 2.02–9.45, $P = 0.00019$), and 1.83 (95% CI = 1.04–3.22, $P = 0.038$) for variants in LSD genes, *GBA*, and mucopolysaccharidosis genes, respectively.

Conclusion: The identification of genetic lesions in lysosomal genes increasing PD risk may have important implications in terms of patient stratification for future therapeutic trials. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson Movement Disorder Society.

Key Words: Parkinson's disease; *GBA;* lysosomal genes; mutation burden

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ORIGINAL ARTICLE

The Italian tremor Network (TITAN): rationale, design and preliminary findings

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Abstract

Introduction The recently released classification has revised the nosology of tremor, defining essential tremor (ET) as a syndrome and fueling an enlightened debate about some newly conceptualized entities such as ET-plus. As a result, precise information of demographics, clinical features, and about the natural history of these conditions are lacking.

Methods The ITAlian tremor Network (TITAN) is a multicenter data collection platform, the aim of which is to prospectively assess, according to a standardized protocol, the phenomenology and natural history of tremor syndromes.

Results In the first year of activity, 679 patients have been recruited. The frequency of tremor syndromes varied from 32% of ET and 41% of ET-plus to less than 3% of rare forms, including focal tremors (2.30%), task-specific tremors (1.38%), isolated rest tremor (0.61%), and orthostatic tremor (0.61%). Patients with ET-plus were older and had a higher age at onset than ET, but a shorter disease duration, which might suggest that ET-plus is not a disease stage of ET. Familial aggregation of tremor and movement disorders was present in up to 60% of ET cases and in about 40% of patients with tremor combined with dystonia. The body site of tremor onset was different between tremor syndromes, with head tremor being most commonly, but not uniquely, associated with dystonia.

Conclusions The TITAN study is anticipated to provide clinically relevant prospective information about the clinical correlates of different tremor syndromes and their specific outcomes and might serve as a basis for future etiological, pathophysiological, and therapeutic research.

Keywords Dystonic tremor · Prevalence · Rest tremor · Essential tremor · Classification

Introduction

Tremor is deemed to be the commonest movement disorder. A population study performed in Northern Italy found tremor syndromes to be the most frequent movement disorder with a prevalence of 14.5% in people aged > 50 years, followed by restless legs syndrome (10.8%) and parkinsonism (6.95%) [1]. Different disorders can present with tremor and they span from very common conditions, including enhancement of physiological tremor (EPT), which is usually transient and non-symptomatic [2], to rare forms of tremor [3]. Probably being the commonest form of tremor seen in clinical practice, Essential Tremor (ET) has an estimated prevalence of 1% of the general population and has been formerly construed to be a monosymptomatic condition with an autosomal dominant pattern of inheritance and characterized by a slow progression of tremor intensity with age [4]. Despite its relative frequency, research efforts into the identification of key pathophysiologic markers and of a defined genetic etiology have been mostly inconclusive [5]. This probably owes to the fact ET has been over-diagnosed with the inclusion of

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Declarations

Competing interests Roberto Erro receives royalties from publication of Case Studies in Movement Disorders—Common and Uncommon Presentations (Cambridge University Press, 2017) and of Paroxysmal Movement Disorders (Springer, 2020). He has received consultancies from Sanofi and honoraria for speaking from the International Parkinson's Disease and Movement Disorders Society. Paolo Barone received consultancies as a member of the advisory board for Zambon, Lundbeck, UCB, Chiesi, Abbvie, and Acorda. Anna De Rosa received consultancies from Lundbeck, and from Bial as a member of advisory board. Andrea Pilotto received grant support from Ministry of Education, Research and University (MIUR) and IMI H2020 Initiative (IDEA-FAST project- MI2-2018-15-06), received research support from Zambon SrL Italy and Bial italy; he received speaker honoraria from Abbvie, Biomarin, Bial and Zambon Pharmaceuticals. Alessandro Padovani received grant support from Ministry of Health (MINSAL) and Ministry of Education, Research and University (MIUR), from CARIPLO Foundation; personal compensation as a consultant/scientific advisory board member for Biogen 2019-2020-2021 Roche 2019-2020 Nutricia 2020-2021 General Healthcare (GE) 2019; he received honoraria for lectures at meeting ADPD2020 from Roche, Lecture at meeting of the Italian society of Neurology 2020 from Biogen and from Roche, Lecture at meeting AIP 2020 and 2021 from Biogen and from Nutricia, Educational Consulting 2019-2020-2021 from Biogen. Lazzaro di Biase has received a speaker honoraria from Bial, consultant honoraria from Abbvie and research funding from Zambon, is the scientific director and one of the shareholders of Brain Innovations Srl, a University spinoff of Campus Bio-Medico University of Rome. All other authors have nothing to disclose.

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

ARTICLE INFO

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Review

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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Article

β-Glucocerebrosidase Deficiency Activates an Aberrant Lysosome-Plasma Membrane Axis Responsible for the Onset of Neurodegeneration

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Abstract: β-glucocerebrosidase is a lysosomal hydrolase involved in the catabolism of the sphingolipid glucosylceramide. Biallelic loss of function mutations in this enzyme are responsible for the onset of Gaucher disease, while monoallelic β-glucocerebrosidase mutations represent the first genetic risk factor for Parkinson's disease. Despite this evidence, the molecular mechanism linking the impairment in β-glucocerebrosidase activity with the onset of neurodegeneration in still unknown. In this frame, we developed two in vitro neuronal models of β-glucocerebrosidase deficiency, represented by mouse cerebellar granule neurons and human-induced pluripotent stem cells-derived dopaminergic neurons treated with the specific β-glucocerebrosidase inhibitor conduritol B epoxide. Neurons deficient for β-glucocerebrosidase activity showed a lysosomal accumulation of glucosylceramide and the onset of neuronal damage. Moreover, we found that neurons react to the lysosomal impairment by the induction of their biogenesis and exocytosis. This latter event was responsible for glucosylceramide accumulation also at the plasma membrane level, with an alteration in lipid and protein composition of specific signaling microdomains. Collectively, our data suggest that β-glucocerebrosidase loss of function impairs the lysosomal compartment, establishing a lysosome–plasma membrane axis responsible for modifications in the plasma membrane architecture and possible alterations of intracellular signaling pathways, leading to neuronal damage.

Keywords: GBA1; glucosylceramide; Gaucher disease; lysosomes; plasma membrane; lipid rafts

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Activation of lncRNA NEAT1 leads to survival advantage of multiple myeloma cells by supporting a positive regulatory loop with DNA repair proteins

by Elisa Taiana, Cecilia Bandini, Vanessa Katia Favasuli, Domenica Ronchetti, Ilaria Silvestris, Noemi Puccio, Katia Todoerti, Silvia Erratico, Domenica Giannandrea, Niccolò Bolli, Nicola Amodio, Alessia Ciarrocchi, Raffaella Chiaramonte, Yvan Torrente, Roberto Piva, and Antonino Neri

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Activation of lncRNA NEAT1 leads to survival advantage of multiple myeloma cells by supporting a positive regulatory loop with DNA repair proteins

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Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

Effective high-throughput isolation of enriched platelets and circulating pro-angiogenic cells to accelerate skin-wound healing

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Abstract

Delayed wound healing and chronic skin lesions represent a major health problem. Over the past years, growth factors mediated by platelet-rich plasma (PRP) and cell-based therapies were developed as effective and affordable treatment able to improve wound healing capacity. We have advanced existing concepts to develop a highly efficient high-throughput protocol with proven application for the isolation of PRP and pro-angiogenic cells (Angio^{PRP}). This protocol outlines the effectiveness of Angio^{PRP} in promoting the critical healing process including wound closure, re-epithelialization, granulation tissue growth, and blood vessel regeneration. We coupled this effect with normalization of mechanical properties of rescued mouse wounds, which is sustained by a correct arrangement of elastin and collagen fibers. Proteomic analysis of treated wounds demonstrated a fingerprint of Angio^{PRP} based on the up-regulation of detoxification pathway of glutathione metabolism, correlated to a decrease in inflammatory response. Overall, these results have enabled us to provide a framework for how Angio^{PRP} supports wound healing, opening avenues for further clinical advances.

Keywords Epithelialization · PRP · Angiogenic potential · Skin remodeling · Proteomics

Introduction

Wound healing is a dynamic and orchestrated sequence of events requiring the interaction of soluble mediators, blood cells and extracellular matrix that result in the restoration of skin integrity and homeostasis [1]. Wound repair proceeds in three overlapping and functionally distinct phases characterized first by infiltration of neutrophils and macrophages, [2] followed by angiogenesis, fibroblasts and keratinocytes proliferation [3] that allows granulation tissue formation and extracellular matrix remodeling [4, 5]. An interruption in the normal wound healing process can lead to the development of non-healing chronic wounds, a typical complication of several diseases, such as foot ulcer from diabetes and pressure ulcer resulting from spinal cord injuries [6]. As wound healing impairment represents a major health problem, the complexity of cell and molecular events required for appropriate repair constitute a major research focus [7, 8]. In this regard, different dressing and ointments, such as hydrocolloids, alginates, foams, sulfadiazine silver patches, and honey gauzes, have been described to promote chronic wound healing [9]. Nevertheless, the systematic review [10] of local interventions do not support conclusive evidences for ulcer healing. Other evidences suggest that hyperbaric oxygen and negative pressure wound therapy systems can induce and accelerate wound healing [11]; however these interventions are limited by reduced availability, patients'

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Article

Immunoproteasome Inhibition Ameliorates Aged Dystrophic Mouse Muscle Environment

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Abstract: Muscle wasting is a major pathological feature observed in Duchenne muscular dystrophy (DMD) and is the result of the concerted effects of inflammation, oxidative stress and cell senescence. The inducible form of proteasome, or immunoproteasome (IP), is involved in all the above mentioned processes, regulating antigen presentation, cytokine production and immune cell response. IP inhibition has been previously shown to dampen the altered molecular, histological and functional features of 3-month-old mdx mice, the animal model for DMD. In this study, we described the role of ONX-0914, a selective inhibitor of the PSMB8 subunit of immunoproteasome, in ameliorating the pathological traits that could promote muscle wasting progression in older, 9-month-old mdx mice. ONX-0914 reduces the number of macrophages and effector memory T cells in muscle and spleen, while increasing the number of regulatory T cells. It modulates inflammatory markers both in skeletal and cardiac muscle, possibly counteracting heart remodeling and hypertrophy. Moreover, it buffers oxidative stress by improving mitochondrial efficiency. These changes ultimately lead to a marked decrease of fibrosis and, potentially, to more controlled myofiber degeneration/regeneration cycles. Therefore, ONX-0914 is a promising molecule that may slow down muscle mass loss, with relatively low side effects, in dystrophic patients with moderate to advanced disease.

Keywords: immunoproteasome; muscle mass; inflammation; sarcopenia; aging

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1. Introduction

Duchenne muscular dystrophy (DMD) is a fatal disease caused by mutations in the dystrophin gene. In DMD, inflammation and muscle invasion by several immune cells are triggered by damage-associated molecular patterns (DAMPs) released by injured myofibers, oxidative stress and defective calcium handling, and underlie muscular degeneration. The asynchronous cycles of muscle fiber regeneration exacerbate muscle infiltration by macrophages and lymphocytes and their secretion of pro-inflammatory cytokines, leading to the replacement of myofibers with connective and adipose tissue, which becomes more evident with age progression [1]. Senescence, oxidative stress and inflammation—together with altered mitochondrial activity and proteostasis—are common features in aged organisms, and are the main pathogenetic mechanisms leading to muscle wasting in sarcopenia, which shares multiple features with DMD [2]. Moreover, detailed proteomic analysis of skeletal muscles from aged individuals highlighted a downregulation of proteins related to energetic metabolism and mitochondrial function and, conversely, an overexpression of signaling molecules regulating proteostasis, autophagy and innate/adaptive immunity [3].

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ARTICLE OPEN

Inhibition of the immunoproteasome modulates innate immunity to ameliorate muscle pathology of dysferlin-deficient BlAJ mice

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Muscle repair in dysferlinopathies is defective. Although macrophage (Mø)-rich infiltrates are prominent in damaged skeletal muscles of patients with dysferlinopathy, the contribution of the immune system to the disease pathology remains to be fully explored. Numbers of both pro-inflammatory M1 Mø and effector T cells are increased in muscle of dysferlin-deficient BlAJ mice. In addition, symptomatic BlAJ mice have increased muscle production of immunoproteasome. In vitro analyses using bone marrow-derived Mø of BlAJ mice show that immunoproteasome inhibition results in C3aR1 and C5aR1 downregulation and upregulation of M2-associated signaling. Administration of immunoproteasome inhibitor ONX-0914 to BlAJ mice rescues muscle function by reducing muscle infiltrates and fibro-adipogenesis. These findings reveal an important role of immunoproteasome in the progression of muscular dystrophy in BlAJ mouse and suggest that inhibition of immunoproteasome may produce therapeutic benefit in dysferlinopathy.

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INTRODUCTION

Mutations in dysferlin gene (DYSF, MIM*603009) are responsible for recessively inherited dysferlinopathy which is most pronounced in the pelvic and shoulder girdle muscles (Limb girdle muscular dystrophy R2-LGMDR2, formerly LGMD2B), or distal myopathy with onset in gastrocnemius and soleus muscles in cases of Miyoshi myopathy (MM or MMD1), or distal myopathy with onset in the tibialis anterior (DMAT) (also referred to as DACM for distal anterior compartment myopathy) [1, 2]. Dysferlin is a transmembrane proteins, that is implicated in protein vesicle fusion and trafficking [3]: it is prevalently expressed in skeletal muscle but it is also present in macrophages (Mø), adipocytes, smooth muscle cells [4]. Dysferlin also interacts with Ca^{2+} handling proteins for excitation-contraction (EC) coupling at the transverse-tubules (T-tubules) in skeletal muscle [5, 6]. Moreover, dysferlin was detected in blood vessels and dysferlin-null mice displayed impaired angiogenic response compared to control mice [7]. LGMDR2 muscles are characterized by enhanced infiltration of macrophages and CD4+ T-cells in the perimysium [8] and the involvement of innate immune system [9–11].

The complement immune system including its activated anaphylatoxins, C3a and C5a, facilitate innate immune response [12]. Both C3a and C5a mediate vasodilation, increased vascular permeability, chemotaxis, and inflammation by innate immune cells through interaction with their specific receptors (C3aR, C5aR) [13]. Murine C3aR was mainly detected on Mø, but not on circulating neutrophils, T cells, and B cells [14], highlighting the potential of anti-inflammatory properties of C3a/C3aR axis. Consistently, C3a receptor signaling has been reported to be involved in Mø recruitment and muscle regeneration [15]. In addition, C3aR expression in aortic tissues confers protection from atherosclerosis through modulation of Mø toward the anti-inflammatory phenotype [16]. Muscle fibers of both animal models and LGMDR2 patients present abnormal activation of complement factors C4 and C5 together with the downregulation of the complement inhibitory factor CD55, the upregulation of major histocompatibility complex I (MHC-I) and the formation of the membrane attack complex (MAC, C5b-9) on their surface [11, 17, 18]. The lack of CD55 enhances the susceptibility of skeletal muscle to complement attack [19], leading to over-expression of inflammatory pathways dependent on heat shock proteins and HMGB1 [20]. This scenario is worsened by HMGB1 secretion from necrotic cells and by activation of macrophages toward a pro-inflammatory phenotype through a HMGB1-C1q signaling [21, 22]. Indeed, C1q can bind to PTX3 to activate the classical component cascade and together modulate Mø M1/M2 polarization [23]. Moreover, complement can enhance the release of metalloproteinases (MMPs) [24] and favor the expression of MMP2 through the C3a-C3aR complex [25].

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EMBO Molecular Medicine

Microbiota dysbiosis influences immune system and muscle pathophysiology of dystrophin-deficient mice

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Abstract

Duchenne muscular dystrophy (DMD) is a progressive severe muscle-wasting disease caused by mutations in *DMD*, encoding dystrophin, that leads to loss of muscle function with cardiac/respiratory failure and premature death. Since dystrophic muscles are sensed by infiltrating inflammatory cells and gut microbial communities can cause immune dysregulation and metabolic syndrome, we sought to investigate whether intestinal bacteria support the muscle immune response in mdx dystrophic murine model. We highlighted a strong correlation between DMD disease features and the relative abundance of *Prevotella*. Furthermore, the absence of gut microbes through the generation of mdx germ-free animal model, as well as modulation of the microbial community structure by antibiotic treatment, influenced muscle immunity and fibrosis. Intestinal colonization of mdx mice with eubiotic microbiota was sufficient to reduce inflammation and improve muscle pathology and function. This work identifies a potential role for the gut microbiota in the pathogenesis of DMD.

Keywords Duchenne muscular dystrophy; gut microbiota; immunity; skeletal muscle metabolism; T-lymphocytes

Subject Categories Digestive System; Microbiology, Virology & Host Pathogen Interaction; Musculoskeletal System

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Introduction

Duchenne muscular dystrophy (DMD) is an X-linked disease caused by mutations in the *DMD* gene and loss of the dystrophin protein, leading to myofiber membrane fragility and necrosis with weakness and contractures. Affected DMD boys typically die in their second or third decade of life due to either respiratory failure or cardiomyopathy (Emery, 2002). Although the primary defects rely on skeletal muscle structure, a multitude of secondary defects exist involving deregulated metabolic and inflammatory pathways. Immune cell infiltration into skeletal muscle is, indeed, a typical feature of DMD pathophysiology and is strongly associated with disease severity (Farini *et al*, 2009). In the dystrophic dystrophin-deficient mdx murine model, we recently found the presence of activated T lymphocytes and the overexpression of immunoproteasome (IP), an enzymatic complex that cleaves peptides to produce epitopes for antigen presentation to T lymphocytes. We have demonstrated that IP inhibition improved dystrophic muscle functions by reducing the number of both circulating and infiltrating activated T cells, confirming a pathogenic role of immune cells (Farini *et al*, 2016).

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Lab Resource: Single Cell Line

Reprogramming of dermal fibroblasts from a Duchenne muscular dystrophy patient carrying a deletion of exons 45–50 into an induced pluripotent stem cell line (CCMi005-A)

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A B S T R A C T

Duchenne muscular dystrophy (DMD) is an X-linked syndrome that affects skeletal and cardiac muscle and is caused by mutation of the dystrophin gene. Induced pluripotent stem cells (iPSCs) were generated from dermal fibroblasts by electroporation with episomal vectors containing the reprogramming factors (OCT4, SOX2, LIN28, KLF4, and L-MYC). The donor carried an out-of-frame deletion of exons 45–50 of the dystrophin gene. The established iPSC line exhibited normal morphology, expressed pluripotency markers, had normal karyotype and possessed trilineage differentiation potential.

Resource Table:

Unique stem cell line identifier	CCMi005-A
Alternative name(s) of stem cell line	DMD4 C3
Institution	Centro Cardiologico Monzino-IRCCS
Contact information of distributor	Davide Rovina; davide.rovina@ccfm.it
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Age: 10 years old (at biopsy) Sex: Male Ethnicity if known: Caucasian
Cell Source	Dermal fibroblasts
Clonality	Clonal
Associated disease	Duchenne Muscular Dystrophy
Gene/locus	*DMD* gene, Xp21.2-p21.1
Date archived/stock date	June 2021
Cell line repository/bank	https://hpscreg.eu/cell-line/CCMi005-A
Ethical approval	The study was approved by the ethical committee of the European Institute of Oncology and Monzino Heart Centre (Istituto Europeo di Oncologia e dal Centro Cardiologico Monzino, IEO-CCM, CEA20150411, ammed. 20,190,528 AN/sd). Informed consent was given to donate biopsy material for use in research to The Telethon Biobank or The Eurobiobank which were accessed via Grant No GTB12001 and GUP13013 respectively.

(continued on next column)

(continued)

1. Resource utility

This iPSC line carrying a *DMD*-causing mutation will be very useful in studying the pathophysiological mechanisms underlying dystrophin deficiency and discovering new therapeutic compounds.

2. Resource details

X-linked Duchenne muscular dystrophy is a neuromuscular disorder that affects both skeletal and cardiac muscle functions (D'Amario et al., 2018). Dystrophin localizes below the sarcolemma and links the actin cytoskeleton and plasma membrane to the extracellular matrix through the dystrophin-associated protein complex (DAPC) (Rovina et al., 2020). DMD is caused by mutations that lead to absence of full-length

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Original research

Comparison of clinical rating scales in genetic frontotemporal dementia within the GENFI cohort

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ABSTRACT

Background Therapeutic trials are now underway in genetic forms of frontotemporal dementia (FTD) but clinical outcome measures are limited. The two most commonly used measures, the Clinical Dementia Rating (CDR)+National Alzheimer's Disease Coordinating Center (NACC) Frontotemporal Lobar Degeneration (FTLD) and the FTD Rating Scale (FRS), have yet to be compared in detail in the genetic forms of FTD.

Methods The CDR+NACC FTLD and FRS were assessed cross-sectionally in 725 consecutively recruited participants from the Genetic FTD Initiative: 457 mutation carriers (77 microtubule-associated protein tau (*MAPT*), 187 *GRN*, 193 *C9orf72*) and 268 family members without mutations (non-carrier control group). 231 mutation carriers (51 *MAPT*, 92 *GRN*, 88 *C9orf72*) and 145 non-carriers had available longitudinal data at a follow-up time point.

Results Cross-sectionally, the mean FRS score was lower in all genetic groups compared with controls: *GRN* mutation carriers mean 83.4 (SD 27.0), *MAPT* mutation carriers 78.2 (28.8), *C9orf72* mutation carriers 71.0 (34.0), controls 96.2 (7.7), p<0.001 for all comparisons, while the mean CDR+NACC FTLD Sum of Boxes was significantly higher in all genetic groups: *GRN* mutation carriers mean 2.6 (5.2), *MAPT* mutation carriers 3.2 (5.6), *C9orf72* mutation carriers 4.2 (6.2), controls 0.2 (0.6), p<0.001 for all comparisons. Mean FRS score decreased and CDR+NACC FTLD Sum of Boxes increased with increasing disease severity within each individual genetic group. FRS and CDR+NACC FTLD Sum of Boxes scores were strongly negatively correlated across all mutation carriers (r_s=−0.77, p<0.001) and within each genetic group (r_s=−0.67 to −0.81, p<0.001 in each group). Nonetheless, discrepancies in disease staging were seen between the scales, and with each scale and clinician-judged symptomatic status. Longitudinally, annualised change in both FRS and CDR+NACC FTLD Sum of Boxes scores initially increased with disease severity level before decreasing in those with the most severe disease: controls −0.1 (6.0) for FRS, −0.1 (0.4) for CDR+NACC FTLD Sum of Boxes, asymptomatic mutation carriers −0.5 (8.2), 0.2 (0.9), prodromal disease −2.3 (9.9), 0.6 (2.7), mild disease −10.2 (18.6), 3.0 (4.1), moderate disease −9.6 (16.6), 4.4 (4.0), severe disease −2.7 (8.3), 1.7 (3.3). Sample sizes were calculated for a trial of prodromal mutation carriers: over 180 participants per arm would be needed to detect a moderate sized effect (30%) for both outcome measures, with sample sizes lower for the FRS.

Conclusions Both the FRS and CDR+NACC FTLD measure disease severity in genetic FTD mutation carriers throughout the timeline of their disease, although the FRS may be preferable as an outcome measure. However, neither address a number of key symptoms in the FTD spectrum, for example, motor and neuropsychiatric deficits, which future scales will need to incorporate.

INTRODUCTION

Frontotemporal dementia (FTD) is a spectrum of heterogenous disorders characterised by neurodegeneration of the frontal and temporal lobes. A total of 20%–30% of FTD cases are genetic,[1 2] with the majority caused by autosomal dominant mutations in three genes[3]: chromosome 9 open reading frame 72 (*C9orf72*),[4] progranulin (*GRN*)[5] and microtubule-associated protein tau (*MAPT*).[6] Clinical syndromes span changes in behaviour (behavioural variant FTD, bvFTD),[7] language (primary progressive aphasia, PPA)[8] and motor function (progressive supranuclear palsy, PSP, corticobasal syndrome, CBS and FTD with amyotrophic lateral sclerosis, FTD-ALS).[9–11] Age of symptom onset, and disease progression and duration vary between and within genetic groups.[12]

The ability to accurately evaluate disease stage and track clinical change in FTD across the spectrum of phenotypes is critical for the design of future trials of disease-modifying therapies. Two candidate global severity measures specific to FTD are the Clinical Dementia Rating (CDR) Dementia

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PERSPECTIVE

Conceptual framework for the definition of preclinical and prodromal frontotemporal dementia

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Abstract

The presymptomatic stages of frontotemporal dementia (FTD) are still poorly defined and encompass a long accrual of progressive biological (preclinical) and then clinical (prodromal) changes, antedating the onset of dementia. The heterogeneity of clinical presentations and the different neuropathological phenotypes have prevented a prior clear description of either preclinical or prodromal FTD. Recent advances in

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APPENDIX A

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Caregiver Tele-Assistance for Reduction of Emotional Distress During the COVID-19 Pandemic. Psychological Support to Caregivers of People with Dementia: The Italian Experience

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Abstract.
Background: COVID-19 pandemic worsened vulnerability of patients with dementia (PWD). This new reality associated with government restriction and isolation worsened stress burden and psychological frailties in PWD caregivers.
Objective: To give tele-psychological support to caregivers and evaluate the effect of this intervention by quantifying stress burden and quality of life during the first COVID-19 lockdown.
Methods: 50 caregivers were divided into two groups: "Caregiver-focused group" (Cg) and "Patient-focused group" (Pg). Both groups received telephone contact every 2 weeks over a 28-week period, but the content of the call was different: in Cg, caregivers answered questions about the state of the PWD but also explored their own emotional state, stress burden, and quality of life. In Pg instead, telephone contacts were focused only on the PWD, and no evaluation regarding the caregiver mood or state of stress was made. Psychometric scales were administered to evaluate COVID-19 impact, stress burden, and quality of life.
Results: Considering the time of intervention, from baseline (W0) to W28, Zarit Burden Interview and Quality of Life-caregiver questionnaires remained unchanged in Cg as compared with baseline ($p > 0.05$), whereas they worsened significantly in Pg ($p < 0.01$), showing increased stress over time and decreased quality of life in this group. Moreover, Impact on Event Scale values improved over the weeks in Cg ($p = 0.015$), while they remained unchanged in Pg ($p = 0.483$).
Conclusion: Caregivers who received telephone support about their mood and stress burden did not worsen their psychological state during the time of intervention, as did instead those who did not get such support.

Keywords: Caregiver, COVID-19 pandemic, people with dementia, quality of life, stress burden, tele-psychological support

INTRODUCTION

Currently, more than 50 million people have dementia worldwide [1]. Dementia is associated with varying degrees of cognitive decline and behavioral changes, which have a huge impact on the quality

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cohorts would be needed to confirm our preliminary data. Unfortunately, in this study the average of drop out was high. Nevertheless, this would be hard to avoid in a population with a considerable burden of activity, linked to its own life and PWD care out of our control. In addition, it cannot be excluded that the sample participating in the study was not biased by other variables (number of family members, social condition, economic status. etc.), therefore confirmatory future studies on larger and better-defined populations would be needed.

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Stratifying the Presymptomatic Phase of Genetic Frontotemporal Dementia by Serum NfL and pNfH: A Longitudinal Multicentre Study

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BRAIN
ORIGINAL ARTICLE

A data-driven disease progression model of fluid biomarkers in genetic frontotemporal dementia

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Several CSF and blood biomarkers for genetic frontotemporal dementia have been proposed, including those reflecting neuroaxonal loss (neurofilament light chain and phosphorylated neurofilament heavy chain), synapse dysfunction [neuronal pentraxin 2 (NPTX2)], astrogliosis (glial fibrillary acidic protein) and complement activation (C1q, C3b). Determining the sequence in which biomarkers become abnormal over the course of disease could facilitate disease staging and help identify mutation carriers with prodromal or early-stage frontotemporal dementia, which is especially important as pharmaceutical trials emerge. We aimed to model the sequence of biomarker abnormalities in presymptomatic and symptomatic genetic frontotemporal dementia using cross-sectional data from the Genetic Frontotemporal dementia Initiative (GENFI), a longitudinal cohort study.

Two-hundred and seventy-five presymptomatic and 127 symptomatic carriers of mutations in *GRN*, *C9orf72* or *MAPT*, as well as 247 non-carriers, were selected from the GENFI cohort based on availability of one or more of the aforementioned biomarkers. Nine presymptomatic carriers developed symptoms within 18 months of sample collection ('converters'). Sequences of biomarker abnormalities were modelled for the entire group using discriminative event-based modelling (DEBM) and for each genetic subgroup using co-initialized DEBM. These models estimate probabilistic biomarker abnormalities in a data-driven way and do not rely on previous diagnostic information or biomarker cut-off points. Using cross-validation, subjects were subsequently assigned a disease stage based on their position along the disease progression timeline.

CSF NPTX2 was the first biomarker to become abnormal, followed by blood and CSF neurofilament light chain, blood phosphorylated neurofilament heavy chain, blood glial fibrillary acidic protein and finally CSF C3b and C1q. Biomarker orderings did not differ significantly between genetic subgroups, but more uncertainty was noted in the

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C9orf72 and *MAPT* groups than for *GRN*. Estimated disease stages could distinguish symptomatic from presymptomatic carriers and non-carriers with areas under the curve of 0.84 (95% confidence interval 0.80–0.89) and 0.90 (0.86–0.94) respectively. The areas under the curve to distinguish converters from non-converting presymptomatic carriers was 0.85 (0.75–0.95).

Our data-driven model of genetic frontotemporal dementia revealed that NPTX2 and neurofilament light chain are the earliest to change among the selected biomarkers. Further research should investigate their utility as candidate selection tools for pharmaceutical trials. The model's ability to accurately estimate individual disease stages could improve patient stratification and track the efficacy of therapeutic interventions.

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Letters

Practice effects in genetic frontotemporal dementia and at-risk individuals: a GENFI study

INTRODUCTION

Frontotemporal dementia (FTD) is a heterogeneous group of neurodegenerative diseases with an onset usually before the age of 65 years even if it can appear also in older ages.[1]

On cognitive tests, patients with FTD show deficits in executive functions, social cognition and language, whereas the initial performances in memory and visuoconstruction tasks usually are preserved.[1] The general approach to detect cognitive decline in dementia is to repeat cognitive testing and observe changes over time. However, exposure to similar tasks could improve performance as the individual gets familiar with both the tasks themselves and the test setting (ie, practice effect or learning effect).[2 3]

Different attempts to adjust for practice effects in repeated testing have been proposed.[4] However, recent research suggests that the phenomenon of practice effects can provide useful information. Patients with neurological and psychiatric conditions show lower practice effects than healthy controls, and individuals with mild cognitive impairment (MCI) that do not show practice effects are more likely to develop Alzheimer disease (AD) within a year than individuals with MCI that have preserved practice effects.[3] In addition to the findings of lower practice effects in patients with dementia, Hassenstab *et al*[5] found that preclinical individuals who later progressed to AD had substantially reduced practice effects in episodic memory compared with cognitively stable individuals. Thus, absence of practice effects might serve as an early marker for cognitive decline.

To our knowledge, practice effects have never been investigated in FTD before. The aim of this study was to examine practice effects in the GENetic Frontotemporal dementia Initiative (GENFI) cohort. More specifically, we investigated whether there is a difference in practice effects between presymptomatic mutation carriers (PMC) and mutation non-carriers (NC).

MATERIALS AND METHODS

Participants

All participants (317 NC, 327 PMC and 159 affected mutation carriers (AMC)) were recruited through GENFI from January 2012 to March 2018 (online supplemental table 1). Of the 803 participants, 471 had two visits; 249 had three visits; and 108 had four visits. After the fourth visit, the number of participants rapidly decreased and only 12 had six test occasions (online supplemental figure 1).

Statistics

A global cognitive score was calculated including the mean z-scores of all tests in the standardised GENFI neuropsychological battery. Additionally, practice effects for different cognitive domains were explored. A linear mixed-effects model was applied to examine potential practice effects. Further details including neuropsychological tests, composite score calculation and model selection criteria are described in the online supplemental materials.

RESULTS

Practice effects

An increase in mean global cognitive test scores was seen in NC over the first five visits (online supplemental figure 2). When investigating different cognitive domains, practice effects were found across visits 1–3 in all domains except for visuoconstruction (online supplemental table 2). The largest practice effect was observed in memory and social cognition. After the third visit, there was a plateau, and the practice effects between visits 3 and 4 as well as visits 4 and 5 were not statistically significant. In contrast, a progressive decline in the mean global score was identified longitudinally in AMC, as could be expected (online supplemental figure 2). PMC carrying a *C9orf72* expansion and with less than 5 years to expected symptom onset (PMC-C9 in proximity to onset) showed no practice effect on their global test score and had the same mean performance at all three visits (figure 1A and online supplemental table 3). Furthermore, PMC-C9 with more than 5 years to expected onset had a lower practice effect between visits 1 and 2 than NC; however, the total practice effect (visits 1–3) was not significantly different from NC.

Similar to PMC-C9, there was a lower practice effect across visits 1–3 in PMC with a proganulin (*GRN*) mutation in proximity to onset compared with NC. However, PMC-GRN in proximity to onset appear to initially have a practice effect but subsequently do not improve their performance at the third visit (figure 1B).

PMC with a *MAPT* mutation (PMC-MAPT) had a similar trajectory in mean cognitive test score across visits 1–3 as NC (figure 1C).

DISCUSSION

In this study, we explored practice effects due to repeated cognitive assessments in

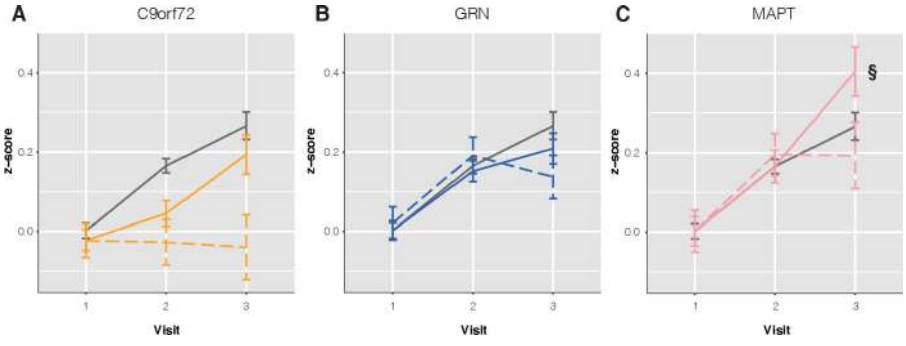

Figure 1 Trajectories of global cognitive test scores in NC and PMC by mutated gene.(A) PMC-C9 and NC (grey line, NC; yellow solid line, PMC-C9 with >5 years to expected symptom onset; yellow dashed line, PMC-C9 with <5 years to expected symptom onset). (B) PMC-GRN and NC (grey line, NC; blue solid line, PMC-GRN with >5 years to expected symptom onset; blue dashed line, PMC-GRN with <5 years to expected symptom onset). (C) PMC-MAPT and NC (grey line, NC; pink solid line, PMC-MAPT with >5 years to expected symptom onset; pink dashed line, PMC-MAPT with <5 years to expected symptom onset). All lines are fitted from the same linear mixed-effect model but plotted in A–C to simplify visualisation. Error bars represent the SEs of the means. §The difference between PMC-MAPT with >5 years to expected symptom onset and NC is no longer observed when PMC-MAPTs are compared with age-matched and family-matched controls. C9, chromosome 9 open reading frame 72; GRN, progranulin; MAPT, microtubule-associated protein tau; NC, non-carrier; PMC, presymptomatic mutation carrier.

a large cohort of individuals with genetic presymptomatic or symptomatic FTD as well as non-mutation carrier family members. Practice effects have been suggested to provide useful information of the progression of cognitive decline but have never been studied in the context of FTD before. Compared with their baseline test scores, NC improved in global cognition at each visit (visits 2 and 3). Presymptomatic individuals carrying the *C9orf72* expansion or a *GRN* mutation had significantly lower practice effects than NC, and this difference was most apparent in PMC-C9 within 5 years of expected symptom onset. However, it is not possible to know if the stable performance over time in PMC in proximity to onset is due to lower practice effects per se or an actual cognitive decline that is masked by practice effects. The question of genuine practice effects applies also to AMC, who showed a progressive decline in global cognitive test scores at each visit. The scores measured after repeated testing in AMC might include a 'hidden' practice effect, and therefore the true cognitive dysfunction would in fact be greater than what was captured in the test scores. Cognitive functions in FTD are expected to decline over the test interval used in this study (mean 1.3 years). Consequently, a potential absence of practice effects in clinical FTD, as reported in AD,[3] cannot be evaluated with the current setup but could be addressed if the retest is performed within days or weeks of the first assessment. Besides the PMC in proximity to onset, also PMC-C9 with more than 5 years to expected symptom onset had lower practice effects than NC which could not be explained by early conversion into a symptomatic stage. Progression of brain atrophy in *C9orf72* expansion carriers can be slow, and some patients have been described with a remarkably long disease duration.[1] Pathological changes in the brain of *C9orf72* expansion carriers are present already in early adulthood, and the potential neurodevelopmental effects could lead to a long prodromal phase in PMC-C9. Previous findings show that cognitive performance in PMC is not different from NC until very close to the disease onset,[1] which is in line with the results of the current study. Nevertheless, an inability to use acquired skills from previous tests might be a marker for very early disease development in PMC-C9. However, the diagnostic potential of practice effects and whether they can be used for differentiating PMC-C9 from NC are yet to be explored.

As the field of FTD research is greatly evolving and treatment opportunities are emerging, knowledge about different stages of the disease is highly required. As we are preparing for clinical trials, several initiatives have been searching for both fluid biomarkers as surrogate endpoints as well as clinical and neuropsychological tests used to evaluate a future treatment response. Practice effects can have implications for the interpretation of longitudinal changes in cognitive performance as it could impact estimations of treatment effects after an intervention, particularly early in the disease course. Furthermore, one could speculate that identifying individuals with lower-than-expected practice effects would be a cost-effective approach for inclusion into clinical trials.[3] The presence of practice effects should thus be considered in future clinical trials especially if neuropsychological measures are included as end points.

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Correction notice This article has been corrected since it was first published online. The 'Results' heading has been added in the text.

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ORIGINAL ARTICLE

Association of rs3027178 polymorphism in the circadian clock gene *PER1* with susceptibility to Alzheimer's disease and longevity in an Italian population

Maria Giulia Bacalini · Flavia Palombo · Paolo Garagnani · Cristina Giuliani · Claudio Fiorini · Leonardo Caporali · Michelangelo Stanzani Maserati · Sabina Capellari · Martina Romagnoli · Sara De Fanti · Luisa Benussi · Giuliano Binetti · Roberta Ghidoni · Daniela Galimberti · Elio Scarpini · Marina Arcaro · Enrica Bonanni · Gabriele Siciliano · Michelangelo Maestri · Biancamaria Guarnieri · Italian Multicentric Group on clock genes, actigraphy in AD · Morena Martucci · Daniela Monti · Valerio Carelli · Claudio Franceschi · Chiara La Morgia · Aurelia Santoro

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Abstract Many physiological processes in the human body follow a 24-h circadian rhythm controlled by the circadian clock system. Light, sensed by retina, is the predominant "zeitgeber" able to synchronize the circadian rhythms to the light-dark cycles. Circadian rhythm dysfunction and sleep disorders have been associated with aging and neurodegenerative diseases including mild cognitive impairment (MCI) and Alzheimer's disease (AD). In the present study, we aimed at investigating the genetic variability of clock genes in AD patients compared to healthy controls from Italy. We also included a group of Italian centenarians, considered as super-controls in association studies given their extreme phenotype of successful aging. We analyzed the exon sequences of eighty-four genes related to circadian rhythms, and the most significant variants identified in this first discovery phase were further assessed in a larger independent cohort of AD patients by matrix assisted laser desorption/ionization-time of flight mass spectrometry. The results identified a significant

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association between the rs3027178 polymorphism in the *PER1* circadian gene with AD, the G allele being protective for AD. Interestingly, rs3027178 showed similar genotypic frequencies among AD patients and centenarians. These results collectively underline the relevance of circadian dysfunction in the predisposition to AD and contribute to the discussion on the role of the relationship between the genetics of age-related diseases and of longevity.

Keywords Aging · Alzheimer's disease · Centenarians · CLOCK genes · Polymorphism · Circadian rhythms

Introduction

The circadian clock is an evolutionary-conserved internal time-keeping system, able to control various physiological processes through the generation of approximately 24-h circadian rhythms in gene expression, which are translated into rhythms of metabolism, sleep, body temperature, blood pressure, cardiovascular, immune, endocrine and renal functions [1, 2]. Two major components include a central clock, residing in the suprachiasmatic nucleus (SCN) of the hypothalamus, and the peripheral clocks, present in nearly every tissue and organ system. Both central and peripheral clocks can be reset by environmental signals, also known as "zeitgebers", the predominant of which for the central clock is light, sensed by retina and synchronizing the circadian rhythms to the light-dark cycles [3, 4]. The central clock entrains the peripheral ones through neuronal and hormonal signals, body temperature and feeding-related stimuli, ultimately aligning all clocks with the external light/dark cycle.

In mammals, the regulation of circadian oscillators occurs through a series of positive/negative transcriptional-translational feedback loops including at least nine core circadian genes [5]. Among them, period homolog (PER1, PER2 and PER3) and cryptochrome (CRY1 and CRY2) clock proteins form complexes to negatively inhibit the nuclear transcription activities of the heterodimers formed by the transcription factors circadian locomotor output cycles kaput (CLOCK) [6] with aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL; also known as BMAL1) [7, 8]. Circadian gene regulation is a complex, temporally orchestrated process that involves not only the main circadian factors mentioned above but also a growing list of secondary or cell type-specific transcription factors, transcription co-regulators and epigenetic activities [4].

The synchronization of the endogenously generated circadian clocks to the light-dark cycle is possible thanks to the projections of the retinal ganglion cells expressing the photopigment melanopsin (mRGCs) to the SCN through the

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Unravelling the Association Between Amyloid-PET and Cerebrospinal Fluid Biomarkers in the Alzheimer's Disease Spectrum: Who Really Deserves an A+?

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Abstract.
Background: Association between cerebrospinal fluid (CSF)-amyloid-β $(Aβ)_{42}$ and amyloid-PET measures is inconstant across the Alzheimer's disease (AD) spectrum. However, they are considered interchangeable, along with $Aβ_{42/40}$ ratio, for defining 'Alzheimer's Disease pathologic change' (A+).
Objective: Herein, we further characterized the association between amyloid-PET and CSF biomarkers and tested their agreement in a cohort of AD spectrum patients.
Methods: We included 23 patients who underwent amyloid-PET, MRI, and CSF analysis showing reduced levels of $Aβ_{42}$ within a 365-days interval. Thresholds used for dichotomization were: $Aβ_{42} < 640$ pg/mL ($Aβ_{42}$+); pTau > 61 pg/mL (pTau+); and $Aβ_{42/40} < 0.069$ (AD_{ratio}+). Amyloid-PET scans were visually assessed and processed by four pipelines (SPM_{CL}, SPM_{AAL}, FS_{GM}, FS_{WC}).
Results: Different pipelines gave highly inter-correlated standardized uptake value ratios (SUVRs) (rho = 0.93–0.99). The most significant findings were: pTau positive correlation with SPM_{CL} SUVR (rho = 0.56, $p = 0.0063$) and $Aβ_{42/40}$ negative correlation with SPM_{CL} and SPM_{AAL} SUVRs (rho = –0.56, $p = 0.0058$; rho = –0.52, $p = 0.0117$ respectively). No correlations between CSF-$Aβ_{42}$ and global SUVRs were observed. In subregion analysis, both pTau and $Aβ_{42/40}$ values significantly correlated with cingulate SUVRs from any pipeline ($R^2 = 0.55$–0.59, $p < 0.0083$), with the strongest associations observed for the posterior/isthmus cingulate areas. However, only associations observed for $Aβ_{42/40}$ ratio were still significant in linear regression models. Moreover, combining pTau with $Aβ_{42}$ or using $Aβ_{42/40}$, instead of $Aβ_{42}$ alone, increased concordance with amyloid-PET status from 74% to 91% based on visual reads and from 78% to 96% based on Centiloids.
Conclusion: We confirmed that, in the AD spectrum, amyloid-PET measures show a stronger association and a better agreement with CSF-$Aβ_{42/40}$ and secondarily pTau rather than $Aβ_{42}$ levels.

Keywords: Alzheimer's disease, $Aβ_{42/40}$ ratio, amyloid, amyloid-PET, biomarkers, centiloids, cerebrospinal fluid, Florbetaben, standardized uptake value ratio, tau

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One further consideration speaks in favor of $A\beta_{42}$+/aPET- being false positive cases, though not unequivocally: the majority of $A\beta_{42}$+/aPET- patients did not develop typical AD, defined as an amnestic syndrome of the hippocampal type, at follow-up (Table 1). However, clinical diagnoses, particularly in highly specialized centers, are recursively linked to biomarker status and should thus not be taken as per se evidence of disease.

A major limitation of this study is the lack of neuropathological confirmatory data, though amyloid PET data show very strong agreement with post-mortem plaque measurements. Another limitation is the rather low number of patients included. However, all data were collected from clinical practice, in which two main issues arise: amyloid-PET is often used as an alternative to CSF biomarkers in patients who cannot undergo LP (e.g., anticoagulation) or as a confirmatory exam in equivocal diagnoses after a period of follow-up; MRI studies are often performed in peripheral centers and thus not always available for analysis. For these reasons, many patients lacked either CSF or MRI in the pre-established one-year interval from amyloid-PET. This was especially true for $A\beta_{42}$-/aPET+ patients. In fact, though we recognize the possibility of 'PET-first' pathway toward established Aβ pathology [60, 61], a more inclusive study design incorporating also $A\beta_{42}$-/aPET+ cases was not possible because most of them did not satisfy the 365-days-interval criterion, consistently with a clinical scenario in which CSF- patients need an adequate span of time to accumulate detectable amount of Aβ by PET.

The main strength of our study is that all CSF analyses were performed according to the same routine and analyzed in the same laboratory and all PET images were acquired at the same PET scanner with the same standardized protocol, conferring high homogeneity to data. Also, four different pipelines for the quantification of SUVR were used, significantly reducing the risk of SUVR quantification bias.

CONCLUSIONS

Our study suggests that, within the AD spectrum, amyloid-PET measures show a stronger association with CSF $A\beta_{42/40}$ and secondarily pTau rather than $A\beta_{42}$ levels. CSF-$A\beta_{42}$ and PET provide partially independent and not interchangeable information [16], the latter sharing with $A\beta_{42/40}$ ratio [19, 65] a greater concordance in defining A+ status. Since $A\beta_{42}$, $A\beta_{42/40}$, and amyloid-PET are all included in the "A" category of the ATN classification system, different operationalizations of the AT(N) system could have important effects on category prevalence [60,70], which would in turn negatively affect patients selection and results comparability in future clinical trials.

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SUPPLEMENTARY MATERIAL

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Cognitive composites for genetic frontotemporal dementia: GENFI-Cog

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Abstract

Background: Clinical endpoints for upcoming therapeutic trials in frontotemporal dementia (FTD) are increasingly urgent. Cognitive composite scores are often used as endpoints but are lacking in genetic FTD. We aimed to create cognitive composite scores for genetic frontotemporal dementia (FTD) as well as recommendations for recruitment and duration in clinical trial design.

Methods: A standardized neuropsychological test battery covering six cognitive domains was completed by 69 *C9orf72*, 41 *GRN*, and 28 *MAPT* mutation carriers with CDR® plus NACC-FTLD ≥ 0.5 and 275 controls. Logistic regression was used to identify the combination of tests that distinguished best between each mutation carrier group and controls. The composite scores were calculated from the weighted averages of test scores in the models based on the regression coefficients. Sample size estimates were calculated for individual cognitive tests and composites in a theoretical trial aimed at preventing progression from a prodromal stage (CDR® plus NACC-FTLD 0.5) to a fully symptomatic stage (CDR® plus NACC-FTLD ≥ 1). Time-to-event analysis was performed to determine how quickly mutation carriers progressed from CDR® plus NACC-FTLD = 0.5 to ≥ 1 (and therefore how long a trial would need to be).

Results: The results from the logistic regression analyses resulted in different composite scores for each mutation carrier group (i.e. *C9orf72*, *GRN*, and *MAPT*). The estimated sample size to detect a treatment effect was lower for composite scores than for most individual tests. A Kaplan-Meier curve showed that after 3 years, ~ 50% of individuals had converted from CDR® plus NACC-FTLD 0.5 to ≥ 1, which means that the estimated effect size needs to be halved in sample size calculations as only half of the mutation carriers would be expected to progress from CDR® plus NACC FTLD 0.5 to ≥ 1 without treatment over that time period.

Discussion: We created gene-specific cognitive composite scores for *C9orf72*, *GRN*, and *MAPT* mutation carriers, which resulted in substantially lower estimated sample sizes to detect a treatment effect than the individual cognitive

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JMP, KMM, JN, and JDR contributed to the conception and design of the work and the analysis of the data. JMP drafted the original work. All authors contributed to the acquisition and interpretation of the data and revised the work. All authors read and approved the final manuscript.

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An Automated Toolbox to Predict Single Subject Atrophy in Presymptomatic *Granulin* Mutation Carriers

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RESEARCH ARTICLE

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Data-driven staging of genetic frontotemporal dementia using multi-modal MRI

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Amyloid PET imaging and dementias: potential applications in detecting and quantifying early white matter damage

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Abstract

Purpose: Positron emission tomography (PET) with amyloid tracers (amy-PET) allows the quantification of pathological amyloid deposition in the brain tissues, including the white matter (WM). Here, we evaluate amy-PET uptake in WM lesions (WML) and in the normal-appearing WM (NAWM) of patients with Alzheimer's disease (AD) and non-AD type of dementia.

Methods: Thirty-three cognitively impaired subjects underwent brain magnetic resonance imaging (MRI), $A\beta_{1\text{-}42}$ (Aβ) determination in the cerebrospinal fluid (CSF) and amy-PET. Twenty-three patients exhibiting concordant results in both CSF analysis and amy-PET for cortical amyloid deposition were recruited and divided into two groups, amyloid positive (A+) and negative (A−). WML quantification and brain volumes' segmentation were performed. Standardized uptake values ratios (SUVR) were calculated in the grey matter (GM), NAWM and WML on amy-PET coregistered to MRI images.

Results: A+ compared to A− showed a higher WML load ($p = 0.049$) alongside higher SUVR in all brain tissues ($p < 0.01$). No correlations between CSF Aβ levels and WML and NAWM SUVR were found in A+, while, in A−, CSF Aβ levels were directly correlated to NAWM SUVR ($p = 0.04$). CSF Aβ concentration was the only predictor of NAWM SUVR (adj $R^2 = 0.91$; $p = 0.04$) in A−. In A+ but not in A− direct correlations were identified between WM and GM SUVR ($p < 0.01$).

Conclusions: Our data provide evidence on the role of amy-PET in the assessment of microstructural WM injury in non-AD dementia, whereas amy-PET seems less suitable to assess WM damage in AD patients due to a plausible amyloid accrual therein.

Keywords: amy-PET, Amyloid, Alzheimer's disease, Non-AD dementias, White matter

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder and the main cause of dementia [1]. The hallmarks of AD pathology are the cortical deposition of beta-amyloid (Aβ) and the aggregation of tau protein into neurofibrillary tangles [2]. In addition to grey matter (GM) pathology, white matter (WM) changes were recently recognized as an important

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quantification accuracy when dealing with brain PET imaging, mainly due to the limited spatial resolution. Some previous studies have used partial volume correction (PVC) for white matter SUVR quantification. However, quantitative amy-PET imaging is usually conducted without PVC, due to the lack of a standardized and widely accepted PVC method, and some authors reported worse results and comparability using PVC as compared to native images. We applied an iterative spatial resolution reconstruction algorithm (TrueX) to images before performing SUVR quantification. Although TrueX cannot be fully equated to a PVC method, it already reduces significantly the partial volume effect.

Conclusions

This study provides evidence on the role of amy-PET in the assessment of microstructural WM injury in non-AD dementia, whereas amy-PET seems less suitable to assess WM damage in AD patients due to a plausible amyloid accrual therein. Therefore, a specific study on AD patients is worth to be specifically performed. A replication in a larger cohort of patients is required to confirm these preliminary data.

Authors' contributions

AMP and AC designed the study, analysed and interpreted the data and drafted the manuscript. TC and LC contributed to the analysis and interpretation of the data. CF performed CSF analyses. AA, MAD and GGF added a minor contribution to the analysis of the data. GM acquired and analysed the PET data. MC, MB, EAS and DG drafted and revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used in this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Consent for publication

Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that they have no competing interests.

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Behavioural Neurology

Examining empathy deficits across familial forms of frontotemporal dementia within the GENFI cohort

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ABSTRACT

Background: Reduced empathy is a common symptom in frontotemporal dementia (FTD). Although empathy deficits have been extensively researched in sporadic cases, few studies have explored the differences in familial forms of FTD.

Methods: Empathy was examined using a modified version of the Interpersonal Reactivity Index (mIRI) in 676 participants from the Genetic FTD Initiative: 216 mutation-negative controls, 192 *C9orf72* expansion carriers, 193 *GRN* mutation carriers and 75 *MAPT* mutation carriers. Using global scores from the CDR® plus NACC FTLD, mutation carriers were divided into three groups, asymptomatic (0), very mildly symptomatic/prodromal (.5), or fully symptomatic (1 or more). The mIRI Total score, as well as the subscores of Empathic Concern (EC) and Perspective Taking (PT) were assessed. Linear regression models with bootstrapping were used to assess empathy ratings across genetic groups, as well as across phenotypes in the symptomatic carriers. Neural correlates of empathy deficits were examined using a voxel-based morphometry (VBM) analysis.

Results: All fully symptomatic groups scored lower on the mIRI Total, EC, and PT when compared to controls and their asymptomatic or prodromal counterparts (all $p < .001$). Prodromal *C9orf72* expansion carriers also scored significantly lower than controls on the mIRI Total score ($p = .046$). In the phenotype analysis, all groups (behavioural variant FTD, primary progressive aphasia and FTD with amyotrophic lateral sclerosis) scored significantly lower than controls (all $p < .007$). VBM revealed an overlapping neural correlate of the mIRI Total score across genetic groups in the orbitofrontal lobe but with additional

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Structural brain splitting is a hallmark of *Granulin*-related frontotemporal dementia

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ORIGINAL COMMUNICATION

Anomia is present pre-symptomatically in frontotemporal dementia due to *MAPT* mutations

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Abstract

Introduction A third of frontotemporal dementia (FTD) is caused by an autosomal-dominant genetic mutation in one of three genes: microtubule-associated protein tau (*MAPT*), chromosome 9 open reading frame 72 (*C9orf72*) and progranulin (*GRN*). Prior studies of prodromal FTD have identified impaired executive function and social cognition early in the disease but few have studied naming in detail.

Methods We investigated performance on the Boston Naming Test (BNT) in the GENetic Frontotemporal dementia Initiative cohort of 499 mutation carriers and 248 mutation-negative controls divided across three genetic groups: *C9orf72*, *MAPT* and *GRN*. Mutation carriers were further divided into 3 groups according to their global CDR plus NACC FTLD score: 0 (asymptomatic), 0.5 (prodromal) and 1 + (fully symptomatic). Groups were compared using a bootstrapped linear regression model, adjusting for age, sex, language and education. Finally, we identified neural correlates of anomia within carriers of each genetic group using a voxel-based morphometry analysis.

Results All symptomatic groups performed worse on the BNT than controls with the *MAPT* symptomatic group scoring the worst. Furthermore, *MAPT* asymptomatic and prodromal groups performed significantly worse than controls. Correlates of anomia in *MAPT* mutation carriers included bilateral anterior temporal lobe regions and the anterior insula. Similar bilateral anterior temporal lobe involvement was seen in *C9orf72* mutation carriers as well as more widespread left frontal atrophy. In *GRN* mutation carriers, neural correlates were limited to the left hemisphere, and involved frontal, temporal, insula and striatal regions.

Conclusion This study suggests the development of early anomia in *MAPT* mutation carriers, likely to be associated with impaired semantic knowledge. Clinical trials focused on the prodromal period within individuals with *MAPT* mutations should use language tasks, such as the BNT for patient stratification and as outcome measures.

Keywords Frontotemporal dementia · Tau · Progranulin · C9orf72 · Naming · Cognition

Introduction

Frontotemporal dementia (FTD) is a heterogeneous neurodegenerative disorder presenting with distinct changes in behaviour, language and motor function [1]. A third of cases are caused by an autosomal-dominant genetic mutation in one of three genes: microtubule-associated protein tau (*MAPT*), chromosome 9 open reading frame 72 (*C9orf72*)

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outcome measure for international clinical trials in presymptomatic *MAPT* mutation carriers, and in helping differential diagnosis and severity staging by understanding the sources of naming difficulty.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00415-022-11068-0.

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OPEN ACCESS

Original research

Development of a sensitive trial-ready poly(GP) CSF biomarker assay for *C9orf72*-associated frontotemporal dementia and amyotrophic lateral sclerosis

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ABSTRACT

Objective A GGGGCC repeat expansion in the *C9orf72* gene is the most common cause of genetic frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). As potential therapies targeting the repeat expansion are now entering clinical trials, sensitive biomarker assays of target engagement are urgently required. Our objective was to develop such an assay.

Methods We used the single molecule array (Simoa) platform to develop an immunoassay for measuring poly(GP) dipeptide repeat proteins (DPRs) generated by the *C9orf72* repeat expansion in cerebrospinal fluid (CSF) of people with *C9orf72*-associated FTD/ALS.

Results and conclusions We show the assay to be highly sensitive and robust, passing extensive qualification criteria including low intraplate and interplate variability, a high precision and accuracy in measuring both calibrators and samples, dilutional parallelism, tolerance to sample and standard freeze–thaw and no haemoglobin interference. We used this assay to measure poly(GP) in CSF samples collected through the Genetic FTD Initiative (N=40 *C9orf72* and 15 controls). We found it had 100% specificity and 100% sensitivity and a large window for detecting target engagement, as the *C9orf72* CSF sample with the lowest poly(GP) signal had eightfold higher signal than controls and on average values from *C9orf72* samples were 38-fold higher than controls, which all fell below the lower limit of quantification of the assay. These data indicate that a Simoa-based poly(GP) DPR assay is suitable for use in clinical trials to determine target engagement of therapeutics aimed at reducing *C9orf72* repeat-containing transcripts.

Key messages

⇒ Accurate measurement of dipeptide repeat proteins (DPRs) generated by the frontotemporal dementia and amyotrophic lateral sclerosis-causing repeat expansion in *C9orf72* will be a key tool for assessing target engagement of repeat/DPR lowering strategies in clinical trials.

⇒ Immunoassays have been developed that can detect the poly(GP) DPR in patient cerebrospinal fluid (CSF), but as some patients' poly(GP) levels are close to background, enhanced sensitivity may be needed.

⇒ We report the development of an ultrasensitive CSF poly(GP) detection assay that is fit-for-purpose for clinical trials. This should allow target engagement to be assessed in the vast majority of trial participants, including those with low poly(GP) levels.

INTRODUCTION

A GGGGCC repeat expansion in the first intron of *C9orf72* is the most common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), accounting for 38% and 25% of familial cases, respectively.[1] Healthy individuals most commonly have two repeats,[2] while people with a *C9orf72* repeat expansion (C9FTD/ALS) can carry hundreds to thousands of repeats.[3–6] The repeats are transcribed in both sense and antisense direction, leading to the formation of RNA aggregates termed RNA foci.[7–10] In

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combinations. In our experience not all polyclonal antibodies behave the same, even when the same peptide sequence was used for antigen. We tested the performance of a monoclonal antibody as both capture and detector in a Homebrew Simoa assay. Unfortunately, the monoclonal antibody tested here did not perform as well as a detector antibody as the polyclonal antibodies, with much higher predicted LLOQs. The reason for this difference is unclear, but the different polyclonal antibodies may recognise different secondary structures of poly(GP).

We used our qualified poly(GP) assay to analyse CSF from a small cohort of CSF samples provided by GENFI, including 15 healthy controls and 40 *C9orf72* expansion carriers. Similar to previously published studies,[17 24 37] our assay was able to distinguish controls and *C9orf72* expansion carriers. In this cohort we had 100% sensitivity and 100% specificity with poly(GP) measured in CSF from all *C9orf72* expansion carriers, while controls either measured below detection (13/15) or below limit of quantification (2/15), determined at 1 pg/mL. *C9orf72* expansion carriers had a range of poly(GP) from 6 to 148 pg/mL, with all positive sample signals at least eightfold higher than control signals, showing a clear separation of controls from *C9orf72* expansion samples. We did not detect poly(GP) above LLOQ in Alzheimer's disease or patients with non-*C9orf72* FTD. All previous studies used MSD immunoassays and reported the average CSF polyGP signal to be in the low nanogram range,[17 37] while our assay gives average polyGP levels in the low–medium picogram range. This difference may be attributed to the different calibrators used in the studies, as we have noted that the same antibody can report different concentrations depending on the calibrator used. The use of different calibrators precludes a direct comparison of the different assays. Simoa technology allows detection of single molecules by converting signal from individual beads into a digital output, which we predict will provide higher sensitivity than the MSD assays that rely on an analogue output from each sample well. Although Simoa assays will not be more sensitive than MSD assays in all cases, as this will depend on the specific antibodies used, we do observe higher sensitivity compared with our standard polyGP MSD assay.[11 38 39] A limitation of our study is that we did not carry out robustness analysis, defined as the capacity of the assay to withstand small but deliberate changes in method parameters such as incubation times, temperatures and buffer pH.[40]

In our cohort of samples we found, similar to previous studies,[17 24] that compared with presymptomatic carriers, symptomatic carriers had higher levels of poly(GP) comparing mean levels, but this difference was not significant. As we observed a trend towards higher polyGP levels with increasing age at donation, the older age of symptomatic carriers may contribute to this effect, although we note that polyGP levels were shown to remain stable on longitudinal testing over 18–24 months.[17] Meeter *et al*[37] found levels in symptomatic carriers were significantly higher.[37] This may be due to the larger cohort size tested with more symptomatic donors with higher than average poly(GP) levels included. Within our small cohort there was one symptomatic *C9orf72* carrier with much higher poly(GP) levels than the rest. Age at onset (66 years) and age at donation (68 years) were both within 1 SD from the mean of other symptomatic donors, indicating no effect of higher levels of poly(GP) on these parameters. We did not have repeat length data for this cohort, although given the variability in repeat length between different tissues in the body it would be difficult to interpret repeat length data determined from blood DNA. Lehmer *et al* found no correlation between repeat size and CSF poly(GP) levels in 11 cases where DNA was available.[24] Should postmortem tissue become available from donors in this cohort, it would be interesting to determine repeat length from brain tissue as well as measure propensity of DPR aggregates in the brain to see if poly(GP) CSF levels reflected aggregate burden.

Similar to previous studies we found no correlations between CSF poly(GP) levels and clinical features including; gender, age of onset or brain volume, analysing either total *C9orf72* cases or just symptomatic *C9orf72* carriers.[17 24 37] We did observe a correlation between CSF poly(GP) levels and age at donation, which is potentially consistent with a relationship between *C9orf72* expansion length and age at DNA sample collection.[41] We analysed NfL levels in a subset of donor matched plasma samples. As expected, symptomatic carriers had higher NfL plasma levels than presymptomatic or controls. As in previous studies that measured NfL in CSF,[24 37] NfL plasma levels did not correlate with poly(GP) CSF levels. Despite the ability of the Simoa assays to detect at single-molecule levels, we were unable to measure poly(GP) in donor matched plasma samples. Signals for all samples were below quantification and did not correlate with poly(GP) CSF levels. If poly(GP) produced in the brain is present in plasma it will require a more sensitive assay platform and a better understanding of potential matrix effects. In summary, we show utility of the Simoa HD-X platform for detecting poly(GP) in the CSF of people with a *C9orf72* expansion, with assay reliability good enough to be used for target engagement analysis in clinical trials directly targeting *C9orf72* repeat containing transcripts.

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RESEARCH ARTICLE

CSF glial markers are elevated in a subset of patients with genetic frontotemporal dementia

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Adjusted mean differences, 95% bootstrapped confidence intervals, and *p*-values from the linear regression models (adjusted for age and sex): (A) TREM2, (B) YKL-40, (C) CHIT1. PS is presymptomatic, S is symptomatic.

Table S2. Mean (standard deviation) concentrations of the microglial activation markers in each decade of life within the controls (excluding the two undetectable concentrations of CHIT1 in controls). Spearman correlation of each measure with age was as follows: TREM2 $r = 0.42$, $p = 0.0008$, YKL-40 $r = 0.71$, $p < 0.0001$, CHIT1 $r = 0.21$, $p = 0.1013$.

Figure S1. Partial correlations (adjusting for age) of CHIT1 with Mini-Mental State Examination in GRN mutation carriers (A) presymptomatic and (B) symptomatic.

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Article

A Novel Automated Chemiluminescence Method for Detecting Cerebrospinal Fluid Amyloid-Beta 1-42 and 1-40, Total Tau and Phosphorylated-Tau: Implications for Improving Diagnostic Performance in Alzheimer's Disease

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Abstract: Recently, a fully automated instrument for the detection of the Cerebrospinal Fluid (CSF) biomarker for Alzheimer's disease (AD) (low concentration of Amyloid-beta 42 (Aβ42), high concentration of total tau (T-tau) and Phosphorylated-tau (P-tau181)), has been implemented, namely CLEIA. We conducted a comparative analysis between ELISA and CLEIA methods in order to evaluate the analytical precision and the diagnostic performance of the novel CLEIA system on 111 CSF samples. Results confirmed a robust correlation between ELISA and CLEIA methods, with an improvement of the accuracy with the new CLEIA methodology in the detection of the single biomarkers and in their ratio values. For Aβ42 regression analysis with Passing–Bablok showed a Pearson correlation coefficient r = 0.867 (0.8120; 0.907% 95% CI $p < 0.0001$), T-tau analysis: r = 0.968 (0.954; 0.978% 95% CI $p < 0.0001$) and P-tau181: r = 0.946 (0.922; 0.962 5% 95% CI $p < 0.0001$). The overall ROC AUC comparison between ROC in ELISA and ROC in CLEIA confirmed a more accurate ROC AUC with the new automatic method: T-tau AUC ELISA = 0.94 (95% CI 0.89; 0.99 $p < 0.0001$) vs. AUC CLEIA = 0.95 (95% CI 0.89; 1.00 $p < 0.0001$), and P-tau181 AUC ELISA = 0.91 (95% CI 0.85; 0.98 $p < 0.0001$) vs. AUC CLEIA = 0.98 (95% CI 0.95; 1.00 $p < 0.0001$). The performance of the new CLEIA method in automation is comparable and, for tau and P-tau181, even better, as compared with standard ELISA. Hopefully, in the future, automation could be useful in clinical diagnosis and also in the context of clinical studies.

Keywords: CSF; biomarkers; Alzheimer's disease; ELISA; CLEIA

1. Introduction

Several studies report the usefulness of cerebrospinal fluid (CSF) biomarkers in the diagnostic setting of Alzheimer's disease (AD) [1] and recent evidence underline an important association between CSF biomarkers such as Amyloid-beta 1-42 (Aβ42), tau and AD neuropathological changes (ADNC) [2].

The biomarker pattern, commonly referred to as the "AD signature", typically displays decreased concentration of Aβ42 and increased concentration of total tau (T-tau) and Phosphorylated-tau (P-tau181). In particular, by combining CSF Aβ42, T-tau and P-tau181, a higher diagnostic accuracy for identification of AD from non-AD dementia, as well as the prediction of progression to AD in patients with Mild Cognitive Impairment (MCI), can be

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Article

Circulating Non-Coding RNA Levels Are Altered in Autosomal Dominant Frontotemporal Dementia

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Abstract: Frontotemporal Dementia (FTD) represents a highly heritable neurodegenerative disorder. Most of the heritability is caused by autosomal dominant mutations in the Microtubule-Associated Protein Tau (*MAPT*), Progranulin (*GRN*), and the pathologic exanucleotide expansion of *C9ORF72* genes. At the pathological level, either the tau or the TAR DNA-binding protein (TDP-43) account for almost all cases of FTD. Pathogenic mechanisms are just arising, and the emerging role of non-coding RNAs (ncRNAs), such as microRNAs (miRNA) and long non-coding RNAs (lncRNAs), have become increasingly evident. Using specific arrays, an exploratory analysis testing the expression levels of 84 miRNAs and 84 lncRNAs has been performed in a population consisting of 24 genetic FTD patients (eight *GRN*, eight *C9ORF72*, and eight *MAPT* mutation carriers), eight sporadic FTD patients, and eight healthy controls. The results showed a generalized ncRNA downregulation in patients carrying *GRN* and *C9ORF72* when compared with the controls, with statistically significant results for the following miRNAs: miR-155-5p (Fold Change FC: 0.45, $p = 0.037$ FDR = 0.52), miR-15a-5p (FC: 0.13, $p = 0.027$, FDR = 1), miR-222-3p (FC: 0.13, $p = 0.027$, FDR = 0.778), miR-140-3p (FC: 0.096, $p = 0.034$, FRD = 0.593), miR-106b-5p (FC: 0.13, $p = 0.02$, FDR = 0.584) and an upregulation solely for miR-124-3p (FC: 2.1, $p = 0.01$, FDR = 0.893). Conversely, *MAPT* mutation carriers showed a generalized robust upregulation in several ncRNAs, specifically for miR-222-3p (FC: 22.3, $p = 7 \times 10^{-6}$, FDR = 0.117), miR-15a-5p (FC: 30.2, $p = 0.008$, FDR = 0.145), miR-27a-3p (FC: 27.8, $p = 6 \times 10^{-6}$, FDR = 0.0005), miR-223-3p (FC: 18.9, $p = 0.005$, FDR = 0.117), and miR-16-5p (FC: 10.9, $p = 5.26 \times 10^{-5}$, FDR = 0.001). These results suggest a clear, distinctive pattern of dysregulation among ncRNAs and specific enrichment gene pathways between mutations associated with the TDP-43 and tau pathologies. Nevertheless, these preliminary results need to be confirmed in a larger independent cohort.

Keywords: frontotemporal disease; microRNA; long non-coding RNA; *GRN*; *MAPT*; *C9ORF72*; TDP-43; tau

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1. Introduction

Frontotemporal dementia (FTD) represents the most common cause of presenile dementia, usually affecting people under 60 years old [1]. Clinically, patients present with changes in either behavior or personality. Up to 40% of patients have a history of familial transmission, with nearly 10% of patients showing an autosomal dominant inheritance pattern [1]. The majority of familial FTD patients carry mutations in the Microtubule-Associated Protein Tau (*MAPT*) and Progranulin (*GRN*) genes, and the pathologic expansion of the hexanucleotide G_4C_2 repeats in the first intron of the *C9ORF72* gene [2]. At

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Primary progressive aphasia and motor neuron disease: A review

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Background: This study aims at reviewing, within the framework of motor neuron disease-frontotemporal degeneration (MND-FTD)-*spectrum* disorders, evidence on the co-occurrence between primary progressive aphasia (PPA) and MND in order to profile such a complex at pathological, genetic and clinical levels.

Methods: This review was pre-registered (osf.io/ds8m4) and performed in accordance with the 2020 PRISMA guidelines. Case reports/series and group studies were included if addressing (1) progressive non-fluent aphasia (PNFA) or semantic dementia (SD) with MND or (2) MND patients with co-morbid PNFA/SD.

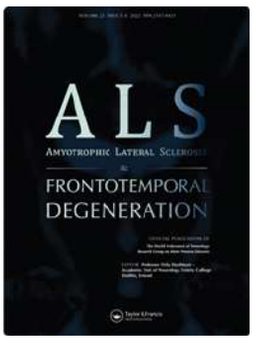
Results: Out of 546 initial records, 56 studies were included. As to case reports/series (N = 35), which included 61 PPA-MND patients, the following findings yielded: (1) PNFA is more frequent than SD in PPA-MND; (2) in PPA-MND, the most prevalent motor phenotypes are amyotrophic lateral sclerosis and predominant-upper MND, with bulbar involvement being ubiquitous; (3) extrapyramidal features are moderately frequent in PPA-MND; (4) PPA-MND patients usually display frontotemporal, left-greater-than-right involvement; (5) TDP-43-B is the typical pathological substrate of PPA-MND; (6) *TBK1* mutations represent the most frequent genetic risk factors for PPA-MND.

As to group studies, including 121 patients, proportional meta-analytic procedures revealed that: (1) the lifetime prevalence of MND in PPA is 6%; (2) PPA occurs in 19% of patients with co-morbid MND and FTD; (3) MND is more frequent in PNFA (10%) than in SD patients (3%).

Discussion: Insights herewith delivered into the clinical, neuropathological and genetic features of PPA-MND patients prompt further investigations aimed at improving clinical practice within the MND-FTD *spectrum*.

KEYWORDS

primary progressive aphasia, motor neuron disease, frontotemporal degeneration, amyotrophic lateral sclerosis, language



Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

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Reliable change indices for the Italian Edinburgh Cognitive and Behavioral ALS Screen (ECAS)

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REPORT

Reliable change indices for the Italian Edinburgh Cognitive and Behavioral ALS Screen (ECAS)

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Abstract

This study aimed at providing standardized regression-based (SRB) reliable change indices (RCIs) for the Italian Edinburgh Cognitive and Behavioral ALS Screen (ECAS). Thirty-one consecutive ALS patients undergoing the ECAS were followed-up (T1) at 6.5 ± 1 months (*range* = 5–8). Ceiling/floor effects, practice effect, and test–retest reliability were assessed. Each ECAS measure was regressed by stepwise-entering as predictors demographics, respective T0 scores, T0 disease duration and ALSFRS-R, retest interval, and progression rate (ΔFS) – i.e., (48 - ALSFRS-R_{T0})/disease $duration_{T0}$ in months. Ceiling effects were infrequently detected, no practice effect emerged and all ECAS measures were reliable at retest (except for Language and Visuo-spatial subscales). T0 scores predicted all ECAS measures except for the Visuo-spatial subscale. The availability of RCIs for the Italian ECAS will aid ALS-related clinical practice and research within the longitudinal dimension.

Keywords: *Reliable change index, Edinburgh Cognitive and Behavioral ALS Screen, amyotrophic lateral sclerosis, neuropsychology, psychometrics*

1. Background

Frontotemporal-*spectrum* cognitive deficits are highly incident in ALS (1) and may worsen with disease progression (2) – negatively affecting patients' prognosis (1). Thereupon, it is recommended that ALS patients undergo periodical cognitive screenings, ideally every 6 months (3).

However, when repeatedly assessing cognition over time, multiple sources of systematic error variance might enter test scores – these being both test (i.e., reliability, practice effect, and ceiling/floor effects), context (i.e., retest interval and regression to the mean), and patient-related (i.e., baseline demographic, cognitive, and motor-functional status) (4). It is thus a matter of interest to identify clinically meaningful changes in patients' cognition net of such confounders (5).

Regression-based approaches to derive reliable change indices (RCIs) for cognitive tests allow to determine whether an individual difference between repeated measurements actually reflects a systematic, true (i.e., reliable) variation of the underlying, target construct (i.e., cognition) net of test-, context-, or patient-related intervening variables (4).

Since such methods are regarded as the current gold-standard to the above scope (4) and have been previously applied to the cognitive section of

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The Frontal Assessment Battery (FAB) effectively discriminates between MCI and dementia within the clinical *spectrum* of neurochemically confirmed Alzheimer's disease

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Background: This study aimed at testing the ability of the frontal assessment battery (FAB) to differentiate between patients with mild cognitive impairment (MCI) and dementia due to Alzheimer's disease (AD), as well as comparing its discriminative power to that of the Mini-Mental State Examination (MMSE).

Methods: The present retrospective cohort included N = 107 Aβ-positive patients diagnosed with either MCI due to AD (N = 40) or probable AD dementia (ADD; N = 67). A two-step multiple logistic regression (MLR) was run to predict an MCI vs. ADD diagnosis based on FAB scores. Within the baseline step, demographics, disease duration, MMSE scores, and information on cognitive phenotypes were entered, with the FAB being added within the second step. Receiver-operating characteristics analyses were also run to derive intrinsic and post-test diagnostics.

Results: Within the baseline MLR step, only lower MMSE scores predicted the occurrence of ADD; by adding the FAB, which likewise was able to discriminate between MCI and ADD (p = 0.016), a significant increase in model fit was detected (p = 0.007). The diagnostic efficiency of the FAB (AUC = 0.85) was comparable (p = 0.583) to that of the MMSE (AUC = 0.82),

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Validity and diagnostics of the Reading the Mind in the Eyes Test (RMET) in non-demented amyotrophic lateral sclerosis (ALS) patients

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Background: The aim of this study was to explore the construct validity and diagnostic properties of the Reading the Mind in the Eyes Test (RMET) in non-demented patients with amyotrophic lateral sclerosis (ALS).

Materials: A total of 61 consecutive patients and 50 healthy controls (HCs) were administered the 36-item RMET. Additionally, patients underwent a comprehensive assessment of social cognition *via* the Story-Based Empathy Task (SET), which encompasses three subtests targeting Causal Inference, Emotion Attribution (SET-EA), and Intention Attribution (SET-IA), as well as global cognitive [the Edinburgh Cognitive and Behavioral ALS Screen (ECAS)] and behavioral screening [the Frontal Behavioral Inventory (FBI); the Dimensional Apathy Scale (DAS); the Beck Depression Inventory (BDI); and the State and Trait Anxiety Inventory-Y]. The construct validity of the RMET was tested by regressing it within a stepwise model that encompassed as predictors the abovementioned cognitive and behavioral measures, covarying for demographic and motor confounders. Receiver-operating characteristics (ROC) analyses allowed exploring intrinsic and post-test properties of the RMET both in discriminating patients from HCs and in identifying patients with a defective SET-EA performance.

Results: The RMET was solely predicted by the SET-EA (p = 0.003) and SET-IA (p = 0.005). RMET scores showed high accuracy both in discriminating patients from HCs (AUC = 0.81) and in identifying patients with a defective SET-EA score (AUC = 0.82), with adequate-to-optimal both intrinsic and post-test properties.

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Diagnostics and clinical usability of the Montreal Cognitive Assessment (MoCA) in amyotrophic lateral sclerosis

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Background: The present study aimed at (1) assessing the diagnostic properties of the Montreal Cognitive Assessment (MoCA) in non-demented ALS patients and at (2) exploring the MoCA administrability according to motor-functional status.

Materials: *N*=348 patients were administered the MoCA and Edinburgh Cognitive and Behavioural ALS Screen (ECAS). Administrability rates and prevalence of defective MoCA scores were compared across King's and Milano-Torino clinical stages. Regression models were run to test whether the non-administrability of the MoCA and a defective score on it were predicted, net of the ECAS-Total, by disease duration, ALS Functional Rating Scale-Revised (ALSFRS-R) and progression rate, computed as (48: ALSFRS-R)/disease duration. Intrinsic and post-test diagnostics were tested against a below-cut-off ECAS-total score.

Results: The 79.9% of patients successfully underwent the MoCA, whose administrability rates decreased with advanced clinical stages, at variance with its defective score prevalence. The probability of the FAB not being administrable was predicted only by lower ALSFRS-R-bulbar and-upper-limb scores; no motor features, but the ECAS-Total, predicted a defective MoCA performance. The MoCA showed high accuracy (AUC=0.82) and good intrinsic and post-test properties—being slightly more specific than sensitive.

Discussion: In non-demented ALS patients, the MoCA is featured by optimal diagnostics as a screener for cognitive impairment, especially for ruling-out its occurrence, as long as patients are in the early stages of the disease and have sufficiently spared bulbar and upper-limb functions.

KEYWORDS

Montreal Cognitive Assessment, amyotrophic lateral sclerosis, cognitive screening, diagnostics, psychometrics

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ORIGINAL ARTICLE

Feasibility and diagnostics of the Frontal Assessment Battery (FAB) in amyotrophic lateral sclerosis

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Abstract

Background Thepresent study aimed at evaluating the diagnostic properties of the Frontal Assessment Battery (FAB) in non-demented ALS patients by addressing the Edinburgh Cognitive Behavioural ALS Screen (ECAS) as the gold standard, as well as by examining the association between its administrability and scores with motor-functional measures.

Materials $N = 348$ consecutive patients were administered the ECAS and FAB. Disease severity (ALSFRS-R), duration, progression rate (ΔFS), and stages (via King's and Milano-Torino systems) were considered. Administrability rates and prevalence of below-cut-off FAB scores were compared across clinical stages; regression models allowed to test whether, net of the ECAS-Total, motor features predicted the probability of the FAB not being administrable and of a defective FAB score. Intrinsic and post-test diagnostics were explored against a combined defective ECAS-Executive and ECAS-Fluency scores.

Results 85.3% of patients managed to complete the FAB. FAB administrability rates decreased with advanced clinical stages, whereas the prevalence of below-cut-off FAB scores did not. The probability of the FAB not being administrable was predicted only by lower ALSFRS-R-bulbar and ALSFRS-R-upper-limb scores; no motor features, but the ECAS-Total, predicted a below-cut-off performance on the FAB. Raw and adjusted FAB scores showed high accuracy (AUC = .85 and .81, respectively) and good intrinsic and post-test properties.

Discussion The FAB is featured by optimal diagnostics for detecting executive deficits in ALS, provided that it can be administered according to its original, standardized procedure, and thus that patients have sufficiently spared motor abilities to complete the test.

Keywords Frontal assessment battery · Amyotrophic lateral sclerosis · Cognitive screening · Executive · Diagnostics · Psychometrics

Background

In ALS patients, the feasibility of the Frontal Assessment Battery (FAB) [1] as a screener for deficits of executive functioning (EF)—which are highly prevalent/incident in this population [2]—has been historically questioned due to its heavy reliance on motor-/verbal-mediated responses, and thus, the possibility of upper-limb disabilities/dysarthric features undermining test execution and/or confounding test scores [3].

Notwithstanding that disease-specific cognitive screeners [4] undisputedly come with the highest level of recommendation for use in both clinical practice [5] and research [6] as addressed to ALS patients, the FAB still appears to be a rather widespread test to screen for EF deficits in this population [7], being also supported by seemingly sound clinimetric evidence [8].

However, available information on the diagnostics of the FAB in ALS patients has the intrinsic downfall of coming from studies that compared it against gold standard measures that were disease-nonspecific [9, 10]. Analogously, those reports that focused on its feasibility in this population, albeit to the noble aim of accommodating motor disabilities, included off-label adjustments

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ORIGINAL ARTICLE

Clinimetrics of the cognitive section of the Italian ALS Cognitive Behavioral Screen (ALS-CBS™)

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Abstract

Background The present study aimed at (1) providing further validity and reliability evidence for the Italian version of the cognitive section of the ALS Cognitive Behavioral Screen (ALS-CBS™) and (2) testing its diagnostics within an Italian ALS cohort, as well as at (3) exploring its capability to discriminate patients from healthy controls (HCs).

Methods $N = 293$ non-demented ALS patients were administered the cognitive sections of the ALS-CBS™ and Edinburgh Cognitive and Behavioural ALS Screen (ECAS). $N = 96$ HCs demographically matched with $N = 96$ patients were also administered the cognitive section of the ALS-CBS™. In patients, factorial and construct validity, internal reliability, and diagnostics against a defective score on the cognitive section of the ECAS were tested. Case–control discrimination was assessed via a logistic regression.

Results ALS-CBS™ cognitive subscales were underpinned by a simple, unidimensional structure, internally reliable (McDonald's $\omega = 0.74$), and mostly related with ECAS *executive* and *fluency* scores ($r_s = 0.54$–0.71). Both raw and age- and education-adjusted scores on the cognitive section of the ALS-CBS™ accurately detected ECAS-defined cognitive impairment ($AUC = 0.80$ and .88, respectively), yielding optimal error-based, information-based and unitary diagnostics. A cut-off of < 15.374 was identified on adjusted scores. The test was able to discriminate patients from HCs ($p < 0.001$).

Discussion The cognitive section of the Italian ALS-CBS™ is a valid, reliable, and diagnostically sound ALS-specific screener for detecting frontotemporal, executive-/attentive-based cognitive inefficiency in non-demented ALS patients, being also able to discriminate them from normotypical individuals.

Keywords ALS Cognitive Behavioral Screen · Amyotrophic lateral sclerosis · Cognitive screening · Frontotemporal degeneration · Neuropsychology · Clinimetrics

Background

Cognitive deficits within the frontotemporal degeneration (FTD) *spectrum*—i.e., executive and language dysfunctions—affect up to 50% of non-demented amyotrophic lateral sclerosis (ALS) patients [1], negatively impacting on their prognosis and clinical management [2]. Early detecting FTD-*spectrum* cognitive impairment in this population is thereupon clinically pivotal [3]. Additionally, cognitive measures are addressed as outcomes within clinical trials addressing ALS [4].

To such an aim, disease-specific cognitive screeners—i.e., (1) sampling from those domains/functions typically involved in ALS and (2) controlling for motor disabilities possibly confounding cognitive performances—have been developed, namely the ALS Cognitive Behavioral Screen (ALS-CBS™) [5] and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS) [6]. The cognitive sections of

Edoardo Nicolò Aiello and Lucia Catherine Greco contributed equally; Valeria Ada Sansone and Barbara Poletti contributed equally as well.

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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

Tommaso Bocci and Laura Campiglio equally contributed to the work and are listed in an alphabetical order.

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GRN−/− iPSC-derived cortical neurons recapitulate the pathological findings of both frontotemporal lobar degeneration and neuronal ceroidolipofuscinosis

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ARTICLE INFO

Keywords:
Progranulin
Frontotemporal lobar degeneration
Neuronal ceroidolipofuscinosis
Induced pluripotent stem cells
Cortical neurons
Lysosomes
TDP-43
Fingerprints

ABSTRACT

Heterozygous mutations in the gene coding for progranulin (*GRN*) cause frontotemporal lobar degeneration (FTLD) while homozygous mutations are linked to neuronal ceroidolipofuscinosis (NCL). While both FTLD/NCL pathological hallmarks were mostly investigated in heterozygous *GRN*+/− brain tissue or induced pluripotent stem cell (iPSC)-derived neurons, data from homozygous *GRN*−/− condition are scarce, being limited to a postmortem brain tissue from a single case. Indeed, homozygous *GRN*−/− is an extremely rare condition reported in very few cases. Our aim was to investigate pathological phenotypes associated with FTLD and NCL in iPSC-derived cortical neurons from a *GRN*−/− patient affected by NCL. iPSCs were generated from peripheral blood of a *GRN* wt healthy donor and a *GRN*−/− patient and subsequently differentiated into cortical neurons. Several pathological changes were investigated, by means of immunocytochemical, biochemical and ultrastructural analyses. *GRN*−/− patient-derived cortical neurons displayed both TDP-43 and phospho-TDP-43 mislocalization, enlarged autofluorescent lysosomes and electron-dense vesicles containing storage material with granular, curvilinear and fingerprints profiles. In addition, different patterns in the expression of TDP-43, caspase 3 and cleaved caspase 3 were observed by biochemical analysis at different time points of cortical differentiation. At variance with previous findings, the present data highlight the existence of both FTLD- and NCL-linked pathological features in *GRN*−/− iPSC-derived cortical neurons from a NCL patient. They also suggest an evolution in the appearance of these features: firstly, FTLD-related TDP-43 alterations and initial NCL storage materials were detected; afterwards, mainly well-shaped NCL storage materials were present, while some FTLD features were not observed anymore.

Abbreviations: GRN, gene coding for progranulin; FTLD, Frontotemporal lobar degeneration; NCL, Neuronal ceroidolipofuscinosis; iPSCs, induced pluripotent stem cells; TDP-43, TAR-DNA binding protein-43; LAMP1, Lysosomal-associated membrane protein 1; TFEB, Transcription factor EB; PBMCs, Peripheral blood mononuclear cells; SCF, Stem cell factor; FTL-3, Fms-related tyrosine kinase ligand 3; KLF-4, Krüppel-like factor 4; OCT4, Octamer-binding transcription factor 4; Sox2, SRY-Box transcription factor 2; MOI, Multiplicity of infection; SSEA4, Stage-specific embryonic antigen-4; NGS, Normal goat serum; NSCs, Neural stem cells; NPCs, Neural progenitor cells; BDNF, Brain-derived neurotrophic factor; GDNF, glial cell derived neurotrophic factor; MAP2, Microtubule-associated protein 2; CUX1, cut like homeobox 1; PAX6, Paired box 6; TEM, Transmission electron microscopy; GRODs, Granular osmiophilic deposits.

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Resting state functional brain networks associated with emotion processing in frontotemporal lobar degeneration

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This study investigated the relationship between emotion processing and resting-state functional connectivity (rs-FC) of the brain networks in frontotemporal lobar degeneration (FTLD). Eighty FTLD patients (including cases with behavioral variant of frontotemporal dementia, primary progressive aphasia, progressive supranuclear palsy syndrome, motor neuron disease) and 65 healthy controls underwent rs-functional MRI. Emotion processing was tested using the Comprehensive Affect Testing System (CATS). In patients and controls, correlations were investigated between each emotion construct and rs-FC changes within critical networks. Mean rs-FC of the clusters significantly associated with CATS scoring were compared among FTLD groups. FTLD patients had pathological CATS scores compared with controls. In controls, increased rs-FC of the cerebellar and visuo-associative networks correlated with better scores in emotion-matching and discrimination tasks, respectively; while decreased rs-FC of the visuo-spatial network was related with better performance in the affect-matching and naming. In FTLD, the associations between rs-FC and CATS scores involved more brain regions, such as orbitofrontal and middle frontal gyri within anterior networks (i.e., salience and default-mode), parietal and somatosensory regions within visuo-spatial and sensorimotor networks, caudate and thalamus within basal-ganglia network. Rs-FC changes associated with CATS were similar among all FTLD groups. In FTLD compared to controls, the pattern of rs-FC associated with emotional processing involves a larger number of brain regions, likely due to functional specificity loss and compensatory attempts. These associations were similar across all FTLD groups, suggesting a common physiopathological mechanism of emotion processing breakdown, regardless the clinical presentation and pattern of atrophy.

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INTRODUCTION

Among the social cognitive functions, the perception of social stimuli is a highly developed skill, gathering crucial information for interpersonal communication. The capacity to associate specific patterns of facial musculature contractions to discrete emotions is an universal aspect of social communication, equally recognized across different cultures [1]. To evaluate emotion recognition, the most commonly used stimuli are the Ekman's pictures of facial affect, a collection of photos to investigate an individual's ability to discriminate and label the six basic emotions (disgust, surprise, happiness, anger, fear and sadness) [2]. Defective emotion recognition can lead to altered social interactions, especially in disorders affecting the frontal and the temporal lobes, such as those belonging to the frontotemporal lobar degeneration (FTLD) spectrum. Specifically, patients with the behavioral variant of frontotemporal dementia (bvFTD) [3], agrammatic/non-fluent (nfvPPA) and semantic (svPPA) variants of primary progressive aphasia (PPA) [4, 5], progressive supranuclear palsy syndrome (PSPs) [6] and amyotrophic lateral sclerosis (ALS) [7, 8], all show reduced emotional reaction and/or recognition mainly for negative stimuli [3]. Subtle affect processing failures are already present in presymptomatic *C9orf72* mutation carriers at risk for bvFTD, as compared with both controls and carriers of other mutations [9, 10].

A set of brain regions, involving limbic and primary sensory systems, are crucial for a rapid and automatic evaluation of the perceived emotion and functional MRI (fMRI) studies showed that they are also engaged during non-conscious subliminal perception of affective stimuli [11]. Emotion identification deficits in FTLD patients have been linked to decreased gray matter (GM) volume of amygdala, insula, inferior frontal, medial prefrontal and orbitofrontal cortices, with a prevalent involvement of the right side, as well as with diffusivity abnormalities of the right inferior longitudinal and inferior fronto-occipital fasciculi, and fornix

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Systematic Review

Gaze-Contingent Eye-Tracking Training in Brain Disorders: A Systematic Review

Laura Carelli [1,*,†], Federica Solca [1,†], Sofia Tagini [2,3], Silvia Torre [1], Federico Verde [1,4], Nicola Ticozzi [1,4], Roberta Ferrucci [5,6,7], Gabriella Pravettoni [8,9], Edoardo Nicolò Aiello [1,10], Vincenzo Silani [1,4,5,‡] and Barbara Poletti [1,‡]

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Abstract: Eye movement abnormalities in association with cognitive and emotional deficits have been described in neurological, neurodevelopmental, and psychiatric disorders. Eye-Tracking (ET) techniques could therefore enhance cognitive interventions by contingently providing feedback to patients. Since no consensus has been reached thus far on this approach, this study aimed at systematically reviewing the current evidence. This review was performed and reported according to PRISMA guidelines. Records were searched for in PubMed, Web of Science, and Scopus (1990–2021) through the following string: ('Eye Tracking' OR 'Eye-Tracking' OR 'Oculomotor') AND ('Neuropsychol*' OR 'Cognitive') AND ('Rehabilitation' OR 'Training' OR 'Stimulation'). Study outcomes were thematically classified and qualitatively synthesized. A structured quality assessment was performed. A total of 24 articles were included, addressing neurodevelopmental (preterm infants and children with autism spectrum disorder, Rett syndrome, or ADHD; $N = 14$), psychiatric (mood and anxiety disorders or alcohol dependence; $N = 7$), and neurological conditions (stroke; $N = 3$). Overall, ET gaze-contingent training proved to be effective in improving cognitive and emotional alterations. However, population heterogeneity limits the generalizability of results. ET gaze-contingent protocols allow researchers to directly and dynamically train attentional functions; together with the recruitment of implicit, "bottom-up" strategies, these protocols are promising and possibly integrable with traditional cognitive approaches.

Keywords: eye-tracking; gaze-contingent training; brain disorders; attention; inhibition

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

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Amyotrophic lateral sclerosis
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Functional connectivity

ABSTRACT

In the present study, we aimed to investigate the resting-state functional connectivity (RS-FC) of the globus pallidus (GP) in patients with amyotrophic lateral sclerosis (ALS) compared to healthy controls, and the relationship between RS-FC changes and disgust recognition. Twenty-six pure-motor ALS patients and 52 healthy controls underwent RS functional MRI and a neuropsychological assessment including the Comprehensive Affect Testing System. A seed-based RS-FC analysis was performed between the left and right GP and the rest of the brain and compared between groups. Correlations between RS-FC significant changes and subjects' performance in recognizing disgust were tested. Compared to controls, patients were significantly less able to recognize disgust. In ALS compared to controls, the seed-based analysis showed: reduced RS-FC between bilateral GP and bilateral middle and superior frontal and middle cingulate gyri, and increased RS-FC between bilateral GP and bilateral postcentral, supramarginal and superior temporal gyri and Rolandic operculum. Decreased RS-FC was further observed between left GP and left middle and inferior temporal gyri and bilateral caudate; and increased RS-FC was also shown between right GP and left lingual and fusiform gyri. In patients and controls, lower performance in recognizing disgust correlated with reduced RS-FC between left GP and left middle and inferior temporal gyri. In pure-motor ALS patients, we demonstrated altered RS-FC between GP and the rest of the brain. The reduced left pallidum-temporo-striatal RS-FC may have a role in the lower ability of patients in recognizing disgust.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal and heterogeneous neurodegenerative disease of the motor system and its wider connections in which the possible presence of cognitive and/or behavioural symptoms is a universally known neuropsychological feature (McKenna et al., 2021). In the past few years, a growing literature focused on the study of social cognition in ALS, from emotional processing to others' intention attribution. Emotional and social deficits in ALS have a great clinical impact, since they may influence the quality of life of patients and increase caregiver burden (Caga et al., 2019). Several studies on emotion perception impairment reported that ALS patients have a diminished psychophysiological arousal to emotional stimuli and difficulties in recognizing and attributing emotions (Andrews et al., 2017; Crespi et al., 2014; Girardi et al., 2011; Oh et al., 2016; Savage et al., 2014; Zimmerman et al., 2007), judging socio-emotional stimuli as more

Abbreviations: RS-FC, resting-state functional connectivity; GP, globus pallidus; CATS, Comprehensive Affect Testing System; RS fMRI, RS functional MRI.

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ORIGINAL COMMUNICATION

Motor, cognitive and behavioural profiles of *C9orf72* expansion-related amyotrophic lateral sclerosis

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Abstract

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

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

Results *C9orf72* HRE was detected in 55/960 ALS patients. C9Pos patients showed a younger onset, higher odds of bulbar onset, increased burden of UMN signs, reduced survival and higher frequency of concurrent dementia. We found an inverse correlation between the HRE length and the performance at ECAS ALS-specific tasks ($P=0.031$). Patients also showed higher burden of behavioural disinhibition ($P=1.6\times10^{-4}$), lower degrees of depression ($P=0.015$) and anxiety ($P=0.008$) compared to C9Neg cases.

Conclusions Our study provides an extensive characterization of motor, cognitive and behavioural features of *C9orf72*-related ALS, indicating that the *C9orf72* HRE size may represent a modifier of the cognitive phenotype.

Keywords ALS · Frontotemporal dementia · Genetics · Motor neuron disease

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by a progressive loss of upper (UMN) and lower motor neurons (LMN). Approximately 90% of ALS cases are sporadic (SALS), while the remaining 10% are familial (FALS). Mutations in four main genes (*C9orf72*, *SOD1*, *TARDBP* and *FUS*) are responsible for up to 75% of FALS cases, with variants in > 25 other genes being relatively uncommon [1].

A $(G_4C_2)_n$ hexanucleotide repeat expansion (HRE) in the *C9orf72* gene accounts for 30–50% of FALS, as well as 5–10% of SALS cases [2], and represents the most common genetic defect in ALS and in frontotemporal dementia (FTD) [3, 4].

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Alzheimer's Research & Therapy

RESEARCH Open Access

Italian adaptation of the Uniform Data Set Neuropsychological Test Battery (I-UDSNB 1.0): development and normative data

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Abstract

Background: Neuropsychological testing plays a cardinal role in the diagnosis and monitoring of Alzheimer's disease. A major concern is represented by the heterogeneity of the neuropsychological batteries currently adopted in memory clinics and healthcare centers. The current study aimed to solve this issue.

Methods: Following the initiative of the University of Washington's National Alzheimer's Coordinating Center (NACC), we presented the Italian adaptation of the Neuropsychological Test Battery of the Uniform Data Set (I-UDSNB). We collected data from 433 healthy Italian individuals and employed regression models to evaluate the impact of demographic variables on the performance, deriving the reference norms.

Results: Higher education and lower age were associated with a better performance in the majority of tests, while sex affected only fluency tests and Digit Span Forward.

Conclusions: The I-UDSNB offers a valuable and harmonized tool for neuropsychological testing in Italy, to be used in clinical and research settings.

Keywords: Neuropsychological tests, UDS, Alzheimer's disease, Cognition

Background

Neuropsychological testing plays a central role in the diagnosis of Alzheimer's disease (AD). The concept of AD as a biological diagnosis based on biomarker positivity has a clear relevance for research, but in most clinical settings, the presence of objective cognitive dysfunction is still representing a "gateway" for a decision about biomarker assessment. The presence of a specific profile of neuropsychological impairment, associated

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Authors' contributions
FC and VE contributed to the design of the work, acquisition, analysis, and interpretation of the data and drafted the work. FR contributed to the creation of the new software used in the work. RM, GC, FB, FLA, EC, VC, GM, BP, MDM, ER, AT, AP, FG, and CR contributed to the acquisition of the data. UL and AAG contributed to the analysis of the data. SDT contributed to the acquisition and analysis of the data. DQ and EC: contributed to the design of the work and analysis of the data and revised the work. MF, VS, RL, MSM, AA, CC, MP, SS, and NC revised the work. CM, RP, PT, MC, RF, SW, CM, FT, and SC contributed to the design of the work and revised the work. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the local ethics committees (Ethic committee of Pavia, IRCCS Policlinico "San Matteo", Pavia, Italy) and complied with the provisions of the Declaration of Helsinki. All subjects gave written informed consent to participate (protocol n. 20200061123, Pavia).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

One-year cognitive follow-up of COVID-19 hospitalized patients

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Abstract

Background and purpose: Cognitive dysfunction has been observed following recovery from COVID-19. To the best of our knowledge, however, no study has assessed the progression of cognitive impairment after 1 year. The aim was to assess cognitive functioning at 1 year from hospital discharge, and eventual associations with specific clinical variables.

Methods: Seventy-six patients (aged 22–74 years) who had been hospitalized for COVID-19 were recruited. Patients received neuropsychological assessments at 5 ($n = 76$) and 12 months ($n = 53$) from hospital discharge.

Results: Over half (63.2%) of the patients had deficits in at least one test at 5 months. Compared to the assessment at 5 months, verbal memory, attention and processing speed improved significantly after 1 year (all $p < 0.05$), whereas visuospatial memory did not (all $p > 0.500$). The most affected domains after 1 year were processing speed (28.3%) and long-term visuospatial (18.1%) and verbal (15.1%) memory. Lower PaO_2/FiO_2 ratios in the acute phase were associated with worse verbal long-term memory ($p = 0.029$) and visuospatial learning ($p = 0.041$) at 5 months. Worse visuospatial long-term memory at 5 months was associated with hyposmia ($p = 0.020$) and dysgeusia ($p = 0.037$).

Conclusion: Our study expands the results from previous studies showing that cognitive impairment can still be observed after 1 year. Patients with severe COVID-19 should receive periodic cognitive follow-up evaluations, as cognitive deficits in recovered patients could have social and occupational implications.

KEYWORDS
COVID-19, cognition, long-COVID, neuropsychological evaluation

Neurological Sciences
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BRIEF COMMUNICATION

Progressive motor neuron syndromes with single CNS lesions and CSF oligoclonal bands: never forget solitary sclerosis!

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Abstract

We describe 3 cases of solitary sclerosis (SS), a rare condition characterized by a single inflammatory demyelinating lesion in the white matter of the brain or spinal cord. All patients had progressive limb motor impairment (patient 1, 66-year-old female: left spastic hemiparesis; patient 2, 39-year-old male: right spastic hemiparesis; patient 3, 42-year-old female: proximally predominant left upper limb weakness with amyotrophy and fasciculations). In all patients, MRI disclosed a single small T2-hyperintense demyelinating lesion: in the right anterior paramedian upper medulla, in the median-left paramedian anterior lower medulla, and in the left paramedian anterior cervical spinal cord at C4 level, respectively. In patients 1 and 2, transcranial magnetic stimulation (TMS) demonstrated altered motor evoked potentials (MEPs) and increased central motor conduction time (CMCT) in the affected limbs; in patient 3, needle EMG revealed chronic neurogenic changes in C5–C7 muscles of left upper limb. Patients 1 and 2 had normal brain ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET). CSF analysis demonstrated IgG oligoclonal bands in all patients. In patients 2 and 3, levels of neurofilament light chain (NFL) in CSF and serum, respectively, were within normal limits. The three cases were consistent with the diagnosis of SS. Notably, while the first two cases mimicked Mills' syndrome (the hemiparetic variant of primary lateral sclerosis, PLS), the third one was rather reminiscent of amyotrophic lateral sclerosis (ALS). This suggests including SS in the differential diagnosis not only of PLS, but also of ALS. We also report the first quantification of NFL levels in SS.

Keywords Solitary sclerosis · Demyelinating diseases · Primary lateral sclerosis (PLS) · Motor neuron disease (MND) · Cerebrospinal fluid (CSF) · Oligoclonal bands

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BRIEF COMMUNICATION

Cerebrospinal fluid/serum albumin quotient (Q-Alb) is not increased in Alzheimer's disease compared to neurological disease controls: a retrospective study on 276 patients

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Abstract

Background The cerebrospinal fluid (CSF)/serum albumin quotient (Q-Alb) is a marker of the blood-CSF barrier (BCSFB) and possibly of the blood–brain barrier (BBB). The latter is known to be altered in Alzheimer's disease (AD) based on neuropathological and neuroimaging studies. Following investigations performed on clinically diagnosed cohorts, we aimed at comparing Q-Alb in cognitively impaired patients with neurochemical demonstration of AD pathophysiology and neurological disease controls (NDCs).

Methods We evaluated $N = 144$ AD patients (MCI, $N = 43$; AD dementia — ADD, $N = 101$) and $N = 132$ NDCs. AD patients were all A + according to the A/T/N framework and were neurochemically classified based on T and N parameters.

Results Q-Alb did not significantly differ between AD patients and NDCs. Moreover, it was not associated with disease stage (MCI vs. ADD), MMSE score, or CSF AD biomarkers.

Discussion Our study indicates that BCSFB dysfunction is not a specific feature of AD. When interpreting Q-Alb as a marker of the BBB, the lack of difference from NDCs might be due to BBB dysfunction widely occurring in other neurological, non-degenerative, conditions or — more probably — to low sensitivity of this biochemical parameter towards subtle BBB alterations causing leakage of molecules smaller than albumin. Furthermore, Q-Alb is not associated with the degree of global cognitive deterioration in AD, nor with CSF AD neurochemical biomarkers.

Keywords Albumin quotient (Q-Alb) · Alzheimer's disease (AD) · Cerebrospinal fluid (CSF) · Blood-brain barrier (BBB) · Blood-cerebrospinal fluid barrier (BCSFB)

Background

The cerebrospinal fluid (CSF)/serum albumin quotient (Q-Alb) provides an estimation of the function of the blood-CSF barrier (BCSFB) [1]. In a broader sense, Q-Alb might be regarded as a marker of the blood–brain barrier (BBB) [2]. Neuropathological and neuroimaging evidence indicates BBB dysfunction in Alzheimer's disease (AD) [3]. Accordingly, Q-Alb has been reported to be elevated in AD, compared to healthy controls [2, 4, 5]. However, this finding has

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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.

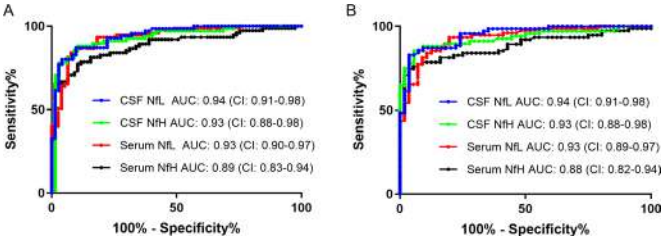

Figure 3 Comparison of neurofilament ROC analyses of ALS versus Con and Con.DD. (A) ROC curves for the discrimination of ALS and Con for CSF and serum NfL and NfH. (B) ROC curves for the discrimination of ALS and Con.DD for CSF and serum NfL and NfH. ALS, amyotrophic lateral sclerosis; AUC, area under 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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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.

AMYOTROPHIC LATERAL SCLEROSIS

Genome-wide study of DNA methylation shows alterations in metabolic, inflammatory, and cholesterol pathways in ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with an estimated heritability between 40 and 50%. DNA methylation patterns can serve as proxies of (past) exposures and disease progression, as well as providing a potential mechanism that mediates genetic or environmental risk. Here, we present a blood-based epigenome-wide association study meta-analysis in 9706 samples passing stringent quality control (6763 patients, 2943 controls). We identified a total of 45 differentially methylated positions (DMPs) annotated to 42 genes, which are enriched for pathways and traits related to metabolism, cholesterol biosynthesis, and immunity. We then tested 39 DNA methylation–based proxies of putative ALS risk factors and found that high-density lipoprotein cholesterol, body mass index, white blood cell proportions, and alcohol intake were independently associated with ALS. Integration of these results with our latest genome-wide association study showed that cholesterol biosynthesis was potentially causally related to ALS. Last, DNA methylation at several DMPs and blood cell proportion estimates derived from DNA methylation data were associated with survival rate in patients, suggesting that they might represent indicators of underlying disease processes potentially amenable to therapeutic interventions.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder characterized by progressive degeneration of motor neurons in the brain and spinal cord (*1*). The disease affects about 1 in 350 people, with death typically occurring within 2 to 5 years after onset. The heritability of ALS is estimated to be around 50% (*2*), showing that a considerable portion of the risk could be conferred by environmental and lifestyle risk factors. However, the identification of these factors has proven difficult because of several challenges such as recall and measurement bias, resulting in a large body of literature with conflicting results and only a few established factors related to ALS risk or patient survival (*3*–*6*). Epigenetic patterns, which act at the interface between genes and environment, can serve as proxies of (past) exposures, therefore enabling the study of these exposures and putative risk factors. Moreover, the identification of ALS-associated epigenetic factors could provide insights into disease etiology and disease processes.

DNA methylation is one of the best characterized and most stable epigenetic modifications and plays an important role in gene regulation, genomic stability, and genomic imprinting (*7*–*9*). The development of standardized assays for quantifying DNA methylation has enabled the systematic analysis of associations between methylomic variation and a wide range of human diseases, including cancer, schizophrenia, and various neurodegenerative diseases (*10*, *11*). DNA methylation in whole blood captures a wide range of putative ALS risk factors at a molecular level, including smoking, alcohol intake, body mass index (BMI), biological age, and various metabolic and inflammatory proteins (*12*–*18*). Leveraging DNA methylation as proxies for these risk factors offers several advantages because it is (i) not prone to recall bias (relevant for smoking and alcohol), (ii) may

capture information not (accurately) captured by the self-report (such as passive and past smoking) and provides a quantifiable measure (*19*), and (iii) is relatively stable in the short term [especially relevant for immunological proteins (*18*)]. Moreover, many risk factor studies have been conducted in small samples (*3*, *6*), whereas our large DNA methylation study can provide a well-powered alternative that jointly considers the molecular correlates of many risk factors. We, therefore, performed a blood-based DNA methylation study of ALS incorporating 9706 samples that passed stringent quality control.

RESULTS

Epigenome-wide association study meta-analysis of ALS identifies 45 DMPs

We quantified genome-wide DNA methylation in whole blood from 10,462 individuals using the Illumina HumanMethylation450 (450 k) array (6275 samples) and the Illumina MethylationEPIC (EPIC) array (4187 samples). We merged individual-level DNA methylation array data from 14 countries into four strata (MinE 450 K, MinE EPIC, AUS1, and AUS2; see Materials and Methods and fig. S1). A total of 6763 patients with ALS and 2943 control individuals passed our stringent quality control, which was followed by normalization of signal intensities in each stratum (Table 1, data file S1, and tables S1 to S5). Samples excluded from our analyses did not show different demographic or clinical characteristics compared to the subset selected for analyses (data file S2).

We performed an epigenome-wide association study (EWAS) in each of the four strata using two methods to adjust for known and unknown confounders. First, we used a linear model adjusting for known confounders and a calibrated number of principal components (PCs) to adjust for unknown confounding factors (fig. S2), followed by correction for residual bias and inflation in test statistics using bacon (hereafter referred to as the LB model) (*20*). Second, we used MOA (mixed linear model–based omic association) as implemented in the OSCA software in which the random effect of total genome-wide DNA methylation captures the correlation structure between probes and directly controls for the genomic inflation (*21*). The MOA algorithm did not converge for the AUS2 stratum, resulting in a total sample size of 9459 for the MOA results. Test statistics across strata were combined using an inverse variance-weighted (IVW) fixed-effects meta-analysis (*22*). Inflation of the test statistics was well controlled in both the LB ($\lambda = 1.046$; Fig. 1) and the MOA results, respectively ($\lambda = 0.984$; Fig. 1), and we observed little heterogeneity between strata (figs. S3 to S5). Various sensitivity analyses indicated that the results were robust to changes in analysis strategy, including adjustment for population stratification (10 genetic PCs), using M values instead of β values, using functional normalization (*23*) instead of dasen (*24*), and excluding specific strata or experimental batches (figs. S6 to S8). Last, application of a method that we recently described (*25*) led to the removal of likely cross-hybridizing probes, including four probes that showed high homology to the *C9orf72* repeat locus (fig. S9). In total, 724,712 positions passed quality control

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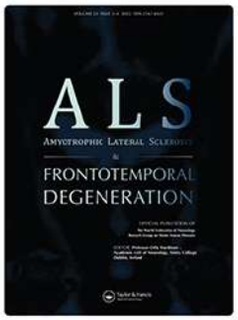

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

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Expanding the phenotype of *TARDBP* mutation in a Tunisian family with clinical phenotype heterogeneity

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SHORT REPORT

Expanding the phenotype of *TARDBP* mutation in a Tunisian family with clinical phenotype heterogeneity

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Abstract

We describe a Tunisian family carrier of the same rare mutation in *TARDBP* but developing different neurodegenerative disease with heterogenous features. We explored the possible genetic modifiers leading to the observed intrafamilial phenotypic variability. Genetic analysis identified *TARDBP* p.G294A mutation among4 members. Additionally, the ALS case was muted in *GBA*. While the three cases of AD were carriers of *PRKN* and *GBA* mutations. Finally, the FTD-parkinsonism patient was mutated for *LRRK2* p.G2019S that might increase his susceptibility to develop Parkinsonism spectrum. Genetic variants of *TARDBP* may influence the clinical manifestation in ALS case.

Keywords: *TARDBP, oligogenic profile, inbreeding, heterogeneity, modulators, biomarker, DNA, genetics*

Introduction

Amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Alzheimer disease (AD) are very prevalent neurodegenerative diseases (NDD) worldwide related to aging (1). Despite the heterogeneity of their clinical features, they share a common pathological process which is the aggregation of specific abnormal proteins (2–4). We present an inbred Tunisian family characterized by wide clinical phenotype heterogeneity.

Clinical report

We considered a large Tunisian family showing multiple cases of consanguineous marriage and a wide clinical heterogeneity among the offspring, including ALS, AD, FTD with Parkinsonism and psychiatric disorders. A genetic screening was conducted using target panel comprised 48 genes associated to the ALS-FTD spectrum, AD and Parkinson's disease namely *ALS2, ANG, DCTN1, FUS, HNRNPA1, HNRNPA2B1, MATR3, NEK1, OPTN, PFN1, SETX, SOD1, SPAST, SPG11, SQSTM1, TARDBP, TBK1, TUBA4A, UBQLN2, VAPB, CHM2B, GRN, MAPT, PRNP, VCP, APOE, APP, PSEN1, PSEN2, TREM2, ATP13A2, DJ1, DNAJ6, EIF4G1, FBXO7, GBA, GCH1, LRRK2, PARK2, PINK1, PLA2G6, PRKRA, SNCA, TAF1, TH, CHL1, VPS13C, VPS35* as well as a two-step protocol was followed for the detection of the hexanucleotide repeat expansion in the *C9orf72* gene. We found the *TARDBP* p.G294Amutation, in 4 family members affected by ALS (IV-1), AD (IV-3 and IV-4), and FTD with parkinsonism (III-5). The NGS analysis revealed additional pathogenic mutations in ALS

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Genotype-phenotype correlation in Tunisian patients with Amyotrophic Lateral Sclerosis

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease. To date, mutations in more than 30 genes have been linked to familial ALS forms. However, no mutational screenings have been reported in African populations so far. We aimed to investigate the presence of rare genetic variants in the 4 most common ALS causative genes among a Tunisian cohort. Patients were screened for mutations in *SOD1* (exons 1-5), *TARDBP* (exon 6), *FUS* (exons 5, 6, 13/14, and 15). Juvenile ALS (JALS) patients were screened also for *ALS2* (exons 3, 10, 28). Analysis of *C9ORF72* was conducted by fluorescent amplicon-length analysis and repeat-primed PCR. We analyzed 197 Tunisian ALS patients, including 11 familial forms (fALS) with 17 ALS cases, 167 sporadic (sALS) and 13 JALS cases. The pathogenic variant *TARDBP* p.G294A mutation was reported among 18 patients. Repeat expansion in *C9orf72* was recorded in 9 patients. Interestingly, 2 unrelated patients carried a double mutation in both *C9orf72* and *TARDBP* genes. Finally, the p.Asp91Val mutation in *SOD1* was identified among 4 cases in homozygous state including 3 sALS and 1 familial JALS with recessive inheritance. No pathogenic variants in *FUS* were identified, nor *ALS2* variants in JALS cases. In our Tunisian cohort the most frequently mutated gene is *TARDBP* (9.4%), followed by *C9orf72* (3.9%) and *SOD1* (2.1%). Our study broadens the mutational spectrum in patients with ALS and defines for the first time the mutational frequency of the main ALS genes in patients of African ethnicity.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, affecting upper and lower motor neurons leading to a progressive weakness and culminating in death typically, in 2–5 years after symptom onset (Al-Chalabi et al., 2016). ALS etiology remains elusive and its complexity is partly due to the wide genetic and phenotypic heterogeneity of the familial and sporadic forms (Chiò et al., 2020).

The mechanisms of neurodegeneration among ALS cases are not fully clear. Indeed, there are several cellular and molecular processes and pathways associated to ALS pathogenesis (Turner et al., 2013). These include toxic protein aggregation that can trigger motoneuron death, such as the nuclear TAR DNA-binding protein 43(TDP-43), superoxide dismutase 1(*SOD1*) and ubiquilin 2(*UBQLN2*). Moreover, the dysregulation of RNA metabolism may leads to abnormalities in RNA processing and transport(*TARDBP* and *FUS*) as well as the formation of pathological RNA foci and dipeptide proteins with nucleolar impairment (*C9orf72*) (Kim et al., 2020).

Therefore, the 4 main ALS-related genes, *SOD1, C9orf72, TARDBP*,and *FUS*, may have an appreciable influence on ALS phenotype (Kim et al., 2020), although their mutational frequency strongly depends on the ancestral origins of ALS patients

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Novel *THAP1* missense variant with incomplete penetrance in a case of generalized young onset dystonia showing good response to deep brain stimulation

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ABSTRACT

We describe a case of young onset generalized dystonia, harboring a previously unreported likely pathogenic *THAP1* missense variant (c.109 G > A; p.Glu37Lys) that was inherited from her unaffected father. Moreover, we report a positive effect of deep brain stimulation, particularly on the cervical component of dystonia.

THAP1 (MIM* 609520) is an established dystonia-associated gene, and several causative variants have been described, including both truncating and missense variants [1]. *THAP1* pathogenic variants are associated with penetrance as low as 10% [2] Herein, we report a case of young onset generalized dystonia with prominent cervical and bulbar involvement, carrying a novel likely pathogenic *THAP1* missense variant with incomplete penetrance, who showed good response to bilateral globus pallidus internus (GPi) deep brain stimulation (DBS), particularly in the cervical district.

A now 27-year-old female patient of Irish and Italian ancestry presented at the age of 14 with cervical dystonia that over the years spread to involve the upper and lower limbs, and orolingual region.

Neurological examination showed left torticollis, right laterocollis and mild retrocollis. Neck posture improved with a sensory trick, i.e. touching her left ear or the back of her head. Her speech was markedly dysarthric, due to jaw tightness and involuntary tongue rolling. There were dystonic posturing of the right arm, shoulder elevation, and abnormal wrist extension when the patient attempted to hold a pen and write. Moreover, inward turning of the right foot could be seen during walking. On the severity subscale of the revised Toronto Spasmodic Torticollis Rating Scale (TWSTRS-2) the patient showed a score of 19 (Table 1).

Because the patient only responded partially to botulinum toxin, she underwent bilateral DBS targeting the GPi at age 25. Following DBS, substantial improvement in the severity of dysarthria and cervical dystonia was observed. Specifically, the patient showed a 7-point decrease of her TWSTRS-2 score (Table 1), mostly due to reduced torticollis and better range of motion. Such improvement was stable two years after surgery.

To investigate a possible genetic etiology, an extensive panel for dystonia was ordered, which identified a previously unreported variant in *THAP1* (NM_018105.2; exon 2 c.109 G > A; p.Glu37Lys), inherited from her unaffected father. Only one carrier (1/125,682) is reported in the genome aggregation database (gnomAD v2.1.1; https://gnomad.broadinstitute.org/). Whole-exome sequencing was additionally performed as reported elsewhere [3]. This analysis excluded additional relevant pathogenic variants in an extended list of genes linked to movement disorders or other *de novo* or bi-allelic variants in novel candidate disease-associated genes (Supplemental Table 1). According to ACMG/AMP guidelines, this variant is classified as likely pathogenic, meeting 2 moderate evidence (PM1, PM2) and 2 supporting evidence criteria (PP3, PP4) [4].

In summary, we report a novel missense variant in *THAP1* in a case with a phenotype highly consistent with that of *THAP1*-related dystonia, including prominent cranio-cervical involvement. Given its low penetrance, determining whether a newly identified variant in *THAP1* is pathogenic with incomplete penetrance or benign can be a complex task. As for this case, the typical clinical presentation, the extreme rarity of the variant in healthy controls, together with a Combined Annotation Dependent Depletion (CADD) score of 24.9 (which puts the variant among the 1% most deleterious in the genome), strongly support its pathogenic role. Moreover, the variant is located within the DNA-binding domain of the protein, where majority of pathogenic variants identified up to date are found. Finally, the absence of additional pathogenic variants in established or novel candidate genes, further reinforces the likely pathogenic role of this variant.

While positive responses to GPi DBS are consistently reported in cases with *TOR1A* or *KMT2B* -related dystonia [3,5], DBS outcome in cases with *THAP1*-related dystonia is less predictable. The reason of this variability is still unclear but can be partially explained by its allelic heterogeneity, and the fact that dystonia affecting the bulbar region, which is often prominent in *THAP1* dystonia, is usually less responsive to DBS treatment [5]. Our case confirms this notion, by showing significant improvement of cervical dystonia without substantial change in oromandibular dystonia.

Declarations of interest

None.

Patient consent

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Table 1
Severity subscale of the revised Toronto Spasmodic Torticollis Rating Scale (TWSTRS-2), showing sustained improvement two years after DBS.

	Before DBS	2 years after DBS
Rotation	3	0
Laterocollis	3	2
Shoulder elevation/displacement	2	1
Duration of CD during exam	4	4
Range of motion	3	1
Time holding head in midline	4	4
TOTAL	19	12

Ethics approval

N/A.

Author contributions

I.J.K.S, A.V., L.K., R.S.A,V.S., S.J.L., D.K., N.E.M. contributed to the conception and design of the study and to the drafting of the text. I.J.K.S, A.V., L.K., N.E.M. contributed to the acquisition of data. I.J.K.S, A.V., L. K., R.S.A,V.S., S.J.L., D.K., N.E.M. revised the manuscript for intellectual content and approved the final article to be submitted.

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D.K. is the Founder and Scientific Advisory Board Chair of Lysosomal Therapeutics Inc. and Vanqua Bio. D.K. serves on the scientific advisory boards of The Silverstein Foundation, Intellia Therapeutics, AcureX and Prevail Therapeutics and is a Venture Partner at OrbiMed. All other authors declare that they do not have competing interests.

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Appendix A. Supplementary data

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Novel bi-allelic *FBXO7* variants in a family with early-onset typical Parkinson's disease

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ABSTRACT

Bi-allelic mutations in *FBXO7* are classically associated with a complex phenotype, known as parkinsonian-pyramidal syndrome. We describe two brothers affected by typical early onset Parkinson's disease (EOPD), who carry novel compound heterozygous variants in *FBXO7*. Our report highlights that typical EOPD can be part of an expanding *FBXO7*-related phenotype.

1. Main text

Early onset Parkinson's Disease (EOPD) is defined by appearance of motor symptoms before the age of 50. A substantial number of EOPD cases has a genetic etiology. *FBXO7* belongs to a group of genes (also including *PLA2G6*, *DNAJC6*), whose variants are usually associated with complex syndromes including parkinsonism plus other features such as generalized dystonia, pyramidal signs, eye movement abnormalities, early cognitive decline, prominent bulbar dysfunction, and an aggressive disease course with limited response to dopaminergic treatments [1]. On the contrary, genes like *PRKN*, *PINK1* and *DJ1* are associated with typical levodopa-responsive parkinsonism presenting with tremor, rigidity, bradykinesia, and occasionally exercise-induced dystonia [1].

Herein, we report the case of two brothers carrying novel compound heterozygous variants in *FBXO7*, who presented with typical DOPA-responsive EOPD.

Patient 1 is a 49-year-old male who presented at the age of 45 with six months of left arm rigidity, tremor, loss of dexterity, and progressive gait unsteadiness. Retrospectively, he reported a history of REM Sleep Behavior Disorder (RBD) since the age of 30. On examination, he exhibited asymmetric parkinsonism with mild left-sided bradykinesia and rigidity, reduced left arm swing, and mildly reduced stride length. His symptoms showed a good motor response to levodopa. Over the following five years, he developed camptocormia and markedly reduced stride length, along with progressive hypomimia, hypophonia, micrographia, and drooling.

Patient 2, the older brother of Patient 1, is a 51-year-old who presented at the age of 45 with two years of progressively slow movements and bilateral loss of hand dexterity. On examination, he had mild hypomimia, mild hypophonia, asymmetric bradykinesia (left greater than right) and rigidity, absent arm swing bilaterally, and slightly reduced stride length bilaterally. Motor symptoms responded well to low-dose levodopa. Over time, he also progressed with respect to his bradykinesia and rigidity. Unlike Patient 1, he denied tremor and RBD symptoms.

Both patients lacked hyposmia, cognitive difficulty, freezing-of-gait, autonomic dysfunction, pyramidal signs, dystonia and levodopa-induced dyskinesias. They are of Northern and Eastern European ancestry, with no history of movement disorders in other family members and no consanguinity in the parents.

Brain MRI was unremarkable in both subjects. Additionally, patient 1 performed (123)I-Ioflupane single-photon emission computed tomography (SPECT) at age 47, which showed decreased radiotracer uptake within the left more than right putamen (data not shown).

Because of the likely genetic etiology, patient 1 underwent genetic testing with a comprehensive parkinsonism panel (Supp. Table 1), which revealed two previously unreported heterozygous variants in *FBXO7*: a missense (NM_012179.3: c.992 G>T; p.G331V) and a 5bp frameshift duplication (NM_012179.3: c.1268_1272dupCATTC; p.Y425HfsX56). Both variants were predicted to be deleterious, as demonstrated by a Combined Annotation Dependent Depletion (CADD) score of 26.6 and 24.8, respectively, and are unreported in the Genome Aggregation Database v2.1 (https://gnomad.broadinstitute.org/). No other relevant variants were observed in other parkinsonism-related genes. Sanger sequencing confirmed the presence of the two variants in both affected siblings, while the unaffected mother carried only the heterozygous frameshift variant, confirming the compound heterozygous state of the variants (see Fig. 1). The father was deceased and not available for testing.

The F-Box Protein 7 (*FBXO7*) gene encodes a protein involved in the ubiquitin-proteasome protein-degradation pathway. It is highly expressed in the cerebral cortex, globus pallidum, and substantia nigra. Moreover, it plays a crucial role in promoting mitophagy through its direct interaction with *PRKN* and *PINK1* [2].

To date, only nine *FBXO7* bi-allelic pathogenic variants in eleven families have been described. Homozygous variants in *FBXO7* were first identified in ten cases from a large Iranian pedigree with a characteristic phenotype known as parkinsonian-pyramidal syndrome (PPS) [3]. All subjects presented in the third decade of life with prominent pyramidal signs and three of them also showed parkinsonian features. Subsequently, other cases with PPS harboring bi-allelic variants in *FBXO7* were reported, expanding the phenotype to include cognitive impairment, upward gaze palsy, dysarthria, dysphagia, dystonia, and consistent response to dopaminergic therapy [2,4].

To date, only two publications have reported *FBXO7* bi-allelic pathogenic variants in EOPD completely lacking any of those atypical

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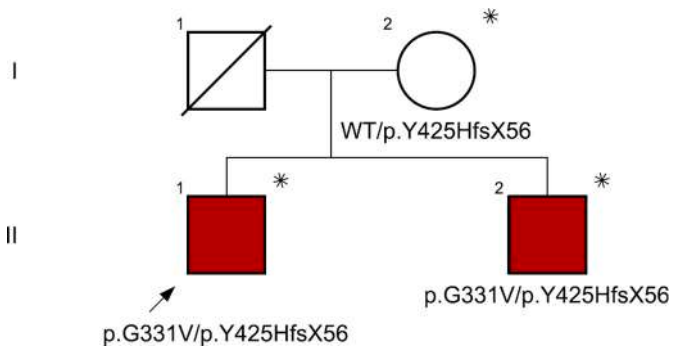

Fig. 1. Family pedigree showing variant segregation. Subjects I2, II1, and II2 were tested with PCR (marked with *). Subject I1 was not available for testing.

features [2,5].

Here, we present two additional related cases of *FBXO7*-related disease, carrying previously unreported likely pathogenic compound heterozygous variants in *FBXO7* presenting with typical EOPD. Other instances of genes generally associated with complex phenotypes that can also underlie typical EOPD are *PLA2G6* and *DNJAC6*. These findings challenge the traditional distinction between typical and atypical monogenic forms of EOPD. Our work suggests that genetic analysis of this group of genes is warranted in the workup of all EOPD cases, regardless of the presence or absence of atypical clinical features.

Declarations of interest

None.

Patient consent

Obtained.

Ethics approval

N/A.

Author contributions

I.J.K.S, M.A., L.K., V.S., R.S.A, T.S., S.J.L., D.K., N.E.M. contributed to the conception and design of the study and to the drafting of the text.

I.J.K.S, M.A., L.K., T.S., N.E.M. contributed to the acquisition of data.

I.J.K.S, M.A., L.K., V.S., R.S.A, T.S., S.J.L., D.K., N.E.M. revised the manuscript for intellectual content and approved the final article to be submitted.

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D.K. is the Founder and Scientific Advisory Board Chair of Lysosomal Therapeutics Inc. and Vanqua Bio. D.K. serves on the scientific advisory boards of The Silverstein Foundation, Intellia Therapeutics, AcureX and Prevail Therapeutics and is a Venture Partner at OrbiMed. All other authors declare that they do not have competing interests.

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Appendix A. Supplementary data

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Structural variation analysis of 6,500 whole genome sequences in amyotrophic lateral sclerosis

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There is a strong genetic contribution to Amyotrophic lateral sclerosis (ALS) risk, with heritability estimates of up to 60%. Both Mendelian and small effect variants have been identified, but in common with other conditions, such variants only explain a little of the heritability. Genomic structural variation might account for some of this otherwise unexplained heritability. We therefore investigated association between structural variation in a set of 25 ALS genes, and ALS risk and phenotype. As expected, the repeat expansion in the *C9orf72* gene was identified as associated with ALS. Two other ALS-associated structural variants were identified: inversion in the *VCP* gene and insertion in the *ERBB4* gene. All three variants were associated both with increased risk of ALS and specific phenotypic patterns of disease expression. More than 70% of people with respiratory onset ALS harboured *ERBB4* insertion compared with 25% of the general population, suggesting respiratory onset ALS may be a distinct genetic subtype.

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INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease predominantly of motor neurons, characterized by progressive weakness of the limbs, trunk, diaphragm, and bulbar musculature, with death occurring from respiratory failure, typically within 3 years of onset. Despite the poor prognosis, there is considerable variation in the survival rate, and up to 10% of people with ALS live more than 8 years from first symptoms[1]. In about 25% of people, the first symptom is difficulty with speaking or swallowing, and in nearly all the rest, it is limb weakness. However, about 1% to 2% of people experience onset with diaphragmatic weakness and early respiratory failure[2,3]. No gene variant has been found to predispose to a specific site of onset without also predisposing to greater risk of ALS. For example, pathological hexanucleotide expansion in the *C9orf72* gene, a cause of ALS, increases the risk of bulbar onset[4]. The possibility that respiratory onset ALS represents a distinct subgroup is supported by the observation that despite early diaphragm involvement, disease progression is in some cases surprisingly slow[5].

Genome-wide association studies have identified ALS risk variants that are relatively common in the population, but such alleles tend to have small effect sizes and can explain only a small proportion of heritability[6,7]. The remaining heritability is presumed to lie in other genomic variation, including rare variants, repeat sequences and structural variants, not easily tagged by SNPs.

Structural variants comprise various forms of genomic imbalance such as insertions, deletions, inversions, duplications and inter-chromosomal translocations[8]. Such variants have been associated with various neurological and psychiatric diseases including Charcot-Marie-Tooth neuropathy[9], schizophrenia[10] and autism[11,12]. Attempts to understand the relationship of structural variation with ALS have been limited by sequencing technology, computational burden, and the small number of samples[13,14]. Measuring the intensity of signals derived from a genotyping array is the most used method in detecting copy number variants[15,16],

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Telomere length analysis in amyotrophic lateral sclerosis using large-scale whole genome sequence data

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Generation of five induced pluripotent stem cells lines from four members of the same family carrying a *C9orf72* repeat expansion and one wild-type member

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Title: Generation of five induced pluripotent stem cells lines from four members of the same family carrying a *C9orf72* repeat expansion and one wild-type member

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Abstract: The most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) is the expansion of a G4C2 hexanucleotide repeat in the C9orf72 gene. The size of the repeat expansion is highly variable and a cut-off of 30 repeats has been suggested as the lower pathological limit. Repeat size variability has been observed intergenerationally and intraindividually in tissues from different organs and within the same tissue, suggesting instability of the pathological repeat expansion. In order to study this genomic instability, we established iPSCs from five members of the same family of which four carried a C9orf72 repeat expansion and one was wild-type.

Resource Table:

Unique stem cell lines identifier	IAIi005-A IAIi006-A IAIi007-A IAIi008-A IAIi009-A
Alternative name(s) of stem cell lines	AC52 (IAIi005-A) BC6 (IAIi006-A) CC5 (IAIi007-A) DC2 (IAIi008-A) EC1 (IAIi009-A)
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Emotional and autonomic response to visual erotic stimulation in patients with functional hypothalamic amenorrhea

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Introduction: Functional hypothalamic amenorrhea (FHA) is a clinical condition associated with high levels of physiological and psychological stress ranging from weight loss to maladaptive behavior and coping skills. A reliable measure of the psychophysiological response to stress and the ability to cope with stimuli is heart rate variability (HRV). Through the sympathetic (SNS) and parasympathetic nervous system (PNS), the autonomic nervous system (ANS) promotes various changes in HRV that reflect the individual's psychophysiological response to stress. FHA patients are characterized by high levels of PNS activation during psychological load, suggesting that parasympathetic hyperactivation could be a pathology marker.

Methods: In the present study, we examine changes in HRV during observation of erotic, neutral, and disgusting images in 10 patients with FHA [(mean ± S.D.) age: 26.8 ± 5.9] and in 9 controls (age: 25.4 ± 6.4; BMI: 22.47 ± 2.97) to assess the differential activation of PNS and SNS between FHA patients and controls matched for age and without other clinical conditions.

Results: Our results showed that FHA patients had significantly higher HRV activation while observing high emotional value images and not during the observation of neutral images confirming a parasympathetic hyperactivation.

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Article

TMEM106B Acts as a Modifier of Cognitive and Motor Functions in Amyotrophic Lateral Sclerosis

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Abstract: The transmembrane protein 106B (*TMEM106B*) gene is a susceptibility factor and disease modifier of frontotemporal dementia, but few studies have investigated its role in amyotrophic lateral sclerosis. The aim of this work was to assess the impact of the *TMEM106B* rs1990622 (A–major risk allele; G–minor allele) on phenotypic variability of 865 patients with amyotrophic lateral sclerosis. Demographic and clinical features were compared according to genotypes by additive, dominant, and recessive genetic models. Bulbar onset was overrepresented among carriers of the AA risk genotype, together with enhanced upper motor neuron involvement and poorer functional status in patients harboring at least one major risk allele (A). In a subset of 195 patients, we found that the homozygotes for the minor allele (GG) showed lower scores at the Edinburgh Cognitive and Behavioral Amyotrophic Lateral Sclerosis Screen, indicating a more severe cognitive impairment, mainly involving the amyotrophic lateral sclerosis-specific cognitive functions and memory. Moreover, lower motor neuron burden predominated among patients with at least one minor allele (G). Overall, we found that *TMEM106B* is a disease modifier of amyotrophic lateral sclerosis, whose phenotypic effects encompass both sites of onset and functional status (major risk allele), motor functions (both major risk and minor alleles), and cognition (minor allele).

Keywords: amyotrophic lateral sclerosis; frontotemporal lobar degeneration; TMEM106B; alleles; cognition; motor neurons

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1. Introduction

Frontotemporal dementia (FTD) is one of the most common causes of early onset dementia, following Alzheimer's disease (AD) and vascular dementia [1]. The spectrum of clinical phenotypes encompasses three subtypes, namely behavioral variant (bvFTD), semantic-variant primary progressive aphasia (svPPA), and nonfluent-variant PPA (nfvPPA) [2]. Neuropathological changes are represented by intranuclear and/or cytoplasmic accumulation of ubiquitinated proteins [3,4], mainly TAR DNA-binding protein 43 (TDP-43) [5,6] and, less frequently, hyperphosphorylated tau [7].

After the discovery that a non-coding hexanucleotide repeat expansion in the chromosome 9 open reading frame 72 (*C9orf72*) gene could result in either FTD, amyotrophic

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ORIGINAL ARTICLE

Upper motor neuron dysfunction is associated with the presence of behavioural impairment in patients with amyotrophic lateral sclerosis

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Abstract

Background and purpose: Increasing evidence shows that approximately half of patients with amyotrophic lateral sclerosis (ALS) display cognitive (ALSci) or behavioural (ALSbi) impairment, or both (ALScbi). The aim of our study was to assess whether the burden of upper and lower motor neuron involvement is associated with the presence of cognitive and behavioural impairment.

Methods: A single-centre retrospective cohort of 110 Italian ALS patients was evaluated to assess correlations between motor and cognitive/behavioural phenotypes. Upper motor neuron regional involvement was measured with the Penn Upper Motor Neuron Score (PUMNS), whilst lower motor neuron signs were assessed using the Lower Motor Neuron Score. The Edinburgh Cognitive and Behavioural ALS Screen—Italian version and the Frontal Behaviour Inventory were administered to evaluate patients' cognitive and behavioural profiles.

Results: The PUMNS at first visit was significantly higher in behaviourally impaired ALS patients (ALSbi and ALScbi) compared to behaviourally unimpaired individuals (ALS and ALSci) (9.9 vs. 6.9, $p = 0.014$). Concerning the different Frontal Behaviour Inventory subdomains, higher PUMNS correlated with the presence of apathy, emotive indifference, inflexibility, inattention, perseveration and aggressiveness.

Conclusion: To our knowledge, this is the first study showing that a clinical prominent upper motor neuron dysfunction is associated with a more significant behavioural impairment in ALS patients, suggesting the hypothesis of a preferential spreading of the pathology from the motor cortex to the ventromedial prefrontal and orbitofrontal cortex in this group of patients.

KEYWORDS
amyotrophic lateral sclerosis, behavioural impairment, ECAS, motor phenotype, upper motor neuron

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The *SOD1*-mediated ALS phenotype shows a decoupling between age of symptom onset and disease duration

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Superoxide dismutase (SOD1) gene variants may cause amyotrophic lateral sclerosis, some of which are associated with a distinct phenotype. Most studies assess limited variants or sample sizes. In this international, retrospective observational study, we compare phenotypic and demographic characteristics between people with *SOD1*-ALS and people with ALS and no recorded *SOD1* variant. We investigate which variants are associated with age at symptom onset and time from onset to death or censoring using Cox proportional-hazards regression. The *SOD1*-ALS dataset reports age of onset for 1122 and disease duration for 883 people; the comparator population includes 10,214 and 9010 people respectively. Eight variants are associated with younger age of onset and distinct survival trajectories; a further eight associated with younger onset only and one with distinct survival only. Here we show that onset and survival are decoupled in *SOD1*-ALS. Future research should characterise rarer variants and molecular mechanisms causing the observed variability.

In 1993, variants in the gene *superoxide dismutase 1 (SOD1*, [NM_000454]*)* were identified as a causal factor in people with amyotrophic lateral sclerosis (ALS), through analysis of 13 different families with 11 different *SOD1* missense mutations[1]. *SOD1* variants are reported in 15% of people with familial ALS in European populations, 30% of people with familial ALS in Asian populations, and 1–2% of people with apparently sporadic ALS in both populations[2]. Limited information is available on other populations.

SOD1-mediated ALS is characterised by distinct features related to the clinical and pathological phenotype. Since the discovery that variants in *SOD1* can cause ALS, over 180 variants have been identified and they are distributed throughout the gene and protein[3]. This is in contrast to other genetic determinants of ALS, for example mutations in *FUS*, *C9orf72* and *TARDBP*, where variants are concentrated in specific functional domains of the protein[4–6]. In *SOD1*-mediated ALS there is very little reported association with cognitive impairment, which, depending on cut-offs for neuropsychological deficits is estimated to occur in up to 50% of people with sporadic ALS in population-based studies[7]. People with *SOD1*-ALS are often reported to have a lower motor neuron predominant phenotype, with more frequent limb onset than is observed in typical ALS[8]. At the cellular level, TDP-43 protein aggregates, which are the pathological hallmark in >95% of ALS cases, are absent in most people with *SOD1*-mediated ALS implying that a different mechanistic pathway leads to motor neuron death[9,10].

Within the *SOD1* ALS population, certain variants are associated with atypical disease progression compared to ALS as reported in population-based studies. For example, the p.A5V variant is associated with shorter survival and the homozygous p.D91A variant with longer survival[11,12]. Demographic factors also correlate with survival. For example, men with *SOD1-mediated* ALS have shorter survival than women[13]. Other variants, such as p.D125V and p.H44R have been associated with faster disease progression in an Australian population[14]. As gene-specific therapies for ALS are being developed it is important to understand the prognostic implications of specific variants. This was demonstrated in a trial of Tofersen, an anti-sense oligonucleotide targeting the knock down of SOD1 mRNA, where a significant impact on disease progression was noted in a subset of patients carrying the p.A5V variant, who typically have a rapid disease progression[15].

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Additional information

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ORIGINAL RESEARCH
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Parkinsonian Syndromes in Motor Neuron Disease: A Clinical Study

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Background: Parkinsonian syndromes may rarely occur in motor neuron disease (MND). However, previous studies are heterogeneous and mostly case reports or small case series. Therefore, we aimed to identify and characterize patients with concurrent parkinsonian syndromes extracted from a cohort of 1,042 consecutive cases diagnosed with MND at a tertiary Italian Center.

Methods: Diagnosis of Parkinson's disease (PD), progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS) was made according to current criteria. Clinical characterization included: upper and lower motor neuron disease features, typical and atypical parkinsonian features, oculomotor disorders, cognitive testing, MRI features, and, when available molecular neuroimaging. Genetic testing was carried out for major MND and PD-associated genes.

Results: Parkinsonian syndromes were diagnosed in 18/1042 (1.7%) of MND patients (7 PD, 6 PSP, 3 CBS, 2 other parkinsonisms). Based on phenotype, patients could be categorized into amyotrophic lateral sclerosis (ALS)-parkinsonism and primary lateral sclerosis (PLS)-parkinsonism clusters. Across the whole database, parkinsonism was significantly more common in PLS than in other MND phenotypes (12.1 vs. 1.1%, $p = 5.0 \times 10^{-10}$). MND patients with parkinsonian features had older age of onset, higher frequency of oculomotor disorders, cognitive impairment, and family history of parkinsonism or dementia. Two patients showed pathogenic mutations in *TARDBP* and *C9orf72* genes.

Conclusion: Specific patterns in MND-parkinsonism were observed, with PLS patients often showing atypical parkinsonian syndromes and ALS patients more frequently showing typical PD. Systematic clinical, genetic, and neuropathologic characterization may provide a better understanding of these phenotypes.

Keywords: motor neuron disease (MND), parkinsonism, amyotrophic lateral sclerosis, primary lateral sclerosis (PLS), progressive supranuclear palsy

REVIEW

Diffusion Magnetic Resonance Imaging Microstructural Abnormalities in Multiple System Atrophy: A Comprehensive Review

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ABSTRACT: Multiple system atrophy (MSA) is a neurodegenerative disease characterized by autonomic failure, ataxia, and/or parkinsonism. Its prominent pathological alterations can be investigated using diffusion magnetic resonance imaging (dMRI), a technique that exploits the characteristics of water random motion inside brain tissue. The aim of this report was to review currently available literature on the application of dMRI in MSA and to describe microstructural abnormalities, diagnostic applications, and pathophysiological correlates. Sixty-four published studies involving microstructural investigation using dMRI in MSA were included. Widespread microstructural abnormalities of white matter were described, especially in the middle cerebellar peduncle, corticospinal tract, and hemispheric fibers. Gray matter degeneration was identified as well, with diffuse involvement of subcortical structures, especially in the putamina. Diagnostic applications of dMRI were mostly explored for the differential diagnosis between MSA parkinsonism and Parkinson's disease. Recently, machine learning algorithms for image processing and disease classification have demonstrated high diagnostic accuracy, showing potential for translation into clinical practice. To a lesser extent, clinical correlates of microstructural abnormalities have also been investigated, and abnormalities related to motor, ocular, and cognitive impairments were described. dMRI in MSA has contributed to in vivo identification of known pathological abnormalities. Translation into clinical practice of the latest advancements for the differential diagnosis between MSA and other forms of parkinsonism seems feasible. Current limitations involve the possibility of correctly diagnosing MSA in the very early stages, when the clinical diagnosis is most uncertain. Furthermore, pathophysiological correlates of microstructural abnormalities remain understudied. © 2022 International Parkinson and Movement Disorder Society.

Key Words: multiple system atrophy; diffusion; magnetic resonance imaging

Multiple system atrophy (MSA) is a neurodegenerative disorder characterized by autonomic failure and a variable combination of ataxia, parkinsonism, and pyramidal signs.[1] The neuropathological hallmark of MSA is argirophilic oligodendroglial cytoplasmic inclusions (GCIs)[2] containing aggregates of insoluble α-synuclein.[3] Oligodendroglial pathology is in turn associated with myelin pallor and degeneration and neuronal loss; microglial activation and astrogliosis also occur.[4,5] GCIs can be found throughout the brain, but their highest density has been reported in the basal ganglia, especially in the highly myelinated striatopallidal fibers (Wilson pencil fibers) of the putamen.[6] The density of CGIs is also associated with neuronal loss.[4] The areas affected by prominent demyelination and neuronal loss are the central

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Semiology and determinants of apathy across neurodegenerative motor disorders: A comparison between amyotrophic lateral sclerosis, Parkinson's and Huntington's disease

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Background: The semiology and determinants of apathy are largely unknown across amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Huntington's disease (HD), due to both motor and non-motor confounders. This study thus aimed at (1) profiling apathy in ALS, PD, and HD and (2) exploring its clinical determinants.

Materials: Consecutive ALS (*N* = 99), PD (*N* = 73), and HD (*N* = 25) patients underwent a motor-free assessment of apathy (Dimensional Apathy Scale, DAS), global cognition, anxiety and depression. Function was assessed through disease-specific scales. The DAS was also completed by *N* = 101 healthy controls (HCs). Between-group comparisons on DAS scores were implemented by covarying for all applicable confounders. Predictive models on DAS scores were built through multiple, stepwise regressions.

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ORIGINAL ARTICLE

Diagnostic properties of the Italian ECAS Carer Interview (ECAS-CI)

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Abstract

Background This study aimed at providing diagnostic properties and normative cut-offs for the Italian ECAS Carer Interview (ECAS-CI).

Materials $N = 292$ non-demented ALS patients and $N = 107$ healthy controls (HCs) underwent the ECAS-CI and the Frontal Behavioural Inventory (FBI). Two ECAS-CI measures were addressed: (1) the number of symptoms (NoS; *range* = 0–13) and (2) that of individual symptom clusters (SC; *range* = 0–6). Diagnostics were explored against an FBI score ≥ than the 95th percentile of the patients' distribution.

Results Both the NoS and SC discriminated patient from HCs. High accuracy, sensitivity, and specificity were detected for both the NoS and SC; however, at variance with SC, the NoS showed better post-test features and did not overestimate the occurrence of behavioural changes. The ECAS-CI converged with the FBI and diverged from the cognitive section of the ECAS.

Discussion The ECAS-CI is a suitable screener for behavioural changes in ALS patients, with the NoS being its best outcome measure (cut-off: ≥ 3).

Keywords Edinburgh Cognitive and Behavioural ALS Screen · Amyotrophic lateral sclerosis · Frontotemporal degeneration · Behavioural symptom · Psychometrics

Barbara Poletti and Edoardo Nicolò Aiello contributed equally to this work; Nicola Ticozzi and Vincenzo Silani contributed equally as well.

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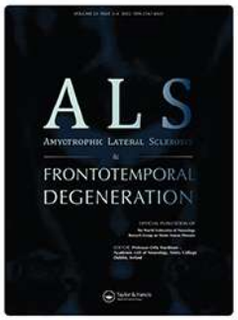

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

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

Antonia Ratti, Silvia Peverelli, Elisabetta D'Adda, Claudia Colombrita, Michele Gennuso, Alessandro Prelle & Vincenzo Silani

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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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Lab Resource: Single Cell Line

Generation of an iPSC line from a patient with spastic paraplegia type 10 carrying a novel mutation in *KIF5A* gene

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A B S T R A C T

We generated an iPSC line from a patient with spastic paraplegia type 10 (SPG10) carrying the novel missense variant c.50G > A (p.R17Q) in the *N*-terminal motor domain of the kinesin family member 5A (*KIF5A*) gene.

This patient-derived *in vitro* cell model will help to investigate the role of different *KIF5A* mutations in inducing neurodegeneration in spastic paraplegia and in other *KIF5A*-related disorders, including Charcot-Marie-Tooth type 2 (CMT2) and amyotrophic lateral sclerosis (ALS).

Resource table

Unique stem cell line identifier	IAIi010-A
Alternative name(s) of stem cell line	KIF5A_C3
Institution	IRCCS Istituto Auxologico Italiano, Milan, Italy
Contact information of distributor	Antonia Ratti, antonia.ratti@unimi.it
Type of cell line	iPSC
Origin	Human
Additional origin info required for human ESC or iPSC	Ethnicity: Caucasian Age: 79 Sex: Female
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	CytoTune iPS 2.0 Sendai Reprogramming Kit
Genetic Modification	NO
Type of Genetic Modification	N/A
Evidence of the reprogramming transgene loss (including genomic copy if applicable)	RT-PCR
Associated disease	Autosomal dominant Spastic Paraplegia type 10 (SGP10)
Gene/locus	KIF5A, chromosome 12q13.13 NM_004984.3: c.50G > A (p.R17Q)
Date archived/stock date	October 2022
Cell line repository/bank	https://hpscreg.eu/user/cellline/edit/IAIi010-A
Ethical approval	Ethical committee Regione Lombardia, sezione Fondazione IRCCS Istituto Neurologico "Carlo Besta", Milan, Italy, Approval n.64

(*continued on next column*)

Resource table (*continued*)

1. Resource utility

Allelic mutations in *KIF5A* gene are associated to different neurodegenerative disorders, such as spastic paraplegia type 10 (SPG10), axonal Charcot-Marie-Tooth type 2 (CMT2), and amyotrophic lateral sclerosis (ALS) as well as to neonatal intractable myoclonus (NEIMY) with distinct mutational hotspots.

We generated an iPSC line from a SPG10 individual carrying the novel missense mutation p.R17Q (c.50G > A) in KIF5A protein motor domain.

This iPSC line represents a new *in vitro* disease model to elucidate, upon differentiation into motoneurons, the pathomechanisms associated with *KIF5A* mutations.

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Diagnostic properties of the Frontal Assessment Battery (FAB) in Huntington's disease

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Background: This study aimed at assessing the diagnostic properties of the Frontal Assessment Battery (FAB) as to its capability to (1) discriminate healthy controls (HCs) from patients with Huntington's disease (HD) and (2) identify cognitive impairment in this population.

Materials: Thirty-eight consecutive HD patients were compared to 73 HCs on the FAB. Patients further underwent the Montreal Cognitive Assessment (MoCA) and the Unified Huntington's Disease Rating Scale (UHDRS). Receiver-operating characteristics (ROC) analyses were run to assess both intrinsic—i.e., sensitivity (Se) and specificity (Sp), and post-test diagnostics, positive and negative predictive values (PPV; NPV) and likelihood ratios (LR^+; LR^-), of the FAB both in a case–control setting and to identify, within the patient cohort, cognitive impairment (operationalized as a below-cut-off MoCA score). In patients, its diagnostic accuracy was also compared to that of the cognitive section of the UHDRS (UHDRS-II).

Results: The FAB and UHDRS-II were completed by 100 and 89.5% of patients, respectively. The FAB showed optimal case–control discrimination accuracy (AUC=0.86–0.88) and diagnostic properties (Se=0.68–0.74; Sp=0.88–0.9; PPV=0.74–0.8; NPV=0.84–0.87; LR^+=5.6–7.68; LR^-=0.36–0.29), performing even better (AUC=0.9–0.91) at identifying cognitive impairment among patients (Se=0.73–1; Sp=0.86–0.71; PPV=0.79–0.71; NPV=0.82–1; LR^+=5.13–3.5; LR^-=0.31–0) and comparably to the UHDRS-II (89% vs. 85% of accuracy, respectively; p=0.46).

Discussion: In HD patients, the FAB is highly feasible for cognitive screening aims, being also featured by optimal intrinsic/post-test diagnostics within both case-control and case-finding settings.