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

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OSPEDALE MAGGIORE POLICLINICO
ISTITUTO DI RICOVERO E CURA A CARATTERE
SCIENTIFICO DI NATURA PUBBLICA

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E

FRONTESPIZI

LAVORI SCIENTIFICI

2024

"CENTRO DINO FERRARI"

Sezione di Neuroscienze
Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti
Università degli Studi di Milano
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BRAIN
REVIEW ARTICLE

Unleashing the potential of mRNA therapeutics for inherited neurological diseases

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Neurological monogenic loss-of-function diseases are hereditary disorders resulting from gene mutations that decrease or abolish the normal function of the encoded protein. These conditions pose significant therapeutic challenges, which may be resolved through the development of innovative therapeutic strategies. RNA-based technologies, such as mRNA replacement therapy, have emerged as promising and increasingly viable treatments. Notably, mRNA therapy exhibits significant potential as a mutation-agnostic approach that can address virtually any monogenic loss-of-function disease. Therapeutic mRNA carries the information for a healthy copy of the defective protein, bypassing the problem of targeting specific genetic variants. Moreover, unlike conventional gene therapy, mRNA-based drugs are delivered through a simplified process that requires only transfer to the cytoplasm, thereby reducing the mutagenic risks related to DNA integration. Additionally, mRNA therapy exerts a transient effect on target cells, minimizing the risk of long-term unintended consequences. The remarkable success of mRNA technology for developing coronavirus disease 2019 vaccines has rekindled interest in mRNA as a cost-effective method for delivering therapeutic proteins. However, further optimization is required to enhance mRNA delivery, particularly to the CNS, while minimizing adverse drug reactions and toxicity.
In this comprehensive review, we delve into past, present and ongoing applications of mRNA therapy for neurological monogenic loss-of-function diseases. We also discuss the promises and potential challenges presented by mRNA therapeutics in this rapidly advancing field. Ultimately, we underscore the full potential of mRNA therapy as a game-changing therapeutic approach for neurological disorders.

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Biallelic variants in *POPDC2* cause a novel autosomal recessive syndrome presenting with cardiac conduction defects and variable hypertrophic cardiomyopathy

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Case report: A novel patient presenting TRIM32-related limb-girdle muscular dystrophy

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Limb-girdle muscular dystrophy autosomal recessive 8 (LGMDR8) is a rare clinical manifestation caused by the presence of biallelic variants in the *TRIM32* gene. We present the clinical, molecular, histopathological, and muscle magnetic resonance findings of a novel 63-years-old LGMDR8 patient of Italian origins, who went undiagnosed for 24 years. Clinical exome sequencing identified two *TRIM32* missense variants, c.1181G > A p.(Arg394His) and c.1781G > A p.(Ser594Asp), located in the NHL1 and NHL4 structural domains, respectively, of the TRIM32 protein. We conducted a literature review of the clinical and instrumental data associated to the so far known 26 *TRIM32* variants, carried biallelically by 53 LGMDR8 patients reported to date in 20 papers. Our proband's variants were previously identified only in three independent LGMDR8 patients in homozygosis, therefore our case is the first in literature to be described as compound heterozygous for such variants. Our report also provides additional data in support of their pathogenicity, since p.(Arg394His) is currently classified as a variant of uncertain significance, while p.(Ser594Asp) as likely pathogenic. Taken together, these findings might be useful to improve both the genetic counseling and the diagnostic accuracy of this rare neuromuscular condition.

KEYWORDS

LGMDR8, TRIM32, limb-girdle muscular dystrophy, clinical exome sequencing, tripartite motif-containing proteins

1 Introduction

The term limb-girdle muscular dystrophy (LGMD) refers to typically non-syndromic childhood- and adult-onset group of muscular dystrophies, affecting primarily skeletal muscles, and usually associated with elevated serum creatine kinase (CK) concentration (1, 2). Patients with LGMD suffer from progressive muscle weakness and wasting, involving proximal more than distal districts, in particular muscles of the shoulder and pelvic girdles (1, 2). However, other muscle groups, such as facial, distal upper and lower limbs, may also be affected (3). In the pre-molecular era, LGMD diagnosis used to be purely clinical, and it could only be confirmed differentially once specific protein testing became available (4, 5) to exclude X-linked recessive neuromuscular disorders, such as Duchenne muscular dystrophy and Becker muscular dystrophy (1). Since the advent of molecular myology, pathogenic variants in 29 genes have been reported in distinct LGMD clinical

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Case report: Dihydropyridine receptor (*CACNA1S*) congenital myopathy, a novel phenotype with early onset periodic paralysis

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Introduction: *CACNA1S* related congenital myopathy is an emerging recently described entity. In this report we describe 2 sisters with mutations in the *CACNA1S* gene and the novel phenotype of congenital myopathy and infantile onset episodic weakness.

Clinical description: Both sisters had neonatal onset hypotonia, muscle weakness, and delayed walking. Episodic weakness started in infancy and continued thereafter, provoked mostly by cold exposure. Muscle imaging revealed fat replacement of gluteus maximus muscles. Next generation sequencing found the missense p.Cys944Tyr variant and the novel splicing variant c.3526-2A>G in *CACNA1S*. Minigene assay revealed the splicing variant caused skipping of exon 28 from the transcript, potentially affecting protein folding and/or voltage dependent activation.

Conclusion: This novel phenotype supports the notion that there are age related differences in the clinical expression of *CACNA1S* gene mutations. This expands our understanding of mutations located in regions of the *CACNA1S* outside the highly conserved S4 segment, where most mutations thus far have been identified.

KEYWORDS

congenital myopathy, episodic weakness, *CACNA1S*, $Ca_V1.1$, DHPR, splice minigene assay, novel phenotype, periodic paralysis

1 Introduction

In skeletal muscle, action potential propagation results in muscle contraction, a process mediated by calcium ions and known as excitation-contraction coupling (ECC) (1). Specialized proteins take part in ECC, including the dihydropyridine receptor (DHPR), a voltage gated calcium channel located on T-tubule membranes and RyR1, located on the sarcoplasmic reticulum (SR). T-tubules tightly associate with terminal cisternae of the SR; the close association between one T-tubule and two terminal cisternae form the triad and the interaction between DHPR and RyR1 upon depolarization activates opening of RyR1 (1).

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Case report: Multiple approach analysis in a case of clinically assessed myotonia congenita

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Myotonia congenita, both in a dominant (Thomsen disease) and recessive form (Becker disease), is caused by molecular defects in *CLCN1* that encodes the major skeletal muscle chloride channel, ClC-1. This channel is important for the normal repolarization of muscle action potentials and consequent relaxation of the muscle, and its dysfunction leads to impaired muscle relaxation after voluntary or evoked contraction and muscle stiffness. More than 300 *CLCN1* pathogenic variants have been found in association with congenital myotonia, inherited as recessive or dominant traits (with complete or incomplete penetrance). In this study, we describe the case of a 44-year-old woman complaining of "leg stiffness" since the age of 20 years and presenting with transient muscle weakness, especially after sitting for several minutes, with grip myotonia and feet myotonia, cold-sensitive and warm-up. The strength was normal, but muscle hypertrophy in the lower limbs was evident. EMG myotonia was detected in all explored muscles. The patient's father had precocious cataract correction but did not show myotonic discharges at EMG. Examination of the patient's sons (aged 18 years and 12 years) was unremarkable. The patient started treatment with mexiletine, with improvement in grip myotonia and limb stiffness, but it was soon interrupted due to gastrointestinal disturbances. Direct sequencing of *CLCN1* identified the previously described heterozygous intronic variant c.1471 + 1G > A, which resulted in the skipping of exon 13 in the *CLCN1* muscle transcript. In addition, the rare heterozygous synonymous nucleotide change c.762C > T p.Cys254Cys was identified and predicted to alter physiological splicing. The detection of multiple splicing abnormalities leading to premature termination codons supported the *in silico* prediction. We developed a Western blot assay to assess the ClC-1 protein in muscle biopsy, and we observed that ClC-1 levels were consistently reduced in the patient's muscle, supporting the pathogenic behavior of the variants disclosed. Overall, we report a novel case of Becker myotonia and highlight the importance of multiple levels of analysis to achieve a firm molecular diagnosis.

KEYWORDS

myotonia congenita, CLCN1, Western blot, splicing, exonic splicing silencer

BRAIN COMMUNICATIONS

Deoxyguanosine kinase deficiency: natural history and liver transplant outcome

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Autosomal recessive pathogenetic variants in the *DGUOK* gene cause deficiency of deoxyguanosine kinase activity and mitochondrial deoxynucleotides pool imbalance, consequently, leading to quantitative and/or qualitative impairment of mitochondrial DNA synthesis. Typically, patients present early-onset liver failure with or without neurological involvement and a clinical course rapidly progressing to death.

This is an international multicentre study aiming to provide a retrospective natural history of deoxyguanosine kinase deficient patients. A systematic literature review from January 2001 to June 2023 was conducted. Physicians of research centres or clinicians all around the world caring for previously reported patients were contacted to provide followup information or additional clinical, biochemical, histological/histochemical, and molecular genetics data for unreported cases with a confirmed molecular diagnosis of deoxyguanosine kinase deficiency.

A cohort of 202 genetically confirmed patients, 36 unreported, and 166 from a systematic literature review, were analyzed. Patients had a neonatal onset (≤ 1 month) in 55.7% of cases, infantile (>1 month and ≤ 1 year) in 32.3%, pediatric (>1 year and ≤18 years) in 2.5% and adult (>18 years) in 9.5%. Kaplan-Meier analysis showed statistically different survival rates ($P < 0.0001$) among the four age groups with the highest mortality for neonatal onset. Based on the clinical phenotype, we defined four different clinical subtypes: hepatocerebral (58.8%), isolated hepatopathy (21.9%), hepatomyoencephalopathy (9.6%), and isolated myopathy (9.6%). Muscle involvement was predominant in adult-onset cases whereas liver dysfunction causes morbidity and mortality in early-onset patients with a median survival of less than 1 year. No genotype–phenotype correlation was identified. Liver transplant significantly modified the survival rate in 26 treated patients when compared with untreated. Only six patients had additional mild neurological signs after liver transplant.

In conclusion, deoxyguanosine kinase deficiency is a disease spectrum with a prevalent liver and brain tissue specificity in neonatal and infantile-onset patients and muscle tissue specificity in adult-onset cases. Our study provides clinical, molecular genetics and biochemical data for early diagnosis, clinical trial planning and immediate intervention with liver transplant and/or nucleoside supplementation.

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CLINICAL RESEARCH ARTICLE

MUSCLE&NERVE WILEY

Six-minute walk test as outcome measure of fatigability in adults with spinal muscular atrophy treated with nusinersen

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Abstract

Introduction/Aims: Fatigue (subjective perception) and fatigability (objective motor performance worsening) are relevant aspects of disability in individuals with spinal muscular atrophy (SMA). The effect of nusinersen on fatigability in SMA patients has been investigated with conflicting results. We aimed to evaluate this in adult with SMA3.

Methods: We conducted a multicenter retrospective cohort study, including adult ambulant patients with SMA3, data available on 6-minute walk test (6MWT) and Hammersmith Functional Motor Scale—Expanded (HFMSE) at baseline and at least at

Abbreviations: 6MWT, six-minute walk test; HFMSE, Hammersmith Functional Rating Scale Expanded; NMJ, neuromuscular junction; RULM, revised upper limb module; SMA, spinal muscular atrophy; SMN, survival motor neuron.

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CONFLICT OF INTEREST STATEMENT

Michela Coccia has received honoraria for speaking, advisory boards and compensation for congress participations from Roche Biogen and Novartis outside the submitted work. Giacomo Pietro Comi attended to advisory boards of Sanofi, Novartis, Roche, Sarepta and PTC Therapeutics. Stefania Corti has participated to scientific advisory board of Novartis, not related to submitted work. Lorenzo Maggi has received honoraria for speaking, advisory boards and compensation for congress participations from Sanofi Genzyme, Roche and Biogen, Amicus Theraputics, Alexion, Janssen, Lupin, outside the submitted work. Rocco Liguori has received honoraria for speaking, advisory boards and compensation for congress participations from Argenx BV, Alexion, UCB Pharma, Amicus Therapeutics, Editree, Summeet, Edra S. p.A., Med Stage. None of the other authors has any conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

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

ETHICS 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.

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REFERENCES

1. Mongiovi P, Dilek N, Garland C, et al. Patient reported impact of symptoms in spinal muscular atrophy (PRISM-SMA). *Neurology*. 2018; 91(13):e1206-e1214.
2. Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. *Neurology*. 2013; 80(4):409-416.
3. de Vries JM, Hagemans ML, Bussmann JB, et al. Fatigue in neuromuscular disorders: focus on Guillain-Barre syndrome and Pompe disease. *Cell Mol Life Sci*. 2010;67(5):701-713.

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REVIEW ARTICLE

Myotonic dystrophies: an update on clinical features, molecular mechanisms, management, and gene therapy

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Abstract
Myotonic dystrophies (DM) encompass a group of complex genetic disorders characterized by progressive muscle weakness with myotonia and multisystemic involvement. The aim of our paper is to synthesize key findings and advancements in the understanding of DM, and to underline the multidisciplinary approach to DM, emphasizing the importance of genetic counseling, comprehensive clinical care, and symptom management. We discuss the genetic basis of DM, emphasizing the role of repeat expansions in disease pathogenesis, as well as cellular and animal models utilized for studying DM mechanisms and testing potential therapies. Diagnostic challenges, such as determining the size of disease expansions and assessing mosaicism, are elucidated alongside emerging genetic testing methods. Therapeutic strategies, mainly for DM1, are also explored, encompassing small molecules, nucleic acid-based therapies (NATs), and genome/transcriptome engineering. The challenges of such a therapeutic delivery and immunogenic response and the importance of innovative strategies, including viral vectors and AAV serotypes, are highlighted within the text. While no curative treatments have been approved, supportive and palliative care remains essential, with a focus on addressing multisystemic complications and maintaining functional independence. Continued exploration of these therapeutic advancements offers hope for comprehensive disease management and potentially curative therapies for DM1 and related disorders.

Keywords DM1 · DM2 · Myotonic dystrophies · Molecular genetics · Management · Update

Introduction

Myotonic dystrophies are the most common muscular dystrophy in adults, autosomal inherited, mainly characterized by muscle weakness and myotonia, with multisystemic involvement. So far 2 distinct entities have been described: Myotonic Dystrophy type 1 (DM1) (OMIM #160900) and Myotonic Dystrophy type 2 (DM2) (OMIM #602668). DM1 and DM2 are clinically similar, sharing core features such as myotonia, muscle weakness and early onset cataract, but there are some important differences allowing the distinction between the two forms [1, 2].

In this article we aim to provide an updated point-by-point review concisely addressing clinical, molecular, therapeutic, and management key aspects of both DM1 and DM2 in a practical and ready-to-use way. This comprehensive overview will delve into the genetic discoveries associated with both conditions, elaborate on their distinct clinical features, unravel the molecular mechanisms underlying their pathogenesis, and discuss the latest available therapeutic interventions and management strategies.

Additionally, the review will incorporate the most recent guidelines for the management of the myotonic dystrophies, ensuring that the information is up to date, reflecting developments in the field until 2024. This comprehensive exploration seeks to enhance our comprehension of these

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MUSCLE DISEASE

Pathogenic *TNNI1* variants disrupt sarcomere contractility resulting in hypo- and hypercontractile muscle disease

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Troponin I (TnI) regulates thin filament activation and muscle contraction. Two isoforms, TnI-fast (*TNNI2*) and TnI-slow (*TNNI1*), are predominantly expressed in fast- and slow-twitch myofibers, respectively. *TNNI2* variants are a rare cause of arthrogryposis, whereas *TNNI1* variants have not been conclusively established to cause skeletal myopathy. We identified recessive loss-of-function *TNNI1* variants as well as dominant gain-of-function *TNNI1* variants as a cause of muscle disease, each with distinct physiological consequences and disease mechanisms. We identified three families with biallelic *TNNI1* variants (F1: p.R14H/c.190-9G>A, F2 and F3: homozygous p.R14C), resulting in loss of function, manifesting with early-onset progressive muscle weakness and rod formation on histology. We also identified two families with a dominantly acting heterozygous *TNNI1* variant (F4: p.R174Q and F5: p.K176del), resulting in gain of function, manifesting with muscle cramping, myalgias, and rod formation in F5. In zebrafish, TnI proteins with either of the missense variants (p.R14H; p.R174Q) incorporated into thin filaments. Molecular dynamics simulations suggested that the loss-of-function p.R14H variant decouples TnI from TnC, which was supported by functional studies showing a reduced force response of sarcomeres to submaximal [Ca^{2+}] in patient myofibers. This contractile deficit could be reversed by a slow skeletal muscle troponin activator. In contrast, patient myofibers with the gain-of-function p.R174Q variant showed an increased force to submaximal [Ca^{2+}], which was reversed by the small-molecule drug mavacamten. Our findings demonstrated that *TNNI1* variants can cause muscle disease with variant-specific pathomechanisms, manifesting as either a hypo- or a hypercontractile phenotype, suggesting rational therapeutic strategies for each mechanism.

INTRODUCTION

The troponin complex is critical for the regulation of muscle contraction [reviewed in (*1*)]. It is composed of three distinct subunits: a Ca^{2+} binding subunit (TnC), a tropomyosin binding subunit (TnT), and the actomyosin adenosine triphosphatase (ATPase) inhibitory subunit (TnI). Upon muscle activation, Ca^{2+} enters the cytosol and binds to TnC, initiating a chain of events that leads to the release of TnI from actin, which allows movement of the tropomyosin dimer strand. This movement exposes myosin binding sites on actin and allows the myosin heads on the thick filament to grab and pull on the

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66. C. A. Ottenheijm, C. C. Witt, G. J. Stienen, S. Labeit, A. H. Beggs, H. Granzier, Thin filament length dysregulation contributes to muscle weakness in nemaline myopathy patients with nebulin deficiency. *Hum. Mol. Genet.* **18**, 2359–2369 (2009).
67. S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W. W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H. L. Rehm; ACMG Laboratory Quality Assurance Committee, Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).

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Cellular and Molecular Life Sciences

ORIGINAL ARTICLE

Targeting STMN2 for neuroprotection and neuromuscular recovery in Spinal Muscular Atrophy: evidence from in vitro and in vivo SMA models

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Abstract

The development of ground-breaking Survival Motor Neuron (SMN) replacement strategies has revolutionized the field of Spinal Muscular Atrophy (SMA) research. However, the limitations of these therapies have now become evident, highlighting the need for the development of complementary targets beyond SMN replacement. To address these challenges, here we explored, in in vitro and in vivo disease models, Stathmin-2 (STMN2), a neuronal microtubule regulator implicated in neurodegenerative diseases like Amyotrophic Lateral Sclerosis (ALS), as a novel SMN-independent target for SMA therapy. Our findings revealed that STMN2 overexpression effectively restored axonal growth and outgrowth defects in induced pluripotent stem cell-(iPSC)-derived motor neurons (MNs) from SMA patients. Intracerebroventricular administration of adeno-associated virus serotype 9 (AAV9) carrying *Stmn2* cDNA significantly ameliorated survival rates, motor functions, muscular and neuromuscular junction pathological features in SMA mice, mirrored by in vitro outcomes. Overall, this pioneering study not only provides insight into the therapeutic potential of STMN2 in SMA, but also suggests its broader applications for MN diseases, marking a substantial step forward in addressing the multifaceted challenges of neurological diseases treatment.

Keywords STMN2 · Motor neurons · SMA mouse · Modifier gene · SMN

Introduction

Spinal Muscular Atrophy (SMA) is still the leading genetic (autosomal-recessive) cause of infant mortality, characterized by the degeneration of spinal motor neurons (MNs) and progressive muscle weakness and atrophy. This degeneration ultimately leads to paralysis and early death [1–7]. SMA arises from mutations in the *Survival Motor Neuron 1* (*SMN1*) gene, which result in reduced SMN protein expression. This deficit is partially offset by the expression of SMN from the paralogous *SMN2* gene. A critical distinction between SMN1 and SMN2 is a single-nucleotide (C-to-T) transition in exon 7 which determines the exclusion of exon 7 during transcription. As a result, the majority of SMN protein produced by *SMN2* is truncated and non-functional, with only about 10% full-length [8]. The number of *SMN2* copies and the overall level of full-length transcripts can approximately predict the severity of SMA, although other modifying genes play a role.

In recent years, there has been a significant advancement in therapeutic approaches for SMA, directed to full-length SMN-level-restoration [9]. Current therapies include small molecules [10–13] and antisense oligonucleotides (ASOs) [14, 15] that modify *SMN2* splicing, thereby promoting

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this way its JNK-mediated degradation, and thus STMN2 levels, and delaying axonal degeneration.

Taken together, our data provide evidence that STMN2 may act as a protective modifier in SMA. The presented research advances our understanding of the pathogenic mechanism of SMA, identifying a possible modifier gene suitable for gene therapy or pharmacological modulation for SMA and other MN diseases.

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Data availability All relevant data are in the manuscript and supplementary information.

Declarations

Ethical approval All animal experiments strictly follow the guidelines of the Italian Ministry of Health in compliance with U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals (718/2022).

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References

1. Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G (1998) A novel function for SMN, the spinal muscular atrophy Disease Gene product, in Pre-mRNA splicing. Cell 95:615–624. https://doi.org/10.1016/S0092-8674(00)81632-3
2. Faravelli I, Nizzardo M, Comi GP, Corti S (2015) Spinal muscular atrophy—recent therapeutic advances for an old challenge. Nat Rev Neurol 11:351–359. https://doi.org/10.1038/nrneurol.2015.77
3. Pearn J (1978) Incidence, prevalence, and gene frequency studies of chronic childhood spinal muscular atrophy. J Med Genet 15:409–413. https://doi.org/10.1136/jmg.15.6.409
4. Sumner CJ, Paushkin S, Ko C-P (2017) Spinal muscular atrophy: disease mechanisms and therapy. Elsevier/Academic, Amsterdam
5. D'Amico A, Mercuri E, Tiziano FD, Bertini E (2011) Spinal muscular atrophy. Orphanet J Rare Dis 6:71. https://doi.org/10.1186/1750-1172-6-71
6. Frugier T, Nicole S, Cifuentes-Diaz C, Melki J (2002) The molecular bases of spinal muscular atrophy. Curr Opin Genet Dev 12:294–298. https://doi.org/10.1016/S0959-437X(02)00301-5
7. Lefebvre S, Bürglen L, Reboullet S et al (1995) Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80:155–165. https://doi.org/10.1016/0092-8674(95)90460-3
8. Ogino S, Wilson RB (2004) Spinal muscular atrophy: molecular genetics and diagnostics. Expert Rev Mol Diagn 4:15–29. https://doi.org/10.1586/14737159.4.1.15
9. Ponomarev AS, Chulpanova DS, Yanygina LM et al (2023) Emerging Gene Therapy approaches in the management of spinal muscular atrophy (SMA): an overview of clinical trials and patent Landscape. IJMS 24:13743. https://doi.org/10.3390/ijms241813743
10. Wang J, Schultz PG, Johnson KA (2018) Mechanistic studies of a small-molecule modulator of SMN2 splicing. Proc Natl Acad Sci U S A 115:E4604–E4612. https://doi.org/10.1073/pnas.1800260115
11. Ratni H, Ebeling M, Baird J et al (2018) Discovery of Risdiplam, a selective survival of Motor Neuron-2 (SMN2) gene splicing modifier for the treatment of spinal muscular atrophy (SMA). J Med Chem 61:6501–6517. https://doi.org/10.1021/acs.jmedchem.8b00741
12. Darras BT, Masson R, Mazurkiewicz-Bełdzińska M et al (2021) Risdiplam-treated infants with type 1 spinal muscular atrophy versus historical controls. N Engl J Med 385:427–435. https://doi.org/10.1056/NEJMoa2102047
13. Mercuri E, Baranello G, Boespflug-Tanguy O et al (2023) Risdiplam in types 2 and 3 spinal muscular atrophy: a randomised, placebo-controlled, dose-finding trial followed by 24 months of treatment. Eur J Neurol 30:1945–1956. https://doi.org/10.1111/ene.15499
14. Finkel RS, Mercuri E, Darras BT et al (2017) Nusinersen versus Sham Control in infantile-onset spinal muscular atrophy. N Engl J Med 377:1723–1732. https://doi.org/10.1056/NEJMoa1702752
15. Van Der Wadman RI, Bosboom WM et al (2019) Drug treatment for spinal muscular atrophy type I. https://doi.org/10.1002/14651858.CD006281.pub5. Cochrane Database of Systematic Reviews
16. Hoy SM (2019) Onasemnogene Abeparvovec: First Global approval. Drugs 79:1255–1262. https://doi.org/10.1007/s40265-019-01162-5
17. Van Alstyne M, Tattoli I, Delestrée N et al (2021) Gain of toxic function by long-term AAV9-mediated SMN overexpression in the sensorimotor circuit. Nat Neurosci 24:930–940. https://doi.org/10.1038/s41593-021-00827-3
18. Chaytow H, Faller KME, Huang Y-T, Gillingwater TH (2021) Spinal muscular atrophy: from approved therapies to future

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Cardiac risk and myocardial fibrosis assessment with cardiac magnetic resonance in patients with myotonic dystrophy

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Introduction: Non-invasive evaluation of myocardial tissue is a major goal of cardiac imaging. This is the case of myocardial fibrosis which is crucial in many myocardial diseases. Cardiac extracellular volume (ECV) was shown to indicate myocardial fibrosis and early cardiac involvement. With this study, our objective is to evaluate ECV measured with cardiac magnetic resonance (CMR) in patients with myotonic dystrophy type 1 (DM1) and 2 (DM2) as potential imaging biomarkers of subclinical cardiac pathology, and its relationship with demographic and clinical parameters, ECG-derived measures of cardiac conduction, and neuromuscular performance status.

Materials and methods: We retrospectively analyzed 18 DM1 patients and 4 DM2 patients without apparent cardiac disease who had CMR at our center. Differences between independent distributions were evaluated using Mann–Whitney U test, while correlations were evaluated using Spearman's ρ.

Results: Global ECV in DM1 patients (median 28.36; IQR 24.81–29.77) was significantly higher ($p = 0.0141$) than in DM2 patients (median 22.93; IQR 21.25–24.35), and than that reported in literature in healthy subjects ($p = 0.0374$; median 25.60; IQR 19.90–31.90). Septal ECV was significantly higher ($p = 0.0074$) in DM1 (median 27.37; IQR 25.97–29.74) than in DM2 patients (median 22.46; 21.57–23.19). Global ECV showed a strong, positive correlation with septal ECV ($\rho = 0.9282$, $p < 0.0001$). We observed that DM1 women showed significantly higher global ($p = 0.0012$) and septal ($p < 0.0001$) ECV values compared to men.

Discussion: We found a significant increase in global and septal cardiac ECV in patients with DM1. These values might thus suggest that DM1 patients present an increased cardiovascular risk, mainly due to cardiac fibrosis, even in absence of overt cardiac pathology at other common cardiovascular exams. DM1 patients may also be at increased risk of early septal fibrosis, with important implications on the risk for fatal arrhythmias. In addition, our results suggest the presence of gender-related differences, with DM1 women being more prone to myocardial fibrosis. Physicians dealing with DM1 may consider CMR as a screening tool for the early identification of patients with increased cardiovascular risk.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Meola G, Cardani R. Myotonic dystrophies: an update on clinical aspects, genetic, pathology, and molecular pathomechanisms. *Biochim Biophys Acta*. (2015) 1852:594–606. doi: 10.1016/J.BBADIS.2014.05.019

2. Liao Q, Zhang Y, He J, Huang K. Global prevalence of myotonic dystrophy: an updated systematic review and meta-analysis. *Neuroepidemiology*. (2022) 56:163–73. doi: 10.1159/000524734

3. Meola G. Myotonic dystrophy type 2: the 2020 update. *Acta Myol*. (2020) 39:222–34. doi: 10.36185/2532-1900-026

4. McNally EM, Mann DL, Pinto Y, Bhakta D, Tomaselli G, Nazarian S, et al. Clinical care recommendations for cardiologists treating adults with myotonic dystrophy. *J Am Heart Assoc*. (2020) 9:e014006. doi: 10.1161/JAHA.119.014006

5. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, et al. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. *N Engl J Med*. (2008) 358:2688–97. doi: 10.1056/NEJMOA062800

6. Freyermuth F, Rau F, Kokunai Y, Linke T, Sellier C, Nakamori M, et al. Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy. *Nat Commun*. (2016) 7:11067. doi: 10.1038/NCOMMS11067

7. Yadava RS, Frenzel-McCardell CD, Yu Q, Srinivasan V, Tucker AL, Puymirat J, et al. RNA toxicity in myotonic muscular dystrophy induces NKX2-5 expression. *Nat Genet*. (2008) 40:61–8. doi: 10.1038/NG.2007.28

8. Mahadevan MS, Yadava RS, Mandal M. Cardiac pathology in myotonic dystrophy type 1. *Int J Mol Sci*. (2021) 22:11874. doi: 10.3390/ijms222111874

9. Cannaò PM, Altabella L, Petrini M, Alì M, Secchi F, Sardanelli F. Novel cardiac magnetic resonance biomarkers: native T1 and extracellular volume myocardial mapping. *Eur Heart J Supplements*. (2016) 18:E64–71. doi: 10.1093/EURHEARTJ/SUW022

10. Ludatscher RM, Kerner H, Amikam S, Gellei B. Myotonia dystrophica with heart involvement: an electron microscopic study of skeletal, cardiac, and smooth muscle. *J Clin Pathol*. (1978) 31:1057–64. doi: 10.1136/JCP.31.11.1057

11. Nguyen HH, Wolfe JT, Holmes DR, Edwards WD. Pathology of the cardiac conduction system in myotonic dystrophy: a study of 12 cases. *J Am Coll Cardiol*. (1988) 11:662–71. doi: 10.1016/0735-1097(88)91547-1

12. Turillazzi E, Neri M, Riezzo I, Di Paolo M, Evangelisti L, Fineschi V. Cardiac fibrosis, arrhythmia and sudden death in myotonic dystrophy type 1: could TGF-ß1 improve the predictive accuracy of patients at risk, opening new therapeutic challenges? *Int J Cardiol*. (2013) 168:4976–8. doi: 10.1016/J.IJCARD.2013.07.135

13. Uemura N, Tanaka H, Niimura T, Hashiguchi N, Yoshimura M, Terashi S, et al. Electrophysiological and histological abnormalities of the heart in myotonic dystrophy. *Am Heart J*. (1973) 86:616–24. doi: 10.1016/0002-8703(73)90338-4

14. Captur G, Lopes LR, Mohun TJ, Patel V, Li C, Bassett P, et al. Prediction of sarcomere mutations in subclinical hypertrophic cardiomyopathy. *Circulation*. (2014) 7:863–71. doi: 10.1161/CIRCIMAGING.114.002411

15. Haaf P, Garg P, Messroghli DR, Broadbent DA, Greenwood JP, Plein S. Cardiac T1 mapping and extracellular volume (ECV) in clinical practice: a comprehensive review. *J Cardiovasc Magn Reson*. (2016) 18:89. doi: 10.1186/S12968-016-0308-4

16. Messroghli DR, Moon JC, Ferreira VM, Grosse-Wortmann L, He T, Kellman P, et al. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2* and extracellular volume: a consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imaging (EACVI). *J Cardiovasc Magn Reson*. (2017) 19:75. doi: 10.1186/s12968-017-0389-8

17. Messroghli DR, Radjenovic A, Kozerke S, Higgins DM, Sivananthan MU, Ridgway JP. Modified look-locker inversion recovery (MOLLI) for high-resolution T 1 mapping of the heart. *Magn Reson Med*. (2004) 52:141–6. doi: 10.1002/mrm.20110

18. Chmielewski L, Bietenbeck M, Patrascu A, Rösch S, Sechtem U, Yilmaz A, et al. Non-invasive evaluation of the relationship between electrical and structural cardiac abnormalities in patients with myotonic dystrophy type 1. *Clin Res Cardiol*. (2019) 108:857–67. doi: 10.1007/S00392-019-01414-0

19. Choudhary P, Nandakumar R, Greig H, Broadhurst P, Dean J, Puranik R, et al. Structural and electrical cardiac abnormalities are prevalent in asymptomatic adults with myotonic dystrophy. *Heart*. (2016) 102:1472–8. doi: 10.1136/HEARTJNL-2015-308517

20. Hermans MC, Faber CG, Bekkers SC, De Die-Smulders CE, Gerrits MM, Merkies IS, et al. Structural and functional cardiac changes in myotonic dystrophy type 1: a cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson*. (2012) 14:54. doi: 10.1186/1532-429X-14-48

21. Petri H, Ahtarovski KA, Vejlstrup N, Vissing J, Witting N, Køber L, et al. Myocardial fibrosis in patients with myotonic dystrophy type 1: a cardiovascular magnetic resonance study. *J Cardiovasc Magn Reson*. (2014) 16:59. doi: 10.1186/S12968-014-0059-Z

22. Tanawuttiwat T, Wagner KR, Tomaselli G, Nazarian S. Left ventricular dysfunction and conduction disturbances in patients with myotonic muscular dystrophy type I and II. *JAMA Cardiol*. (2017) 2:225–8. doi: 10.1001/JAMACARDIO.2016.4145

23. Turkbey EB, Gai N, Lima JAC, Van Der Geest RJ, Wagner KR, Tomaselli GF, et al. Assessment of cardiac involvement in myotonic muscular dystrophy by T1 mapping on magnetic resonance imaging. *Heart Rhythm*. (2012) 9:1691–7. doi: 10.1016/j.hrthm.2012.06.032

24. Alì M, Monti CB, Melazzini L, Cardani R, Fossati B, Cavalli M, et al. Rare disease: cardiac risk assessment with MRI in patients with myotonic dystrophy type 1. *Front Neurol*. (2020) 11:525516. doi: 10.3389/FNEUR.2020.00192/BIBTEX

25. Cardona A, Arnold WD, Kissel JT, Raman SV, Zareba KM. Myocardial fibrosis by late gadolinium enhancement cardiovascular magnetic resonance in myotonic muscular dystrophy type 1: highly prevalent but not associated with surface conduction abnormality. *J Cardiovasc Magn Reson*. (2019) 21:26. doi: 10.1186/S12968-019-0535-6

26. Leali M, Aimo A, Ricci G, Torri F, Todiere G, Vergaro G, et al. Cardiac magnetic resonance findings and prognosis in type 1 myotonic dystrophy. *J Cardiovasc Med*. (2023) 24:340–7. doi: 10.2459/JCM.0000000000001476

27. Luetkens JA, von Landenberg C, Isaak A, Faron A, Kuetting D, Gliem C, et al. Comprehensive cardiac magnetic resonance for assessment of cardiac involvement in myotonic muscular dystrophy type 1 and 2 without known cardiovascular disease. *Circ Cardiovasc Imaging*. (2019) 12:e009100. doi: 10.1161/CIRCIMAGING.119.009100

THERAPEUTIC ADVANCES in
Neurological Disorders

Case Report

A rare association of Guillain–Barré syndrome/Miller–Fisher syndrome overlap syndrome and Herpes Simplex Virus Type 1 infection: trigger or exacerbating factor?

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***Abstract*:** Guillain–Barré syndrome (GBS) and its variants represent a spectrum of acute, immune-mediated polyneuropathies with heterogeneous clinical presentations and underlying etiologies. While infectious triggers are common precursors to these disorders, the association between viral infections and autoimmune neurological conditions remains an area of active investigation. Here, we report a case of GBS/Miller–Fisher syndrome overlap syndrome in an 80-year-old male presenting with dysarthria, dysphonia, ophthalmoplegia, areflexia, and postural instability following an upper respiratory tract infection. Cerebrospinal fluid analysis revealed the unexpected detection of herpes simplex virus type 1 DNA. Treatment with intravenous immunoglobulin therapy and acyclovir resulted in a progressive recovery of neurological symptoms. This case emphasizes the role of viral infections in differential diagnosis or as potential triggers for autoimmune neurological disorders highlighting the efficacy to addressed therapy in such complex cases.

Keywords: case report, Guillain–Barré syndrome, Herpes Simplex Virus 1, HSV-1 encephalitis, inflammatory neuropathy, Miller–Fisher syndrome

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Introduction

Guillain–Barré syndrome (GBS) represents a spectrum of acute, immune-mediated polyneuropathies and one of the major emergencies in the neuromuscular domain. GBS is a multifaceted disorder, characterized by an array of phenotypes, electrophysiological features, and prognostic outcomes.[1]

The recognition of GBS as a spectrum of disorders with discrete, complete, and incomplete forms that sometimes overlap is critical for understanding its various clinical and electrophysiological variants and their underlying mechanisms. This spectrum includes typical presentations as well as atypical forms such as Miller–Fisher syndrome (MFS), which is characterized by ophthalmoplegia, ataxia, and areflexia, often with the presence of anti-GQ1b antibodies.[2,3]

Overlapping clinical features between GBS and MFS, particularly in cases involving limb weakness and motor nerve abnormalities, have led to the classification of GBS/MFS overlap syndromes.[1,4,5] While a variety of noninfectious triggers such as surgical procedures, trauma, certain medications (notably immune checkpoint inhibitors), vaccinations, and systemic illnesses have been identified as antecedents or risk factors for GBS, infectious events are the predominant precursors to its clinical onset.[6,7] This association is highlighted by the frequent occurrence of GBS following respiratory or gastrointestinal infections, with pathogens like *Campylobacter jejuni*, Cytomegalovirus (CMV), and Epstein–Barr virus

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ORIGINAL COMMUNICATION

Exploiting the role of CSF NfL, CHIT1, and miR-181b as potential diagnostic and prognostic biomarkers for ALS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder characterized by relentless and progressive loss of motor neurons. A molecular diagnosis, supported by the identification of specific biomarkers, might promote the definition of multiple biological subtypes of ALS, improving patient stratification and providing prognostic information. Here, we investigated the levels of neurofilament light chain (NfL), chitotriosidase (CHIT1) and microRNA-181b (miR-181b) in the cerebrospinal fluid (CSF) of ALS subjects ($N=210$) as well as neurologically healthy and neurological disease controls ($N=218$, including $N=74$ with other neurodegenerative diseases) from a large European multicentric cohort, evaluating their specific or combined utility as diagnostic and prognostic biomarkers. NfL, CHIT1 and miR-181b all showed significantly higher levels in ALS subjects compared to controls, with NfL showing the most effective diagnostic performance. Importantly, all three biomarkers were increased compared to neurodegenerative disease controls and, specifically, to patients with Alzheimer's disease (AD; $N=44$), with NfL and CHIT1 being also higher in ALS than in alpha-synucleinopathies ($N=22$). Notably, ALS patients displayed increased CHIT1 levels despite having, compared to controls, a higher prevalence of a polymorphism lowering CHIT1 expression. While no relationship was found between CSF miR-181b and clinical measures in ALS (disease duration, functional disability, and disease progression rate), CSF NfL was the best independent predictor of disease progression and survival. This study deepens our knowledge of ALS biomarkers, highlighting the relative specificity of CHIT1 for ALS among neurodegenerative diseases and appraising the potential diagnostic utility of CSF miR-181b.

Keywords ALS · CSF · Biomarker · NfL · CHIT1 · MiR-181b

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REVIEWS

Shaping the Neurovascular Unit Exploiting Human Brain Organoids

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Abstract

Brain organoids, three-dimensional cell structures derived from pluripotent stem cells, closely mimic key aspects of the human brain *in vitro*, providing a powerful tool for studying neurodevelopment and disease. The neuroectodermal induction protocol employed for brain organoid generation primarily gives rise to the neural cellular component but lacks the vital vascular system, which is crucial for the brain functions by regulating differentiation, migration, and circuit formation, as well as delivering oxygen and nutrients. Many neurological diseases are caused by dysfunctions of cerebral microcirculation, making vascularization of human brain organoids an important tool for pathogenetic and translational research. Experimentally, the creation of vascularized brain organoids has primarily focused on the fusion of vascular and brain organoids, on organoid transplantation *in vivo*, and on the use of microfluidic devices to replicate the intricate microenvironment of the human brain *in vitro*. This review summarizes these efforts and highlights the importance of studying the neurovascular unit in a forward-looking perspective of leveraging their use for understanding and treating neurological disorders.

Keywords Pluripotent stem cells · Vascular system · Cerebral organoids · Vascular organoids · Circulation · Microcirculation

Introduction

Organoids are complex three-dimensional (3D) cellular systems derived from pluripotent stem cells (PSCs) or adult stem cells [1, 2]. Concerning the nervous system's organoids, when the originating cells are cultured in suspension under specific conditions that reproduce embryonic development, they form structures that mimic the organ architectures and functions, including the central nervous system (CNS) as a whole (whole-brain organoids) or specific CNS areas (regional organoids) [3]. As cells develop within brain organoids, they follow a developmental timeline that is similar to *in vivo* neurogenesis [3]. Brain organoids are able to generate spontaneous neural activity, form functional synapses, and support interneuron migration or axonal projection, as well as interact and fuse with adjacent organoids giving rise to assembloids, mimicking the architecture and complex interactions of CNS tissues [4, 5]. Additionally, transcriptomic and epigenetic studies confirmed that brain organoids recapitulate the key molecular features of the human embryonic/fetal brain [4].

The main source of cells used for brain organoid generation is induced pluripotent stem cells (iPSCs) [6] and embryonic stem cells (ESCs) [7], both widely employed. iPSCs are derived from somatic cells primarily of the skin or of the blood, which are reprogrammed into embryonic-like states by administration of specific pluripotency

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Sleep and sleep-related breathing disorders in patients with spinal muscular atrophy: a changing perspective from novel treatments?

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Spinal Muscular Atrophy (SMA) is an inherited neuromuscular disorder characterized by progressive muscle weakness and atrophy, resulting from the degeneration of motor neurons in the spinal cord. A critical aspect of SMA is its impact on respiratory function. As the disease progresses, respiratory muscles, in particular intercostal muscles, become increasingly affected, leading to breathing difficulties and respiratory failure. Without intervention, many children with SMA type 1 die from respiratory failure before their second year of life. While assisted ventilation has improved survival, it often results in ventilator dependence. The development of new SMN-augmenting therapies has renewed optimism, but their long-term impact on respiratory function is uncertain, and non-invasive respiratory support remains an important part of SMA management. Despite the importance of respiratory support in SMA, knowledge regarding sleep disorders in this population is limited. This review aims to synthesize existing literature on sleep and sleep-related breathing disorders in patients with SMA, with a focus on SMA type 1. We summarize evidence of sleep-disordered breathing and respiratory failure in SMA, as well as outcomes and survival benefits associated with non-invasive or invasive ventilation with or without pharmacological therapies. We also discuss current knowledge regarding the effects of novel disease-modifying therapies for SMA on respiratory function and sleep. In conclusion, optimal care for children with SMA requires a multidisciplinary approach that includes neurology and respiratory specialists. This review highlights the importance of monitoring sleep and respiratory function in SMA, as well as the potential benefits and challenges associated with assisted ventilation combined with new therapies.

KEYWORDS

spinal muscular atrophy, sleep, respiratory disorders, sleep-related breathing disorders, non-invasive ventilation

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REVIEW

WILEY

Charcot–Marie-Tooth type 2A in vivo models: Current updates

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Abstract

Charcot–Marie-Tooth type 2A (CMT2A) is an inherited sensorimotor neuropathy associated with mutations within the *Mitofusin 2* (*MFN2*) gene. These mutations impair normal mitochondrial functioning via different mechanisms, disturbing the equilibrium between mitochondrial fusion and fission, of mitophagy and mitochondrial axonal transport. Although CMT2A disease causes a significant disability, no resolutive treatment for CMT2A patients to date. In this context, reliable experimental models are essential to precisely dissect the molecular mechanisms of disease and to devise effective therapeutic strategies. The most commonly used models are either in vitro or in vivo, and among the latter murine models are by far the most versatile and popular. Here, we critically revised the most relevant literature focused on the experimental models, providing an update on the mammalian models of CMT2A developed to date. We highlighted the different phenotypic, histopathological and molecular characteristics, and their use in translational studies for bringing potential therapies from the bench to the bedside. In addition, we discussed limitations of these models and perspectives for future improvement.

KEYWORDS
animal model, Charcot–Marie-Tooth type 2A, mitofusin 2, mouse models

1 | INTRODUCTION

Charcot–Marie-Tooth (CMT) type 2A (CMT2A; OMIM 609260) is an inherited sensorimotor axonal neuropathy and is the most frequent subtype of axonal CMT.[1–7] The disease is associated with mutations in the r *Mitofusin 2* (*MFN2*) gene, which encodes for the protein Mitofusin 2 (MFN2).[1] MFN2 is a GTPase that is anchored to the outer mitochondrial membrane which is involved in the maintenance of the equilibrium between mitochondrial fusion and fission, as well as other mitochondrial processes.[3] CMT2A is clinically defined by marked neuropathy, primarily affecting motor function, and in certain instances, accompanied by substantial proprioception loss. This results in progressive muscle weakness and atrophy in the legs and arms, typically onset during childhood and leading to considerable disability.[6–9] More than 100 *MFN2* mutations have been described in affected subjects,[10–13] Nevertheless, although CMT2A is generally associated with autosomal dominant or de novo dominant inheritance, few recessive and semidominant forms have been reported.[1–3,5–7,10,11,13] MFN2 mutations seem to induce the disease through a 'dominant-negative' mechanism, where the expression of the wild-type (WT) MFN2 allele is negatively controlled by the mutant protein.[6,14–17] To date, no clinical

Stefania Corti and Federica Rizzo equally contributed to the work.

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Improved synthesis and application of an alkyne-functionalized isoprenoid analogue to study the prenylomes of motor neurons, astrocytes and their stem cell progenitors

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Keywords:
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ABSTRACT

Protein prenylation is one example of a broad class of post-translational modifications where proteins are covalently linked to various hydrophobic moieties. To globally identify and monitor levels of all prenylated proteins in a cell simultaneously, our laboratory and others have developed chemical proteomic approaches that rely on the metabolic incorporation of isoprenoid analogues bearing bio-orthogonal functionality followed by enrichment and subsequent quantitative proteomic analysis. Here, several improvements in the synthesis of the alkyne-containing isoprenoid analogue C15AlkOPP are reported to improve synthetic efficiency. Next, metabolic labeling with C15AlkOPP was optimized to obtain useful levels of metabolic incorporation of the probe in several types of primary cells. Those conditions were then used to study the prenylomes of motor neurons (ES-MNs), astrocytes (ES-As), and their embryonic stem cell progenitors (ESCs), which allowed for the identification of 54 prenylated proteins from ESCs, 50 from ES-MNs, and 84 from ES-As, representing all types of prenylation. Bioinformatic analysis revealed specific enriched pathways, including nervous system development, chemokine signaling, Rho GTPase signaling, and adhesion. Hierarchical clustering showed that most enriched pathways in all three cell types are related to GTPase activity and vesicular transport. In contrast, STRING analysis showed significant interactions in two populations that appear to be cell type dependent. The data provided herein demonstrates that robust incorporation of C15AlkOPP can be obtained in ES-MNs and related primary cells purified via magnetic-activated cell sorting allowing the identification and quantification of numerous prenylated proteins. These results suggest that metabolic labeling with C15AlkOPP should be an effective approach for investigating the role of prenylated proteins in primary cells in both normal cells and disease pathologies, including ALS.

1. Introduction

Protein prenylation is one example of a broad class of post-translational modifications where proteins are covalently linked to various hydrophobic moieties. Prenylated proteins incorporate either C15 (farnesyl) or C20 (geranylgeranyl) isoprenoids derived from farnesyl (FPP) or geranylgeranyl diphosphate (GGPP), respectively (Fig. 1) [1]. The lipidation reaction is catalyzed by protein prenyltransferases,

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RESEARCH ARTICLE

Early spinal muscular atrophy treatment following newborn screening: A 20-month review of the first Italian regional experience

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Abstract

Objectives: Mandatory newborn screening (NBS) for spinal muscular atrophy (SMA) was implemented for the first time in Italy at the end of 2021, allowing the identification and treatment of patients at an asymptomatic stage. **Methods**: DNA samples extracted from dried blood spot (DBS) from newborns in Apulia region were analysed for SMA screening by using a real-time PCR-based assay. Infants harbouring homozygous deletion of *SMN1* exon 7 confirmed by diagnostic molecular tests underwent clinical and neurophysiological assessment and received a timely treatment. **Results**: Over the first 20 months since regional NBS introduction, four out of 42,492 (0.009%) screened children were found to carry a homozygous deletion in the exon 7 of *SMN1* gene, with an annual incidence of 1:10,623. No false negatives were present. Median age at diagnosis was 7 days and median age at treatment was 20.5 days. Three of them had two copies of *SMN2* and received gene therapy, while the one with three *SMN2* copies was treated with nusinersen. All but one were asymptomatic at birth, showed no clinical signs of disease after a maximum follow-up of 16 months and reached motor milestones appropriate with their age. The minimum interval between diagnosis and the treatment initiation was 9 days. **Interpretation**: The timely administration of disease-modifying therapies prevented presymptomatic subjects to develop disease symptoms. Mandatory NBS for SMA should be implemented on a national scale.

Introduction

Spinal muscular atrophy (SMA) is a relatively prevalent genetic disorder caused by biallelic mutations in *SMN1* gene and characterised by progressive degeneration of lower motor neurons. Primarily affecting infants and young children, its most severe and common form was once associated with early mortality prior to the advent of SMN augmenting therapy.[1] Notably, nearly 50% of SMA cases are categorised as type 1, marked by a severe onset in early infancy and, in most cases, a presence of 2 *SMN2* copies. Without treatment, symptoms manifest within the first 6 months of life, accompanied by the inability to reach the milestones of sitting and walking. Most type 1 patients do not survive beyond the second year of life, often dying of respiratory failure unless mechanical ventilation is provided.[1]

A recent surge of innovative SMN-augmenting therapies has prompted a major transformation in the landscape of SMA treatment, dramatically changing the

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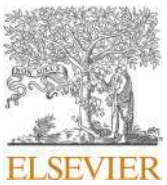

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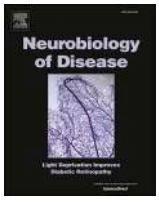

Review

Charcot-Marie-tooth disease type 2A: An update on pathogenesis and therapeutic perspectives

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ABSTRACT

Mutations in the gene encoding MFN2 have been identified as associated with Charcot–Marie–Tooth disease type 2A (CMT2A), a neurological disorder characterized by a broad clinical phenotype involving the entire nervous system. MFN2, a dynamin-like GTPase protein located on the outer mitochondrial membrane, is well-known for its involvement in mitochondrial fusion. Numerous studies have demonstrated its participation in a network crucial for various other mitochondrial functions, including mitophagy, axonal transport, and its controversial role in endoplasmic reticulum (ER)-mitochondria contacts. Considerable progress has been made in the last three decades in elucidating the disease pathogenesis, aided by the generation of animal and cellular models that have been instrumental in studying disease physiology. A review of the literature reveals that, up to now, no definitive pharmacological treatment for any CMT2A variant has been established; nonetheless, recent years have witnessed substantial progress. Many treatment approaches, especially concerning molecular therapy, such as histone deacetylase inhibitors, peptide therapy to increase mitochondrial fusion, the new therapeutic strategies based on MF1/MF2 balance, and SARM1 inhibitors, are currently in preclinical testing. The literature on gene silencing and gene replacement therapies is still limited, except for a recent study by Rizzo et al.(Rizzo et al., 2023), which recently first achieved encouraging results in in vitro and in vivo models of the disease. The near-future goal for these promising therapies is to progress to the stage of clinical translation.

1. Introduction

Charcot-Marie-Tooth disease (CMT) includes a wide spectrum of primary inherited sensory-motor neuropathies, also defined as hereditary sensory and motor neuropathies (HSMN). Characterized by the degeneration of the peripheral nerves' axonal or myelin structures, diseases within this spectrum exhibit pathological and molecular features impacting the motor and sensory neurons (Laurá et al., 2019; Reilly et al., 2011). The overall prevalence of the disease has been estimated at 1/2000-2500, making it the most common inherited neuromuscular disorder (Braathen, 2012). Its transmission is predominantly autosomal dominant, although some subtypes of autosomal recessive axonal and demyelinating CMT have been described. From a clinical perspective, CMT closely overlap with distal hereditary motor neuropathy (dHMN), a pure length-dependent motor nerve syndrome with no sensory involvement. CMTs can be classified based on their neurophysiological properties and inheritance patterns. Demyelinating CMT type 1 is distinguished by a decrease in motor nerve conduction velocity (MNCV), whereas axonal CMT type 2 exhibits normal motor nerve conduction velocity (Laurá et al., 2019). The most common form of CMT is type 2A, caused by mutations in the mitochondrial GTPase mitofusin 2 (OMIM 609260), accounting for around 30-40% of axonal CMT cases and representing 4-7% of all CMTs with a confirmed genetic diagnosis (Braathen, 2012; Fridman et al., 2015; Murphy et al., 2012). CMT2A patients present with a "classic phenotype" characterized by mild weakness and sensory loss during the first two decades of life, with slow progression thereafter. Some of patients experience pure motor neuropathies, while many others have significant loss of proprioception in addition to weakness. This suggests that in some cases, there is involvement of large-diameter sensory axons or their perikaryons, while

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3. Conclusion

We are entering a groundbreaking phase in CMT2A research marked by the elucidation of pathophysiological mechanisms and the advancement of innovative treatments. Understanding the underlying causes and mechanisms driving axonal degeneration is fundamental to finding a cure. A comprehensive understanding of the temporal progression of axonal degeneration will pave the way for implementing molecular therapies like SARM1 inhibitors (Summers et al., 2020; Geisler et al., 2019), capable of preventing degeneration in both in vivo and in vitro disease models.

Recent exploration of the multifaceted roles of the MFN2 protein has revealed its involvement not only in established mechanisms like mitochondrial fusion, axonal mitochondrial transport, and mitophagy but also its role in interorganellar communication between mitochondria and the ER. Additionally, its contentious role in ER-stress induced apoptosis underscores the criticality of preserving the central role of native MFN2 in gene silencing and MFN2 agonists therapies. These discoveries have also uncovered potential new therapeutic targets.

Our review underscores the need for a personalized medicine approach tailored to the diverse mechanisms underlying MFN2-related diseases while targeting the common pathways of axonal degeneration.

Despite the transformative impact of gene therapy on other neuromuscular diseases, its potential benefits have not yet been effectively realized in CMT2A therapy. The pioneering study by Rizzo et al.(Rizzo et al., 2023), focusing on gene silencing and gene replacement therapies, suggests employing a combined RNAi and gene therapy strategy as a potential therapeutic avenue for addressing the broad spectrum of human diseases linked to MFN2 mutations.

As promising treatments advance toward clinical application, the identification of optimal outcome measures, novel biomarkers, and suitable trial designs becomes imperative. These steps are crucial to facilitate the successful testing and validation of these novel treatments for patients affected by CMT2A.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Authors' contributions

EA and CA collected the data and drafted the manuscript. AA, FR, SC and GC revised the manuscript adding important intellectual contributions. CA drafted the figures.

CRediT authorship contribution statement

Claudia Alberti: Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Federica Rizzo:** Writing – review & editing, Funding acquisition, Conceptualization. **Alessia Anastasia:** Writing – review & editing, Investigation, Conceptualization. **Giacomo Comi:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Stefania Corti:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Elena Abati:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

No data was used for the research described in the article.

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References

Abati, E., Manini, A., Velardo, D., Del Bo, R., Napoli, L., Rizzo, F., Moggio, M., Bresolin, N., Bellone, E., Bassi, M.T., D'Angelo, M.G., Comi, G. Pietro, Corti, S., 2022. Clinical and genetic features of a cohort of patients with MFN2-related neuropathy. Sci. Rep. 12, 6181. https://doi.org/10.1038/s41598-022-10220-0.

Abresch, R.T., Carter, G.T., Han, J.J., McDonald, C.M., 2012. Exercise in neuromuscular diseases. Phys. Med. Rehabil. Clin. N. Am. 23, 653–673. https://doi.org/10.1016/j.pmr.2012.06.001.

Amiott, E.A., Lott, P., Soto, J., Kang, P.B., McCaffery, J.M., DiMauro, S., Abel, E.D., Flanigan, K.M., Lawson, V.H., Shaw, J.M., 2008. Mitochondrial fusion and function in Charcot-Marie-tooth type 2A patient fibroblasts with mitofusin 2 mutations. Exp. Neurol. 211, 115–127. https://doi.org/10.1016/j.expneurol.2008.01.010.

Baloh, R.H., Schmidt, R.E., Pestronk, A., Milbrandt, J., 2007. Altered axonal mitochondrial transport in the pathogenesis of Charcot-Marie-tooth disease from Mitofusin 2 mutations. J. Neurosci. 27, 422–430. https://doi.org/10.1523/JNEUROSCI.4798-06.2007.

Bannerman, P., Burns, T., Xu, J., Miers, L., Pleasure, D., 2016. Mice hemizygous for a pathogenic Mitofusin-2 allele exhibit hind limb/foot gait deficits and phenotypic perturbations in nerve and muscle. PloS One 11, e0167573. https://doi.org/10.1371/JOURNAL.PONE.0167573.

Barbullushi, K., Abati, E., Rizzo, F., et al., 2019. Disease modeling and therapeutic strategies in CMT2A: state of the art. Mol. Neurobiol. 56, 6460–6471. https://doi.org/10.1007/s12035-019-1533-2.

Benoy, V., Van Helleputte, L., Prior, R., d'Ydewalle, C., Haeck, W., Geens, N., Scheveneels, W., Schevenels, B., Cader, M.Z., Talbot, K., Kozikowski, A.P., Vanden Berghe, P., Van Damme, P., Robberecht, W., Van Den Bosch, L., 2018. HDAC6 is a therapeutic target in mutant GARS-induced Charcot-Marie-tooth disease. Brain 141, 673–687. https://doi.org/10.1093/brain/awx375.

Bernard-Marissal, N., Médard, J.-J., Azzedine, H., Chrast, R., 2015. Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. Brain 138, 875–890. https://doi.org/10.1093/brain/awv008.

Bernard-Marissal, N., van Hameren, G., Juneja, M., Pellegrino, C., Louhivuori, L., Bartesaghi, L., Rochat, C., El Mansour, O., Médard, J.-J., Croisier, M., Maclachlan, C., Poirot, O., Uhlén, P., Timmerman, V., Tricaud, N., Schneider, B.L., Chrast, R., 2019. Altered interplay between endoplasmic reticulum and mitochondria in Charcot–Marie–tooth type 2A neuropathy. Proc. Natl. Acad. Sci. 116, 2328–2337. https://doi.org/10.1073/pnas.1810932116.

Braathen, G.J., 2012. Genetic epidemiology of Charcot-Marie-tooth disease. Acta Neurol. Scand. Suppl. iv–22. https://doi.org/10.1111/ane.12013.

Calvo, J., Funalot, B., Ouvrier, R.A., Lazaro, L., Toutain, A., De Mas, P., Bouche, P., Gilbert-Dussardier, B., Arne-Bes, M.C., Carrière, J.P., Journel, H., Minot-Myhie, M. C., Guillou, C., Ghorab, K., Magy, L., Sturtz, F., Vallat, J.M., Magdelaine, C., 2009. Genotype-phenotype correlations in Charcot-Marie-tooth disease type 2 caused by Mitofusin 2 mutations. Arch. Neurol. 66, 1511–1516. https://doi.org/10.1001/ARCHNEUROL.2009.284.

Cartoni, R., Martinou, J.-C., 2009. Role of mitofusin 2 mutations in the physiopathology of Charcot–Marie–tooth disease type 2A. Exp. Neurol. 218, 268–273. https://doi.org/10.1016/j.expneurol.2009.05.003.

Cartoni, R., Arnaud, E., Médard, J.-J., Poirot, O., Courvoisier, D.S., Chrast, R., Martinou, J.-C., 2010. Expression of mitofusin 2(R94Q) in a transgenic mouse leads to Charcot-Marie-tooth neuropathy type 2A. Brain 133, 1460–1469. https://doi.org/10.1093/brain/awq082.

Casasnovas Pons, C., Banchs, I., Cassereau, J., Gueguen, N., Chevrollier, A., Martínez-Matos, J.A., Bonneau, D., Volpini, V., 2010. Phenotypic spectrum of MFN2 mutations in the Spanish population. J. Med. Genet. 47, 249–256. https://doi.org/10.1136/JMG.2009.072488.

Chandhok, G., Lazarou, M., Neumann, B., 2018. Structure, function, and regulation of mitofusin-2 in health and disease. Biol. Rev. Camb. Philos. Soc. 93, 933–949. https://doi.org/10.1111/brv.12378.

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Brief Report

Association between *ZASP/LDB3* Pro26Ser and Inclusion Body Myopathy

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Abstract: Inclusion body myositis (IBM) is a slowly progressive disorder belonging to the idiopathic inflammatory myopathies, and it represents the most common adult-onset acquired myopathy. The main clinical features include proximal or distal muscular asymmetric weakness, with major involvement of long finger flexors and knee extensors. The main histological findings are the presence of fiber infiltrations, rimmed vacuoles, and amyloid inclusions. The etiopathogenesis is a challenge because both environmental and genetic factors are implicated in muscle degeneration and a distinction has been made previously between sporadic and hereditary forms. Here, we describe an Italian patient affected with a hereditary form of IBM with onset in his mid-forties. Next-generation sequencing analysis disclosed a heterozygous mutation c.76C>T (p.Pro26Ser) in the PDZ motif of the *LDB3/ZASP* gene, a mutation already described in a family with a late-onset myopathy and highly heterogenous degree of skeletal muscle weakness. In the proband's muscle biopsy, the expression of ZASP, myotilin, and desmin were increased. In our family, in addition to the earlier age of onset, the clinical picture is even more peculiar given the evidence, in one of the affected family members, of complete ophthalmoplegia in the vertical gaze. These findings help extend our knowledge of the clinical and genetic background associated with inclusion body myopathic disorders.

Keywords: ZASP/LDB3; inclusion body myositis; rimmed vacuoles; core-like alterations; next-generation sequencing

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1. Introduction

Sporadic inclusion body myositis (sIBM) represents the most common form of idiopathic inflammatory myopathy among individuals, predominantly males, above 50 years of age [1,2]. Its distinctive clinical features include slowly progressive skeletal muscle weakness and atrophy with a major involvement of quadriceps and long finger flexors [3]. IBM is a degenerative disorder that ultimately leads to loss of ambulation. Also, nutritional and respiratory complications may occur when oropharyngeal muscles are involved [4]. Creatine kinase levels are slightly elevated, and electromyography reveals myogenic abnormalities or mixed neurogenic and myogenic changes [3]. Skeletal muscle morphological observation shows degenerative features—namely, rimmed vacuoles, accumulation of myotoxic proteins/products including amyloid, TDP43 (TAR DNA binding protein 43),

RESEARCH ARTICLE

Clinical, Histopathologic, and Genetic Features of Patients With Myofibrillar and Distal Myopathies

Experience From the Italian Network

Sara Bortolani, MD, PhD, Marco Savarese, PhD, Gaetano Vattemi, MD, Silvia Bonanno, MD, PhD, Yuri M. Falzone, MD, PhD, Alessia Pugliese, MD, Guido Primiano, MD, PhD, Cristina Sancricca, MD, Diego Lopergolo, MD, Giulia Greco, MD, Chiara Gemelli, MD, Sabrina Ravaglia, MD, Roberta P. Bencivenga, MD, Daniele Velardo, MD, Francesca Magri, MD, Maria L. Valentino, MD, Marta Cheli, MD, Eleonora Torchia, MD, Matteo Lucchini, MD, PhD, Antonio Petrucci, MD, Giulia Ricci, MD, Matteo Garibaldi, MD, PhD, Guja Astrea, MD, Anna Rubegni, MD, PhD, Corrado I. Angelini, MD, Alessandra Ariatti, MD, Filippo M. Santorelli, MD, PhD, Alessandra Ruggieri, PhD, Giovanni Antonini, MD, Gabriele Siciliano, MD, PhD, Massimiliano Filosto, MD, PhD, Massimiliano Mirabella, MD, PhD, Rocco Liguori, MD, PhD, Giacomo P. Comi, MD, Lucia Ruggiero, MD, Marina Grandis, MD, PhD, Roberto Massa, MD, PhD, Alessandro Malandrini, MD, Serenella Servidei, MD, PhD, Tiziana E. Mongini, MD, PhD, Carmelo Rodolico, MD, Antonio Toscano, MD, PhD, Stefano C. Previtali, MD, PhD, Paola Tonin, MD, Jordi Diaz-Manera, MD, PhD, Mauro Monforte, MD, PhD, Enzo Ricci, MD, Lorenzo Maggi, MD,* and Giorgio Tasca, MD, PhD*

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Abstract

Background and Objectives

The diagnostic process for myofibrillar myopathies (MFM) and distal myopathies (DM) is particularly complex because of the large number of causative genes, the existence of still molecularly undefined disease entities, and the overlapping features between the 2 categories. This study aimed to characterize a large cohort of patients affected by MFM and DM and identify the most important diagnostic and prognostic aspects of these diseases.

Methods

Patients with either a myopathological diagnosis of MFM or a clinical diagnosis of DM were included in this retrospective multicentric national study. Demographic, genetic, clinical, and histopathologic data of anonymized patients were collected from the neuromuscular centers of the Italian Association of Myology network.

Results

Data regarding 132 patients with MFM (mean age 57.0 ± 15.8 years, 49% female) and 298 patients with DM (mean age 50.7 ± 15.9 years, 40% female) were gathered from 20 neuromuscular centers. 69 patients fulfilled the criteria for both groups (distal myopathies with myofibrillar pathology, DM-MP). Molecular confirmation was achieved in 63% of the patients.

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Guanylate Kinase 1 Deficiency: A Novel and Potentially Treatable Mitochondrial DNA Depletion/Deletions Disease

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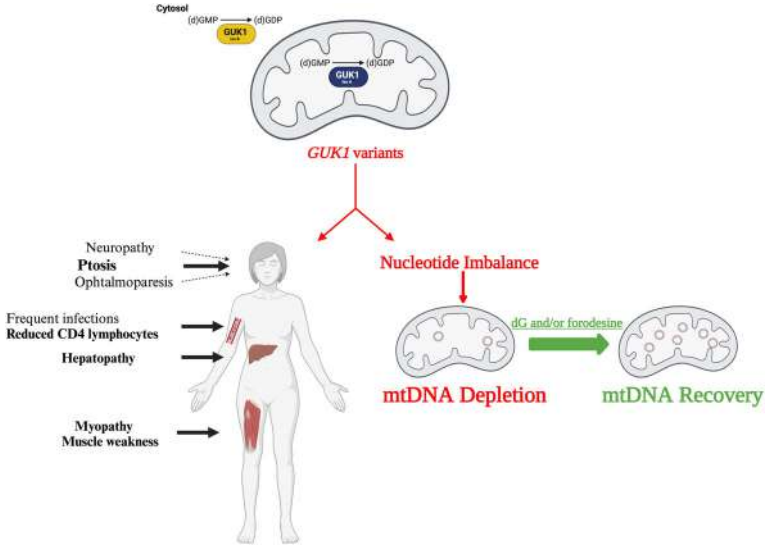

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Case report: A novel *ACTA1* variant in a patient with nemaline rods and increased glycogen deposition

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Background: Congenital myopathies are a group of heterogeneous inherited disorders, mainly characterized by early-onset hypotonia and muscle weakness. The spectrum of clinical phenotype can be highly variable, going from very mild to severe presentations. The course also varies broadly resulting in a fatal outcome in the most severe cases but can either be benign or lead to an amelioration even in severe presentations. Muscle biopsy analysis is crucial for the identification of pathognomonic morphological features, such as core areas, nemaline bodies or rods, nuclear centralizations and congenital type 1 fibers disproportion. However, multiple abnormalities in the same muscle can be observed, making more complex the myopathological scenario.

Case presentation: Here, we describe an Italian newborn presenting with severe hypotonia, respiratory insufficiency, inability to suck and swallow, requiring mechanical ventilation and gastrostomy feeding. Muscle biopsy analyzed by light microscopy showed the presence of vacuoles filled with glycogen, suggesting a metabolic myopathy, but also fuchsinophilic inclusions. Ultrastructural studies confirmed the presence of normally structured glycogen, and the presence of minirods, directing the diagnostic hypothesis toward a nemaline myopathy. An expanded Next Generation Sequencing analysis targeting congenital myopathies genes revealed the presence of a novel heterozygous c.965 T > A p. (Leu322Gln) variant in the *ACTA1* gene, which encodes the skeletal muscle alpha-actin.

Conclusion: Our case expands the repertoire of molecular and pathological features observed in actinopathies. We highlight the value of ultrastructural examination to investigate the abnormalities detected at the histological level. We also emphasized the use of expanded gene panels in the molecular analysis of neuromuscular patients, especially for those ones presenting multiple bioptic alterations.

KEYWORDS

***ACTA1*, skeletal muscle rods, glycogen storage, nemaline myopathy, case report**

Movement Disorders
CLINICAL PRACTICE
RESEARCH ARTICLE

Comparing Essential Tremor with and without Soft Dystonic Signs and Tremor Combined with Dystonia: The TITAN Study

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Abstract: Background: Tremor disorders remain as clinical diagnoses and the rate of misdiagnosis between the commonest non-parkinsonian tremors is relatively high.

Objectives: To compare the clinical features of Essential Tremor without other features (pure ET), ET plus soft dystonic signs (ET + DS), and tremor combined with dystonia (TwD).

Methods: We compared the clinical features of patients with pure ET, ET + DS, and TwD enrolled in The ITAlian tremor Network (TITAN). Linear regression models were performed to determine factors associated with health status and quality of life.

Results: Three-hundred-eighty-three patients were included. Sex distribution was significantly different between the groups with males being more represented in pure ET and females in TwD. The initial site of tremor was different between the groups with about 40% of TwD having head tremor and ET + DS unilateral upper limb tremor at onset. This pattern mirrored the distribution of overt dystonia and soft dystonic signs at examination. Sensory trick, task-specificity, and position-dependence were more common, but not exclusive, to TwD. Pure ET patients showed the lowest degree of alcohol responsiveness and ET + DS the highest. Midline tremor was more commonly encountered and more severe in TwD than in the other groups. Regression analyses demonstrated that tremor severity, sex, age, and to a lesser degree the variable "group", independently predicted health status and quality of life, suggesting the existence of other determinants beyond tremor.

Conclusions: Pure ET and TwD manifest with a phenotypic overlap, which calls for the identification of diagnostic biomarkers. ET + DS shared features with both syndromes, suggesting intra-group heterogeneity.

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TITAN study group are present in Appendix A.

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Correspondence

The p.Val234Met *LRP10* likely pathogenic variant associated with Parkinson's disease: Possible molecular implications

Dear Editor,

The *LRP10* gene has been associated with Lewy Body Diseases, including PD, Parkinson's Disease Dementia (PDD) and Dementia with Lewy Bodies (DLB) and it is characterised by autosomal dominant inheritance. In recent years, multiple studies have linked *LRP10* pathogenic variants to PD, but the role played by the protein in the pathogenetic process is still unclear [1,2]. Studies on patients carrying LRP10 pathological variants showed co-staining between LRP10 and α-synuclein in Levi's Bodies present Substantia nigra pars compacta and in the core of brainstem type, but functional significance of this interaction remains difficult to interpret [3,4].

A wide genetic screening including 24 movement disorder-associated genes was applied to sixty consecutive PD patients, in our Institution. A very rare *LRP10* heterozygos likely pathogenic variant c.700G > A (NM_014045.5) p.Val234Met (GRCh37.p13 chr 14, NC_000014.8:g.23344857G > A, dbSNP, rs371755191; GnomAD: minor allele frequency/MAF = 0.0003) (Fig. 1) was detected in a 53-onset tremor-dominant PD patient with a positive family history (brother, unfortunately not available for assessment). Brain MRI at diagnosis was normal, whereas the dopaminergic imaging showed both putaminal and caudate deficits prominent on the right side. At clinical presentation, the patient showed a classical asymmetric parkinsonism and no atypical motor feature (such as dystonia, early freezing of gait or postural instability). At time of diagnosis he already complained about night sialorrhea, anxiety and insomnia, along with a mild urinary urgency; constipation was not reported. The patient was classified as benignant phenotype according to the lack of presence of REM sleep behavioural disorder, orthostatic hypotension and mild cognitive impairment at time of diagnosis [5]. The patient displayed levodopa responsiveness in a L-dopa challenging test, but considering the young age he was first prescribed rasagiline as monotherapy. During the following years, due to incomplete efficacy of the therapy, pramipexole was added up to the dosage of 2.1 mg per day. L-DOPA was prescribed in September 2016, progressively increased up to 650 mg per day distributed in four administrations. The patient developed motor fluctuations with OFF-related dystonia around eight years into the disease, but only mild and rare dyskinesias were reported (Supplemental Tables 1 and 2).

Regarding non motor symptoms, urinary urgency and sialorrhea remained stable during the years, while insomnia improved with clonazepam. The patient developed early onset dysphagia (from 2016), mainly for solids, and severe dysphonia (from 2017), both treated with logopedia. No behavioural or cognitive changes or hallucinations were reported until follow-up in 2023, confirming the benign PD phenotype after nine years of disease (Fig. 1).

In order to elucidate a possible molecular mechanism, the LRP10 protein was simulated by an online 3D program (Uniprot) and its structure showed to be characterized by 2 extracellular CUB domains and large intracellular tails containing acidic dileucine motif. The CUB domains (derived from for complement C1r/C1s, Uegf, Bmp1) are structural motifs conserved in evolution mainly found in the extracellular part of protein associated with membrane. Valine 234 was identified in the internal and hydrophobic portion of the CUB2 domain β-strand and exhibited an essential role in constituting a totally hydrophobic core in its motif. The β-sheet was composed additionally by Leu232, Phe236, Val276, Val274 on one side and Val249, Val285, Leu224, Ala283 and Val285 on the opposite side.

The novel likely pathogenic variant changed the branched, hydrophobic amino acid Valine in position 234 with the linear, non-polar amino acid Methionine. This Valine is demonstrated to be highly conserved in mammals during the evolution. It is only in zebrafish (*Danio Rerio*) that the amino acid is substituted with a Leucine which is a similar branched amino acid. Therefore, the substitution with a linear Methionine could impair the function of the protein by deforming the structure of its extracellular CUB2 domain probably due to the different steric hindrance of the side chains of the two amino acids, while maintaining the non-polarity of the core (Supplemental Fig. 1).

The role of the CUB2 domain in the protein disfunction is supported by the evidence that the same protein region is affected by the adjacent pathogenic variants p.Arg230Trp and p.Arg235Cys reported in literature as potentially damaging in relation to PD.

The heterozygous pathogenic variant c.703C > T (NM_014045.5) p.Arg235Cys (GRCh37.p13 chr 14, NC_000014.8:g.23344860C > T, dbSNP, rs374479224) was found in a patient diagnosed with PD evolving in a rapidly progressive cognitive impairment and a positive familial history of PDD. Further neuro-biological tests showed that the patient was probably affected by a mixed form of dementia with similarities to DLB and mild signs of an Alzheimer's disease co-pathology. In his family, three relatives were affected by similar neurodegenerative pathologies and the p.Arg235Cys pathogenic variant was found in only two of them while the third tested negative and was characterized by a slower evolution of the disease. A fourth relative tested positive for the variation but manifested no signs or symptoms of the disease probably due to an incomplete penetrance of the pathogenic variant or maybe a late-onset form of the pathology [1].

Further functional studies on the effect of the p.Arg235Cys pathogenic variant were made analyzing brain samples obtained by autopsy from some patients and controls. No particular difference in immunochemistry was found between *LRP10* p.Arg235Cys carriers and controls,

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The other Authors declare that there are no conflicts of interest relevant to this work.

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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.2024.106973.

References

[1] M. Quadri, W. Mandemakers, M.M. Grochowska, R. Masius, H. Geut, E. Fabrizio, G. J. Breedveld, D. Kuipers, M. Minneboo, L.J.M. Vergouw, A. Carreras Mascaro, E. Yonova-Doing, E. Simons, T. Zhao, A.B. Di Fonzo, H.C. Chang, P. Parchi, M. Melis, L. Correia Guedes, C. Criscuolo, A. Thomas, R.W.W. Brouwer, D. Heijsman, A.M. T. Ingrassia, G. Calandra Buonaura, J.P. Rood, S. Capellari, A.J. Rozemuller, M. Sarchioto, H. Fen Chien, N. Vanacore, S. Olgiati, Y.H. Wu-Chou, T.H. Yeh, A.J. W. Boon, S.E. Hoogers, M. Ghazvini, A.S. Ijpma, W.F.J. van Ijcken, M. Onofrj, P. Barone, D.J. Nicholl, A. Puschmann, M. De Mari, A.J. Kievit, E. Barbosa, G. De Michele, D. Majoor-Krakauer, J.C. van Swieten, F.J. de Jong, J.J. Ferreira, G. Cossu, C.S. Lu, G. Meco, P. Cortelli, W.D.J. van de Berg, V. Bonifati, I.P.G. Network, LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study, Lancet Neurol. 17 (7) (2018) 597–608.

[2] A. Cherian, D. K P, A. Vijayaraghavan, Parkinson's disease - genetic cause, Curr. Opin. Neurol. 36 (4) (2023) 292–301.

[3] M.M. Grochowska, A. Carreras Mascaro, V. Boumeester, D. Natale, G.J. Breedveld, H. Geut, W.A. van Cappellen, A.J.W. Boon, A.J.A. Kievit, E. Sammler, P. Parchi, P. Cortelli, D.R. Alessi, W.D.J. van de Berg, V. Bonifati, W. Mandemakers, N.B. Bank, LRP10 interacts with SORL1 in the intracellular vesicle trafficking pathway in non-neuronal brain cells and localises to Lewy bodies in Parkinson's disease and dementia with Lewy bodies, Acta Neuropathol. 142 (1) (2021) 117–137.

[4] A. Carreras Mascaro, M.M. Grochowska, V. Boumeester, N.F.J. Dits, E.N. Bilgiç, G. J. Breedveld, L. Vergouw, F.J. de Jong, M.E. van Royen, V. Bonifati, W. Mandemakers, LRP10 and α-synuclein transmission in Lewy body diseases, Cell. Mol. Life Sci. 81 (1) (2024) 75.

[5] T.A. Mestre, S.M. Fereshtehnejad, D. Berg, N.I. Bohnen, K. Dujardin, R. Erro, A. J. Espay, G. Halliday, J.J. van Hilten, M.T. Hu, B. Jeon, C. Klein, A.F.G. Leentjens, J. Marinus, B. Mollenhauer, R. Postuma, R. Rajalingam, M. Rodríguez-Violante, T. Simuni, D.J. Surmeier, D. Weintraub, M.P. McDermott, M. Lawton, C. Marras, Parkinson's disease subtypes: critical appraisal and recommendations, J. Parkinsons Dis. 11 (2) (2021) 395–404.

[6] N. Song, Y. Wang, L. Zhou, J. Zhang, F. Wu, M. Li, W. Wang, Y. Liu, X. Lu, Q. Chen, N. Zhang, Y. Yan, F. Han, Genetic analysis of the LRP10 gene in Chinese patients with Parkinson's disease, Neurol. Sci. 44 (3) (2023) 905–912.

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Increased glucosylsphingosine levels and Gaucher disease in *GBA1*-associated Parkinson's disease

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RAB32 mutation in Parkinson's disease

We read with great interest the Article by Emil K Gustavsson and colleagues,[1] which provides strong evidence for the *RAB32* variant c.213C>G (Ser71Arg; dbSNP rs200251693) as a cause of monogenic Parkinson's disease. Their initial suggestive finding was the observation of three variant-positive patients among 130 probands from multi-incident Parkinson's disease pedigrees. Subsequent analyses in several databases identified 13 additional patients with this variant. Although the study extensively describes the available data at the patient level, generalisations are hampered by the non-uniformity of information across the diverse resources.

We investigated our data from phase 1 of the Rostock International Parkinson's Disease (ROPAD) study (NCT03866603), an observational clinical study that enrolled patients with Parkinson's disease in Europe, the Americas, and Israel.[2,3] Genome sequencing data were available for 3354 patients, in whom known monogenic causes for Parkinson's disease had been excluded by panel-based sequencing. These patients were selected on the basis of having a positive family history of Parkinson's disease, an age at onset of 55 years or younger, or both, and were representative of the larger idiopathic cohort in terms of sex distribution (62% male and 38% female) and country of origin (the top five countries being the USA, Germany, Israel, Türkiye, and Italy). Nine (0·3%) of the 3354 patients with genome-sequencing data from four countries (Germany, Italy, Spain, and Türkiye) were heterozygous for *RAB32* c.213C>G. This proportion is more than 100 times greater than the 0·002% found in non-Finnish European individuals in the Genome Aggregation Database,[4] which is an appropriate control population. Genome sequencing data are in line with all our patients sharing the *RAB32* Ser71Arg-associated haplotype described by Gustavsson and colleagues.[1] After analysing the ROPAD study case report forms, we found that age at onset was similar in variant-positive (52 years [SD 16]) and variant-negative (52 years [SD 22]) individuals, whereas the proportion of women was significantly higher in variant-positive (78%) than in variant-negative (38%) patients (p=0·032; two-sided Fisher's exact test). Additional differences did not reach significance.

Our data corroborate the association of the *RAB32* c.213C>G variant with Parkinson's disease and support the existence of a founder haplotype. Future studies and meta-analyses investigating whether patients with this variant form a recognisable subgroup characterised by specific clinical features are warranted.

CB received a research grant from the Michael J Fox Foundation, reports a patent entitled *Method for Preparing an RNA Preparation and Use Thereof* (PCT/EP2024/054459), and holds shares in Centogene. KKK holds shares in Centogene. PB is a board member and general manager of, and holds shares in, Centogene. All other authors declare no competing interests. Members of the ROPAD Study Group are listed in the appendix (pp 1–9).

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1 Gustavsson EK, Follett J, Trinh J, et al. *RAB32* Ser71Arg in autosomal dominant Parkinson's disease: linkage, association, and functional analyses. *Lancet Neurol* 2024; **23:** 603–14.
2 Skrahina V, Gaber H, Vollstedt EJ, et al. The Rostock International Parkinson's Disease (ROPAD) study: protocol and initial findings. *Mov Disord* 2021; **36**: 1005–10.
3 Westenberger A, Skrahina V, Usnich T, et al. Relevance of genetic testing in the gene-targeted trial era: The Rostock Parkinson's Disease Study. *Brain* 2024; **147:** 2652–67.
4 Chen S, Francioli LC, Goodrich JK, et al. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature* 2024; **625:** 92–100.

We read with great interest the Article by Emil K Gustavsson and colleagues[1] showing the pathogenicity of the rare *RAB32* c.213C>G (Ser71Arg; dbSNP rs200251693) variant in Parkinson's disease. To assess the role of this variant in patients with Parkinson's disease in Italy, we analysed the *RAB32* gene in 1052 unrelated individuals with Parkinson's disease (whole-exome sequencing data n=802, whole-genome sequencing data n=250; males 61·3%, females 38·7%; mean age at onset 54·9 years; Italian origin 92·2%) and 5553 unrelated individuals without Parkinson's disease (whole-exome sequencing data n=5046, whole-genome sequencing data n=507; males 50·2%, females 49·8%; mean age at sampling 56·2 years, Italian origin 84·9%). The control cohort included neurologically unaffected individuals (n=2622), patients with non-neurodegenerative neurological conditions (n=2065), and neurological patients without parkinsonism (n=866; appendix p 1). We used aggregate data from six Italian centres to conduct our study.

See Online for appendix

In the Parkinson's disease cohort, four (0·4%) of 1052 patients (allele frequency 0·0019) were found to carry the *RAB32* Ser71Arg variant in the heterozygous state. No carriers were found among 5553 control individuals (allele frequency 0). The difference is significant (χ^2 test, p<0·0001). The patients carrying the *RAB32* variant did not harbour any pathogenic or risk variant in other Parkinson's disease genes. We did not find other rare (minor allele frequency <0·1%) variants in the *RAB32* coding region or intron–exon boundaries to be significantly enriched in the participants with Parkinson's disease (appendix p 2).

See Online for appendix

Data from the families of patients with *RAB32* Ser71Arg suggest incomplete penetrance. One patient had sporadic Parkinson's disease and two had relatives with unspecified tremor. In family RE-01, the variant segregated in all three siblings affected by Parkinson's disease, increasing the total number of patients carrying the *RAB32* Ser71Arg variant to six (appendix p 3).

The mean age at Parkinson's disease onset for patients carrying the *RAB32* Ser71Arg variant was 56 years (range 49–68). The phenotype was that of classical Parkinson's disease (bradykinesia plus rigidity, resting tremor, or both) with good levodopa response, motor fluctuations and, in one patient, excellent response to deep brain stimulation. Rapid eye movement sleep behaviour disorder, constipation, orthostatic hypotension, hyposmia, cognitive impairment, and psychosis were not frequent. DaT-SPECT was positive and brain MRI was unremarkable. No relevant common comorbidities were observed, except for arterial hypertension (appendix p 3).

The *RAB32* c.213C>G (Ser71Arg) variant lay in a common haplotype shared by all probands in this study, which encompasses the founder haplotype described by Gustavsson and colleagues,[1] supporting the origin of the variant in a common ancestor (appendix p 4).

In summary, our data corroborate the pathogenic role of the intermediate penetrant *RAB32* Ser71Arg variant in the population of Italian patients with Parkinson's disease.

ADF received fees for advisory board participation from Bial; consulting fees from Bial, Sanofi Genzyme, and Zambon; and support for attending meetings from Sanofi. All other authors declare no competing interests. This study was partially funded by the Italian Ministry of Health (current research IRCCS—ParkNet project) and grants from the Fresco Parkinson Institute. The research centre CMP3 VdA and the Project 5000genomi@ VdA are co-funded by Fondo Europeo di Sviluppo Regionale (FESR) Programma Investimenti per la crescita e l'occupazione 2014/20 (European Social Fund [ESF] and European Regional Development Fund), the Autonomous Region of the Aosta Valley, and the Italian Ministry of Labour and Social Policy (CUPB68H19005520007). This work was also supported by a grant from the EU-ESF, the Autonomous Region of the Aosta Valley, and the Italian Ministry of Labour and Social Policy. See the appendix for a list of ParkNet Study Group members involved in this project (p 5) and a complete list of ParkNet Study Group members (p 6).

See **Online** for appendix

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1 Gustavsson EK, Follett J, Trinh J, et al. *RAB32* Ser71Arg in autosomal dominant Parkinson's disease: linkage, association, and functional analyses. *Lancet Neurol* 2024; **23:** 603–14.

We read with much interest the Article by Emil K Gustavsson and colleagues,[1] which proposes a rare variant in *RAB32* as a novel genetic risk factor for late-onset Parkinson's disease, with reduced penetrance.[1] The authors found that the *RAB32* c.213C>G variant (Ser71Arg; dbSNP rs200251693) co-segregated with autosomal dominant Parkinson's disease in three families (from Tunisia and Canada), and additionally identified five heterozygotes of north American or European origin. A further eight heterozygotes of White, North American, or European descent were identified through bioinformatic analyses of large databases.

Given the variability in the frequency, penetrance, and clinical effect of genetic variants across different ethnicities, we investigated the presence and contribution of this variant to Parkinson's disease in our cohort from southern Spain. We genotyped the variant in 1209 patients diagnosed with Parkinson's disease[2,3] (appendix p 1), recruited since 2008 at the Movement Disorders Unit of the Hospital Universitario Virgen del Rocío in Seville, Spain. Our analysis did not identify the variant in any of the patients from this cohort.

To our knowledge, our study is the first and largest to analyse this variant in southern Spain. Our results do not support an association between *RAB32* Ser71Arg and risk of Parkinson's disease, suggesting a limited role for this variant, at least in our population. Spain's complex demographic history might explain the differences between our participants and those tested by Gustavsson and colleagues,[1] leading to different findings. However, Parkinson's disease is genetically complex and heterogeneous, and we cannot completely rule out the possibility of other pathogenic *RAB32* variants associated with Parkinson's disease in our population. In conclusion, our study does not support a role for the variant *RAB32* Ser71Arg as a risk factor in our cohort and highlights the need for further study in people of diverse ancestries.

We declare no competing interests. We thank the donors and the University Hospital Virgen del Rocio, Biomedical Institute of Seville Biobank (Andalusian Public Health System Biobank and ISCIII-Red de Biobancos PT20/00069) for the human specimens used in this study.

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1 Gustavsson EK, Follett J, Trinh J, et al. *RAB32* Ser71Arg in autosomal dominant Parkinson's disease: linkage, association, and functional analyses. *Lancet Neurol* 2024; **23:** 603–14.
2 Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry* 1988; **51:** 745–52.
3 Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord* 2015; **30:** 1591–601.

We read with interest the Article by Emil K Gustavsson and colleagues,[1] which proposes *RAB32* c.213C>G (Ser71Arg; dbSNP rs200251693)—a variant present in appropriately 0·15% of individuals with Parkinson's disease—as a risk factor for the

Lewy pathology formation in patient-derived *GBA1* Parkinson's disease midbrain organoids

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Abstract

Fibrillary aggregation of α-synuclein in Lewy body inclusions and nigrostriatal dopaminergic neuron degeneration define Parkinson's disease neuropathology. Mutations in *GBA1*, encoding glucocerebrosidase, are the most frequent genetic risk factor for Parkinson's disease. However, the lack of reliable experimental models able to reproduce key neuropathological signatures has hampered the clarification of the link between mutant glucocerebrosidase and Parkinson's disease pathology. Here, we describe an innovative protocol for the generation of human induced pluripotent stem cell-derived midbrain organoids containing dopaminergic neurons with nigral identity that reproduce characteristics of advanced maturation. When applied to patients with *GBA1*-related Parkinson's disease, this method enabled the differentiation of midbrain organoids recapitulating dopaminergic neuron loss and fundamental features of Lewy body pathology observed in human brains, including the generation of α-synuclein fibrillary aggregates with seeding activity that also propagate pathology in healthy control organoids. Still, we observed that the retention of mutant glucocerebrosidase in the endoplasmic reticulum and increased levels of

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Epileptic Disorders

CLINICAL VIGNETTE

Soft cerebellar signs unveil *RARS2*-related epilepsy

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Pathogenic *RARS2* variants, by critically reducing mitochondrial arginyl-tRNA aminoacylation activity, cause a rare autosomal recessive disease, generally presenting as a severe encephalopathy with onset at birth, premature death, microcephaly, drug-resistant epilepsy, and hypotonia.[1–9] Epilepsy was reported in ~90% of the cases, often with myoclonic and clonic seizures.[2] As in other primary mitochondrial diseases, epilepsy may be attributed to ATP depletion, resulting in loss of neuron hyperpolarization (Na^+/K^+ ATPase activity impairment) and increased excitation (loss of GABA-mediated inhibition).[10] The most frequent neuroradiological finding is cerebral atrophy, followed by pontocerebellar hypoplasia.[1–9] We report the clinical and genetic findings of a 16-year-old boy with *RARS2*-related encephalopathy distinguished for later onset, longer survival, and milder phenotype compared to previously reported cases.

A 13-year-old Italian boy without family history of neurological diseases was brought to the emergency room for a sleep-related focal-to-bilateral seizure. His mother reported the following ictal semiology: paroxysmal arousal from sleep, sitting up on the bed, head deviation, drooling, grunting, and asymmetric limb tonic posturing with right upper limb extension and left upper limb flexion (figure 4 sign), without awareness nor response to stimuli, with full recovery in ~10 min. EEG showed a 7 Hz background rhythm with brief trains of synchronous and asynchronous frontal 3 Hz sharp waves, prevalent on the right side (Figure 1A). After a similar second seizure on the following day, levetiracetam 500 mg twice a day was started achieving a seizure-free three-year follow-up and a consistent EEG improvement.

The patient's medical history revealed normal pregnancy, birth, and early motor development with autonomous walking at 15 months. A single episode of febrile seizure at 1 year of age was reported. At 3 years, he was referred to a pediatric neurologist for language delay with increased nonverbal communication; square wave jerks, interrupted pursuit, mild dysmetria, and tandem gait inability were observed at that time. Psychomotor and speech therapy alongside with professional school support were provided with benefit. A brain MRI at the age of 13 years revealed isolated cerebellar vermis atrophy (Figure 1B). At the last neurological examination, at 16 years, the patient displayed mild intellectual disability and cerebellar features including dysarthria, gaze difficulties, postural and kinetic tremor of upper limbs, limbs dysmetria, dysdiadochokinesia, and gait ataxia.

BRAIN COMMUNICATIONS

LETTER TO THE EDITOR

Response to: Are there two disjunct episignatures for KMT2B-related disease?

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We thank Prof. Konrad Oexle for his letter and interest in our work.[1] His concerns are related to the 'proclaimed' occurrence of different epitypes (i.e. allelic episignatures) associated with different sets of variants affecting *KMT2B*. A simple reading of our paper shows that the colleague has clearly misinterpreted our considerations.[2]

In order to 'avoid unnecessary confusion', we address each of the raised main concerns below.

First, we made explicit that the c.1918T>A (p.S640T), c.2909G>A (p.R970Q) and c.7016G>A (p.R2339Q) substitutions were predicted as 'benign' or as having conflicting predictions by various *in silico* tools.[2] Though these tools are generally highly valuable in the assessment of the functional impact of genomic variants, it should be noted that they might misinterpret variant functional relevance and clinical significance in various circumstances, particularly when considering adult-onset diseases with reduced penetrance and variable expressivity. As an example, this is the case of a class of Parkinson's disease risk *GBA1* missense variants (e.g. E326K and T369M) that are improperly predicted as 'benign' by most of these tools.

Regarding Oexle's[1] consideration related to the occurrence of a 'disjunct episignature' linked with *KMT2B* variants associated with/contributing to adult-onset dystonia, as explicitly stated in our article, the tested samples carrying the four *KMT2B* variants were not classified as fitting DYT28 based on the originally identified 'conventional' episignature.[3] Then, we used DNA methylation profiling to explore a differential impact shared by these four variants. Notably, the identified set of differentially methylated probes common to the eight affected individuals was not 'proclaimed' as defining a new 'disjunct episignature'. Instead, our findings highlight a common differential behaviour (in terms of genome-wide DNA methylation) of the eight samples sharing rare missense *KMT2B* variants.

We thank Oexle[1] for suggesting the adoption of analytical approaches using more stringent genome-wide significance thresholds. We note that false discovery rate (FDR) correction of *P*-values represents the most commonly used strategy in controlling errors arising from multiple testing. A long-standing wide consensus about the effectiveness of FDR in managing this specific issue stands in the scientific community.[4] Moreover, the selection of the approach was constrained by the small sample size.

Finally, as stated in the original article, the causal association between dysregulated KMT2B function and adult-onset dystonia requires further confirmatory studies using independent pedigrees and cohorts. While the possibility that the shared DNA methylation pattern might represent a 'contingent effect independent of KMT2B' cannot ruled out *a priori*, by providing the identified differentially methylated probes we encouraged researchers to independently explore this association. An independent validation of our findings would represent only a first step towards the identification of a new disease-specific DNA methylation signature, which, as discussed in the article, necessarily requires a collaborative

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LETTER TO THE EDITOR

A Novel Pathogenic *PSEN1* Variant in a Patient With Dystonia-Parkinsonism Without Dementia

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Dear Editor,

The *PSEN1* gene is located on chromosome 14 and encodes the presenilin 1 protein, which is a key component of the γ-secretase complex that is involved in the cleavage of amyloid precursor protein (APP) in amyloid beta (Aβ) peptides.[1] Recent works have suggested that Aβ peptides may also play a role in the pathophysiology of motor symptoms in Alzheimer's disease (AD) patients carrying *PSEN1* mutations (*PSEN1*-AD) through accumulation in the striatum.[2] Aβ peptides may disrupt the function of the basal ganglia, possibly leading to the development of extrapyramidal symptoms.[3] In addition, *PSEN1* mutations can be associated with the accumulation of other proteins, such as alpha-synuclein and tau, which have also been linked with motor signs.[4] Parkinsonism, ataxia, and spasticity are the most frequently described motor symptoms in *PSEN1*-AD patients.[5] Motor impairment has been reported in the early stages of the disease and may even precede cognitive decline in a small subset of patients.[5] Few cases have been reported of *PSEN1* mutation carriers with parkinsonism as an isolated presenting feature without dementia or significant cognitive decline over time.[6-8] Here, we report a male patient with adult-onset dystonia-parkinsonism with positive AD biomarkers carrying a novel *PSEN1* frameshift variant.

A 52-year-old man came to our observation for painful sustained muscular contractions, cramps, and subjective mild attention deficit. Neurological examination revealed lower limb dystonia and symmetric rigidity causing gait impairment, rest and postural right-hand tremor, mild spontaneous and sensory-induced myoclonus, global and symmetric bradykinesia, mild cerebellar dysarthria and fluctuating diplopia (Supplementary Video 1 in the online-only Data Supplement). He had a positive family history of neurological diseases on the maternal side of the family with a hereditary pattern compatible with autosomal dominant inheritance (i.e., head tremor in his mother and Parkinson's disease evolving to dementia in a second-degree cousin and dementia in a great aunt, both from the maternal side) (Figure 1A).

Blood test results were normal, including systemic autoimmunity, neurological paraneoplastic antibodies, and ceruloplasmin. Brain magnetic resonance imaging (MRI) was unremarkable (Figure 1B, C), while single photon emission computed tomography (SPECT) with ioflupane (123I) showed a moderate reduction in radiotracer uptake in the striatum with a slight right-side prevalence (Figure 1D). In-depth cognitive assessment revealed substantially preserved cognition with selective difficulty in visuospatial short-term memory and learning verbal material

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LETTER TO THE EDITOR

A Case of 18p Chromosomal Deletion Encompassing *GNAL* in a Patient With Dystonia-Parkinsonism

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Dear Editor,

Deletions of the short arm of chromosome 18 encompassing the *GNAL* gene are associated with 18p deletion syndrome; the clinical features of this syndrome include facial dysmorphism, short stature, mental retardation, and movement disorders, particularly dystonia.[1] Heterozygous loss-of-function variants of *GNAL* are known to cause adult-onset cranio-cervical dystonia and/or asymmetric dystonic tremor.[2] GNAL-related dystonia (DYT-GNAL) exhibits an autosomal dominant (AD) inheritance pattern with incomplete penetrance and variable phenotypic expressivity. A limited number of cases of DYT-GNAL have been reported thus far; therefore, its clinical spectrum has yet to be fully explored.[2] In this case report, we describe a patient with dystonia-parkinsonism and evidence of nigrostriatal denervation carrying a chromosome 18p deletion encompassing the entire *GNAL* gene.

A 55-year-old man reported slowness of movement and rigidity of the right side of the body with abnormal posture of the right upper limb, which appeared simultaneously a few months previously. Neurological examination revealed right-sided hemiparkinsonism with concomitant marked dystonic posture of the right upper limb, right lower limb dystonia with slight eversion of the foot, forward trunk flexion, hypomimia and reduced right arm swing during gait (Supplementary Video 1 in the online-only Data Supplement). His past medical history was unremarkable; in particular, no history of a decreased sense of smell, depression or psychiatric disorders, autonomic dysfunction, sleep disturbances, or gastrointestinal symptoms was reported. There was no family history of Parkinson's disease (PD) or other movement disorders. Brain magnetic resonance imaging did not reveal any significant alterations (Figure 1A), while ioflupane [^{123}I] SPECT imaging revealed a moderate reduction in presynaptic dopaminergic uptake in the left caudate nucleus and bilateral putamen (Figure 1B). [^{18}F] Fluoro-

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Movement Disorders
CLINICAL PRACTICE

CASE SERIES

Dominant *VPS16* Pathogenic Variants: Not Only Isolated Dystonia

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Abstract: Background: *VPS16* pathogenic variants have been recently associated with inherited dystonia. Most patients affected by dominant VPS16-related disease display early-onset isolated dystonia with prominent oromandibular, bulbar, cervical, and upper limb involvement, followed by slowly progressive generalization.
Cases: We describe six newly reported dystonic patients carrying *VPS16* mutations displaying unusual phenotypic features in addition to dystonia, such as myoclonus, choreoathetosis, pharyngospasm and freezing of gait. Response to bilateral Globus Pallidus Internus Deep Brain Stimulation (GPi-DBS) is reported in three of them, associated with significant improvement of dystonia but only minor effect on other hyperkinetic movements. Moreover, five novel pathogenic/likely pathogenic variants are described.
Conclusions: This case collection expands the genetic and clinical spectrum of *VPS16*-related disease, prompting movement disorder specialists to suspect mutations of this gene not only in patients with isolated dystonia.

Dominant and recessive *VPS16* pathogenic variants have been associated with inherited dystonia.[1–3] Initially, a homozygous mutation was found to co-segregate with juvenile-onset progressive generalized dystonia in a consanguineous Chinese family.[2] Subsequently, heterozygous *VPS16* deleterious variants were identified in patients affected by autosomal dominant dystonia with incomplete penetrance.[1,4–10]

Most *VPS16* patients display early-onset isolated dystonia with prominent oromandibular, bulbar, cervical and upper limb involvement, followed by slowly progressive generalization, typically retaining the ability to walk in adulthood[1] (Table S1).

We report six patients carrying heterozygous pathogenic *VPS16* variants, five of which are novel. All patients displayed various hyperkinetic features associated with dystonia Table 1.

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Keywords: choreoathetosis, freezing, GPi-DBS, HOPSANDs, myoclonus, pharyngeal spasm.

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Movement Disorders
CLINICAL PRACTICE

BRIEF REPORT

Family History in Parkinson's Disease: A National Cross-Sectional Study

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Abstract: Background: Family history of Parkinson's disease (PD) is a common finding in PD patients. However, a few studies have systematically examined this aspect.

Objectives: We investigated the family history of PD patients, comparing demographic and clinical features between familial PD (fPD) and sporadic PD (sPD).

Methods: A cross-sectional study enrolling 2035 PD patients was conducted in 28 Italian centers. Clinical data and family history up to the third degree of kinship were collected.

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Keywords: familial and sporadic Parkinson's disease, family history, hyposmia, cognitive impairment, depression, bipolar disorder.

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Movement Disorders
CLINICAL PRACTICE
RESEARCH ARTICLE

Comparing Essential Tremor with and without Soft Dystonic Signs and Tremor Combined with Dystonia: The TITAN Study

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Abstract: Background: Tremor disorders remain as clinical diagnoses and the rate of misdiagnosis between the commonest non-parkinsonian tremors is relatively high.

Objectives: To compare the clinical features of Essential Tremor without other features (pure ET), ET plus soft dystonic signs (ET + DS), and tremor combined with dystonia (TwD).

Methods: We compared the clinical features of patients with pure ET, ET + DS, and TwD enrolled in The ITAlian tremor Network (TITAN). Linear regression models were performed to determine factors associated with health status and quality of life.

Results: Three-hundred-eighty-three patients were included. Sex distribution was significantly different between the groups with males being more represented in pure ET and females in TwD. The initial site of tremor was different between the groups with about 40% of TwD having head tremor and ET + DS unilateral upper limb tremor at onset. This pattern mirrored the distribution of overt dystonia and soft dystonic signs at examination. Sensory trick, task-specificity, and position-dependence were more common, but not exclusive, to TwD. Pure ET patients showed the lowest degree of alcohol responsiveness and ET + DS the highest. Midline tremor was more commonly encountered and more severe in TwD than in the other groups. Regression analyses demonstrated that tremor severity, sex, age, and to a lesser degree the variable "group", independently predicted health status and quality of life, suggesting the existence of other determinants beyond tremor.

Conclusions: Pure ET and TwD manifest with a phenotypic overlap, which calls for the identification of diagnostic biomarkers. ET + DS shared features with both syndromes, suggesting intra-group heterogeneity.

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Keywords: essential tremor plus, soft signs, dystonia, quality of life, gender.

TITAN study group are present in Appendix A.

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Movement Disorders
CLINICAL PRACTICE

BRIEF REPORT

Dystonic Tremor as Main Clinical Manifestation of SCA21

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Abstract: Background: Spinocerebellar ataxia type 21 (SCA21) is a rare inherited neurological disorder characterized by motor, cognitive, and behavioral disturbances, caused by autosomal dominant *TMEM240* variants.

Objectives: To identify the genetic cause of a dystonic tremor with autosomal dominant inheritance.

Methods: Six subjects of a multi-generational French family affected by tremor and dystonia were studied. Each patient underwent a comprehensive clinical assessment and a whole-exome sequencing analysis.

Results: All six subjects presented with early-onset prominent hand dystonic tremor and multifocal/generalized dystonia, secondarily developing mild cerebellar ataxia. The younger generation showed more pronounced cognitive and behavioral impairment. The known pathogenic *TMEM240* c.509C>T (p.P170L) variant was found in heterozygosis in all subjects.

Conclusions: Dystonic tremor can represent the core clinical feature of SCA21, even in absence of overt cerebellar ataxia. Therefore, *TMEM240* pathogenic variants should be considered disease-causing in subjects displaying dystonic tremor, variably associated with ataxia, parkinsonism, neurodevelopmental disorders, and cognitive impairment.

Spinocerebellar ataxia 21 (SCA21) is a rare early-onset, slowly progressive, autosomal dominant cerebellar ataxia, first described in a large French family in 2001.[1] So far, 63 cases and 24 families have been reported.[1–12]

Whole-exome sequencing (WES) and linkage analysis have allowed the identification of pathogenic variants in *TMEM240* as the cause of SCA21.[5] Seven pathogenic variants, with two being recurrent (p.P170L and p.G66R), have been reported to date.[8,9] *TMEM240* is expressed in mammal brains and might contribute to organize the cerebellar network.[13–16] Tmem240, the protein encoded by *TMEM240*, localizes at intracellular membranes, and its mutant forms are associated with autophagic-lysosomal degradation impairment in vitro.[14–17]

Besides cerebellar ataxia, cognitive impairment represents an important feature of SCA21. Additional rarely reported neurological features include tremor, bradykinesia, rigidity, pyramidal signs, myoclonus, dystonia, and oculomotor dysfunction. Neurodevelopmental abnormalities, psychiatric symptoms, and epilepsy have been reported as well.[2,3,8–11,18]

Here we report a multigenerational French family with six affected members presenting dystonic tremor as main clinical manifestation of SCA21.

Methods

Six members of a multigenerational family originating from North-East France have been evaluated at the Movement Disorders Center of the Grenoble Alpes University Hospital (Grenoble, France). Detailed medical histories were obtained.

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Keywords: tremor, dystonia, spinocerebellar ataxia, autism spectrum, psychomotor delay.
Vidal Yahya and Claudio Baiata contributed equally to this manuscript (co-first authorship).

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BRIEF COMMUNICATION

Obsessive-compulsive disorder as a first manifestation of Ataxia with Oculomotor Apraxia type 2 due to a novel mutation of SETX gene

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Abstract

Background Ataxia with oculomotor apraxia type 2 (AOA2) is an autosomal recessive disorder presenting with cerebellar ataxia, sensory-motor axonal neuropathy, oculomotor apraxia, cerebellar atrophy and high alpha-fetoprotein (AFP) serum level. AOA2 is due to coding mutations of the SETX gene, mapped to chromosome 9q34. Seldom noncoding mutations affecting RNA processing have been reported too. To date psychiatric symptoms have never been reported in AOA2.

Case presentation A 19 years-old man came to our attention for progressive gait ataxia debuted five years earlier. His past medical history was unremarkable, while his parents were consanguineous. On neurological examination, he had bilateral horizontal gaze-evoked nystagmus with hypometric saccades and saccadic horizontal smooth pursuit, appendicular ataxia, limbs and trunk myoclonic involuntary movements with hands' dystonic postures and dance of the tendons. Psychological evaluation described intrusive and obsessive thoughts experienced by the patient, then diagnosed as obsessive-compulsive disorder. Blood tests detected an elevated AFP level. Brain MRI showed cerebellar atrophy, while electroneuromyography revealed an axonal sensory-motor polyneuropathy. In the suspicion of a pathology belonging to the autosomal recessive cerebellar ataxias (ARCA) spectrum disorder, a direct search of point mutations by whole-exome sequencing was performed revealing a novel biallelic variant in SETX gene (c.6208+2dupT), which was classified as likely pathogenic.

Conclusion The present case expands the genotypic and phenotypic spectrum of AOA2, reporting a novel likely pathogenic SETX mutation (c.6208+2dupT) and highlighting an early psychiatric involvement in AOA2, suggesting the need for psychiatric assessment in these neurologic patients.

Keywords SETX · AOA2 · Obsessive-compulsive disorder · Whole-exome sequencing · Ataxia

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BRIEF COMMUNICATION

Childhood-onset focal epilepsy and acute para-infectious encephalopathy in a patient with biallelic *QARS1* variants

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Abstract

Introduction Biallelic variants in *QARS1*, a house-keeping gene involved in protein synthesis, cause a rare encephalopathy classically characterized by severe developmental delay, drug-resistant neonatal-onset epilepsy, microcephaly, and brain atrophy. We aim to raise awareness on mild *QARS1*-related phenotypes describing a 6-year-old patient.

Case description Epilepsy onset occurred at 3.5 years with a sleep-related focal autonomic seizure, accompanied by interictal occipital spikes at EEG. In the following months, daytime focal impaired awareness seizures appeared. Due to developmental delay and short stature, trio-based whole-exome sequencing was performed, unraveling two compound heterozygous *QARS1* variants: the likely pathogenic c.1304A>G (p.Y435C) and the c.799C>T (p.R267W), extremely rare and predicted deleterious by in silico analysis. At 5 years, the patient had a para-infectious encephalopathy with acute psychomotor slowing, delta-theta activity at EEG, new-onset bilateral subcortical white matter T2-hyperintensities with diffusion restriction at brain MRI, and optimal response to intravenous methylprednisolone administration. At 12-month follow-up, the patient had been seizure-free for a year with levetiracetam monotherapy.

Discussion Mild *QARS1*-related encephalopathies may present with a childhood-onset focal epilepsy accompanied by developmental delay and short stature as red flags of monogenic etiology. The episode of steroid-responsive acute para-infectious encephalopathy, previously reported in another patient harboring the p.Y435C variant, suggests that milder cases might be more susceptible to encephalopathy caused by intercurrent illnesses (e.g., infection). As recommended for other aminoacyl-tRNA synthetase-related diseases, it is important to provide this cohort with an early genetic diagnosis in order to encourage precision medicine and personalized treatment.

Keywords Childhood-onset · Focal epilepsy · Developmental delay · Short stature · Acute encephalopathy · *QARS1*

Introduction

Biallelic *QARS1* variants cause a rare disease, classically characterized by severe developmental delay, drug-resistant neonatal-onset epilepsy, microcephaly, and progressive brain atrophy. Additional features include short stature, hypotonia, spasticity, nystagmus, corpus callosum hypoplasia, and delayed myelination [1–6].

The house-keeping gene *QARS1* encodes glutaminyl-tRNA synthetase, an enzyme with a prominent role in cytosolic and mitochondrial protein synthesis machinery. Since no combination of biallelic null *QARS1* variants has been reported yet, a total loss of QARS1 function is probably incompatible with life. Consistently, phenotype correlates with aminoacylation activity and solubility of the mutated enzyme, as milder clinical features are associated with

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ORIGINAL ARTICLE

Predominant right temporal lobe atrophy: Clinical, neuropsychological and structural differences based on amyloid status

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Abstract

Background: Predominant right temporal atrophy is a radiological sign usually associated with frontotemporal dementia but this sign can also be present in Alzheimer's disease. Given the overlap of clinical symptoms between the two conditions, it is important to know which characteristics allow them to be differentiated.

Objectives: To compare clinical, neuropsychological and structural magnetic resonance imaging (MRI) data of subjects with prominent right anterior temporal atrophy, depending on the status of amyloid biomarkers.

Methods: Among patients followed in the dementia center of Ospedale Maggiore Policlinico, subjects with right anterior temporal atrophy, defined as grade 3 or 4 on the corresponding visual rating scale, were identified. Only subjects with both an MRI scan and amyloid status available were considered. For selected subjects, data were extracted from clinical and neuropsychological records at initial presentation and at last available follow-up. Two raters applied a protocol of eight visual rating scales to compare brain atrophy and white matter hyperintensities.

Results: Of 497 subjects, 17 fulfilled the inclusion criteria: 7 amyloid-positive and 10 amyloid-negative. At initial presentation, executive dysfunction and topographical disorientation were more common in amyloid-positive patients. At follow-up, behavioral symptoms, such as social awkwardness and compulsive attitude, were more frequent in the amyloid-negative patients. Amyloid-positive patients presented an overall worse neuropsychological performance, especially in the language and visuospatial domain, and had higher scores on the right anterior cingulate visual rating scale.

Conclusion: Patients with predominant right temporal atrophy showed clinical, neuropsychological and radiological differences, depending on the status of amyloid biomarkers.

KEYWORDS
Alzheimer's disease, atrophy, biomarkers, frontotemporal dementia, magnetic resonance imaging

Alzheimer's Research & Therapy

RESEARCH

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Diagnostic accuracy of research criteria for prodromal frontotemporal dementia

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Abstract

Background The Genetic Frontotemporal Initiative Staging Group has proposed clinical criteria for the diagnosis of prodromal frontotemporal dementia (FTD), termed mild cognitive and/or behavioral and/or motor impairment (MCBMI). The objective of the study was to validate the proposed research criteria for MCBMI-FTD in a cohort of genetically confirmed FTD cases against healthy controls.

Methods A total of 398 participants were enrolled, 117 of whom were carriers of an FTD pathogenic variant with mild clinical symptoms, while 281 were non-carrier family members (healthy controls (HC)). A subgroup of patients underwent blood neurofilament light (NfL) levels and anterior cingulate atrophy assessment.

Results The core clinical criteria correctly classified MCBMI vs HC with an AUC of 0.79 ($p < 0.001$), while the addition of either blood NfL or anterior cingulate atrophy significantly increased the AUC to 0.84 and 0.82, respectively ($p < 0.001$). The addition of both markers further increased the AUC to 0.90 ($p < 0.001$).

Conclusions The proposed MCBMI criteria showed very good classification accuracy for identifying the prodromal stage of FTD.

Keywords Prodromal, MCBMI, Frontotemporal dementia, Diagnostic criteria, Diagnostic accuracy

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FTD field; the first with the attempt to capture a specific disease phenotype, while the second tries to identify the earliest phases of the global FTD spectrum. Both are reasonable and potentially useful depending on the clinical question, whether in relation to early-stage treatments, particularly for monogenic disease, that target the pathogenetic mechanisms of the disease regardless of the clinical phenotype. However, both approaches comply with a diagnostic tool rather than a screening test, reporting greater specificity than sensitivity.

We acknowledge that the present study entails several limitations. First, we did not include a control group with other neurodegenerative diseases, such as prodromal Alzheimer's disease or non-neurodegenerative psychiatric disorders. This will be mandatory to confirm the validity of these criteria in real-world situations. Second, we did not perform a validation of the MCBMI criteria against a cohort that includes full phenotypes of FTD, as well as sporadic cases. While the criteria demonstrated validity in our specific cohort, further validation in cohorts encompassing a broader spectrum of FTD phenotypes and sporadic cases is crucial to ensure its applicability and validity in various clinical contexts. Third, while the scales used have shown good validity, it will be important in future studies to formally assess both intra- and inter-rater variabilities. Fourth, we acknowledge the limitation of not including premanifest disease carriers and not evaluating the stability of the prodromal status and phenoconversion to symptomatic syndromes which should be further assessed in future longitudinal studies.

The MCBMI criteria have demonstrated potential validity in identifying prodromal FTD within the confines of the present study, though further validation in diverse cohorts is essential to fully establish their validity and utility in clinical settings.

Abbreviations

AUC	Area under the curve
avPPA	Agrammatic variant of primary progressive aphasia
bvFTD	Behavioral variant frontotemporal dementia
C9orf72	Chromosome 9 open reading frame 72
CBS	Corticobasal syndrome
CI	Confidence interval
CDR plus NACC FTLD	CDR® Dementia Staging Instrument plus National Alzheimer's Coordinating Centre behavior and language domains
GENFI	Genetic Frontotemporal Initiative
GRN	Progranulin
HC	Healthy controls
FTD	Frontotemporal dementia
MAPT	Microtubule-associated protein tau
MCBMI	Mild cognitive and/or behavioral and/or motor impairment
MRI	Magnetic resonance imaging
NfL	Neurofilament light
PSP	Progressive supranuclear palsy
ROC	Receiver operating characteristic
svPPA	Semantic variant of primary progressive aphasia

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13195-024-01383-1.

Additional file 1: Table S1. Diagnostic accuracy of each domain of proposed MCBMI criteria in classifying prodromal FTD from healthy controls. **Table S2.** Diagnostic accuracy of proposed criteria in classifying prodromal FTD from healthy controls for each genetic group. **Table S3.** Diagnostic accuracy of each subdomain of proposed MCBMI criteria in classifying prodromal FTD from healthy controls.

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OPEN ACCESS

Short report

NfL reliability across laboratories, stage-dependent diagnostic performance and matrix comparability in genetic FTD: a large GENFI study

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ABSTRACT

Background Blood neurofilament light chain (NfL) is increasingly considered as a key trial biomarker in genetic frontotemporal dementia (gFTD). We aimed to facilitate the use of NfL in gFTD multicentre trials by testing its (1) reliability across labs; (2) reliability to stratify gFTD disease stages; (3) comparability between blood matrices and (4) stability across recruiting sites.

Methods Comparative analysis of blood NfL levels in a large gFTD cohort (GENFI) for (1)–(4), with n=344 samples (n=148 presymptomatic, n=11 converter, n=46 symptomatic subjects, with mutations in *C9orf72*, *GRN* or *MAPT*; and n=139 within-family controls), each measured in three different international labs by Simoa HD-1 analyzer.

Results NfL revealed an excellent consistency (intraclass correlation coefficient (ICC) 0.964) and high reliability across the three labs (maximal bias (pg/mL) in Bland-Altman analysis: 1.12±1.20). High concordance of NfL across laboratories was moreover reflected by high areas under the curve for discriminating conversion stage against the (non-converting) presymptomatic stage across all three labs. Serum and plasma NfL were largely comparable (ICC 0.967). The robustness of NfL across 13 recruiting sites was demonstrated by a linear mixed effect model.

Conclusions Our results underline the suitability of blood NfL in gFTD multicentre trials, including cross-lab reliable stratification of the highly trial-relevant conversion stage, matrix comparability and cross-site robustness.

INTRODUCTION

Genetic frontotemporal dementias (gFTDs) represent a group of progressive neurodegenerative diseases characterised by a progressive decline of executive, behavioural and language functions, frequently resulting from mutations in the genes chromosome open reading frame 72 (*C9orf72*), progranulin (*GRN*) or microtubule-associated protein tau (*MAPT*).[1] Neurofilament light chain (NfL)—an intermediate filament that constitutes part of the neuronal cytoskeleton—is released after neuronal damage into the interstitial fluid, cerebrospinal fluid and blood. Blood-based NfL has an increasing impact as a trial biomarker in gFTD for multiple contexts of use, for example, patient stratification,[2–5] trial inclusion,[6] toxicity monitoring and treatment-response capture,[7] and has now been approved by the U.S. Food and Drug Administration as a surrogate endpoint contributing to approval of novel drugs (tofersen).[8] However, its wider use in multicentre trials—as well as in real-world clinical settings—has been questioned due to potential cross-laboratory heterogeneity in analytical approaches and blood sample matrices that might lead to different, non-comparable concentrations of blood NfL.[9 10]

Leveraging a large gFTD cohort, we here aimed to facilitate the use of blood NfL in gFTD multicentre trials and real-world clinical settings by testing: (1) its reliability across laboratories, measured at different time points, by different end-user devices and kits; (2) cut-off values maximising stratification accuracy of the trial relevant gFTD disease stages (conversion stage, symptomatic stage), with cut-off values validated across labs; (3) comparability between blood matrices and (4) robustness across recruiting sites.

METHODS

Cohort and NFL measurements

Subjects were patients with FTD caused by mutations in the genes *C9orf72*, *GRN* or *MAPT* (symptomatic

The high robustness of NfL across 13 recruiting sites was shown by a linear mixed effect model, as the categorial variable 'recruiting site' did not explain any variance (estimate 0.001, SE 0.001, Wald-Z 1.403, significance 0.161).

DISCUSSION

Blood NfL has an increasing impact as a trial biomarker in gFTD for multiple contexts of use[5 7] and is now being increasingly acknowledged by the FDA as a surrogate endpoints in drug approval processes.[8] However, its wider use in multicentre trials and real-world clinical settings is limited by lack of larger data demonstrating cross-lab reliability, cross-lab validated cut-off values and cross-lab validated comparability between blood matrices in gFTD. Leveraging a large genetic FTD, our findings show that blood NfL is a biomarker in gFTD with high reliability across labs—even if assessed at different time points, and by partly different kits (NF-Light Advantage Kit vs Neurology 4-Plex A Kit). This finding confirms and extends earlier findings showing a good cross-lab reliability of blood NfL, which so far, however, has been limited to smaller sample sets and non-gFTD cohorts.[16] Given, however, that all three labs in our study still used the same type of platform (Simoa HD-1), future studies need to investigate a potential decrease in cross-lab reliability if different measurement platforms are being used for blood NfL (eg, Ella,[17] Uman,[18] Atellica[19]). A pilot study on this showed promising results.[20]

Reliable cut-off values of blood NfL for accurately stratifying different gFTD disease stages are key for its use as a molecular stratification marker of gFTD subjects into treatment trials.[3 5 7] In particular, reliable blood-based stratification of subjects close to conversion to the symptomatic phase of the disease will be of extremely high value to identify and recruit subjects into upcoming mechanistic treatment trials tailored to prevent neurodegeneration by early intervention.[5 21] Extending earlier findings on blood NfL cut-offs in gFTD,[3] our findings now indicate that these cut-off values can be provided by blood NfL for gFTD even with a high reliability across labs. In addition, they also show that NfL levels in converting carriers are already more similar to symptomatic carriers than (non-converting) presymptomatic carriers. Nevertheless, in the absence of a certified reference material, value assigned by a certified reference method, the reported cut-offs remain preliminary and prospective laboratory-specific validation remains required.

Multicentre use of blood NfL—whether in trials or real-world clinical settings—is inherently characterised by cross-centre variability in preanalytical sample handling. Our data from a large set of different sites (n=13) suggest that this variability might not exert a substantial effect on multicentre blood NfL values—even despite the fact that no strictly enforced cross-centre harmonised standard operating procedure or centralised biosampling monitoring had been employed across centres. These data corroborate blood NfL as a very stable biomarker that is resistant to most types of clinically relevant variation in preanalytical sample handling.[22] Future studies with larger sample batches per centre and testing more extreme variabilities in preanalytical sample handling are warranted to further investigate and specify the limits of this cross-centre comparability.

Real-world clinical multicentre use of blood NfL moreover often faces the challenge that samples come from different blood matrices (eg, serum vs plasma).[9] While our findings confirm differences in the absolute blood NfL concentrations between serum and plasma, they at the same time show a high consistency between both blood matrices, allowing comparability of both matrices. The calculated median ratio serum/plasma might be a first coarse help when comparing results derived from these different matrices. However, its use might be limited to Simoa-based blood NfL measurements, and further larger in-depth studies in independent cohorts are required to confirm this factor.

Our study has several limitations. First, although leveraging the largest gFTD cohort existing so far, the sample size is partly limited by the requirement to measure each sample in three labs, leading to limited sample sizes in particular for some gFTD subcohorts (eg, converters). Second, the construct and wording of 'cut-offs' suggest a separating dichotomy where in fact a biological continuum of NfL levels and disease progression exists.

Despite these limitations, our results underline the suitability of blood NfL as a fit-for-purpose biomarker in gFTD multicentre trials.

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RESEARCH

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Utility of visual rating scales in primary progressive aphasia

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Abstract

Introduction Differential diagnosis among subjects with Primary Progressive Aphasia (PPA) can be challenging. Structural MRI can support the clinical profile. Visual rating scales are a simple and reliable tool to assess brain atrophy in the clinical setting.

The aims of the study were to establish to what extent the visual rating scales could be useful in the differential diagnosis of PPA, to compare the clinical diagnostic impressions derived from routine MRI interpretations with those obtained using the visual rating scale and to correlate results of the scales in a voxel-based morphometry (VBM) analysis.

Method Patients diagnosed with primary progressive aphasia (PPA) according to current criteria from two centers—Ospedale Maggiore Policlinico of Milan and Hospital Clínic de Barcelona—were included in the study. Two blinded clinicians evaluated the subjects MRIs for cortical atrophy and white matter hyperintensities using two protocols: routine readings and the visual rating scale. The diagnostic accuracy between patients and controls and within PPA subgroups were compared between the two protocols.

Results One hundred fifty Subjects were studied. All the scales showed a good to excellent intra and inter-rater agreement. The left anterior temporal scale could differentiate between semantic PPA and all other variants.

The rater impression after the protocol can increase the accuracy just for the logopenic PPA. In the VBM analysis, the scores of visual rating scales correlate with the corresponding area of brain atrophy.

Conclusion The Left anterior temporal rating scale can distinguish semantic PPA from other variants. The rater impression after structured view improved the diagnostic accuracy of logopenic PPA compared to normal readings. The unstructured view of the MRI was reliable for identifying semantic PPA and controls. Neither the structured nor the unstructured view could identify the nonfluent and undetermined variants.

Keywords Primary progressive aphasia, Dementia, Visual rating scales, Atrophy, Biomarkers, Magnetic resonance imaging

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Abbreviations

AC	Anterior cingulate rating scale
AT	Anterior Temporal rating scale
AUC	Area under the Receiver operating characteristic curve
CSF	Cerebrospinal fluid
DP	Dorsal Parietal rating scale
FAZ PV	Fazekas periventricular rating scale
FAZ WMH	Fazekas deep white matter hyperintensities rating scale
FI	Fronto-insula rating scale
GM	Gray matter
HC	Healty controls
lvPAA	logopenic variant primary progressive aphasia
MMSE	Mini Mental state examination
MTA	Medial Temporal rating scale
nfvPPA	nonfluent/agrammatic variant primary progressive aphasia
OF	Orbitofrontal rating scale
PA	Posterior rating scale
PCS	Posterior cingulate rating scale
POS	Parieto-occipital rating scale
PPA	Primary progressive aphasias
PRE	Precuneus rating scale
svPPA	Semantic variant primary progressive aphasia
uPPA	Undetermined primary progressive aphasia
VBM	Voxel based morphometry
WM	White matter

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Authors' contributions
GGF and NF designed the study, analysed, interpreted the data and drafted the manuscript. AA and GC analysed and interpreted the data. TC and LS contributed to the analysis of the data. FT and DG revised the manuscript for intellectual content. RSV 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 data supporting the findings of this study are available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate
This study was approved by the Local Ethical Committee on human studies and written informed consent from all subjects was obtained.

Consent for publication
Not applicable.

Competing interests
RSV reports consultancy or speaker fees from Ionis, AviadoBio, NovoNordisk, Pfizer, Neuraxpharm, Roche diagnosis. The other authors have no conflict of interest to report.

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References

1. Ashburner J. A fast diffeomorphic image registration algorithm. NeuroImage. 2007. https://doi.org/10.1016/j.neuroimage.2007.07.007.
2. Bisenius S, Mueller K, Diehl-Schmid J, Fassbender K, Grimmer T, Jessen F, Kassubek J, Kornhuber J, Landwehrmeyer B, Ludolph A, Schneider A, Anderl-Straub S, Stuke K, Danek A, Otto M, Schroeter ML. Predicting primary progressive aphasias with support vector machine approaches in structural MRI data. NeuroImage Clin. 2017;14:334–43. https://doi.org/10.1016/j.nicl.2017.02.003.
3. Falgàs N, Balasa M, Bargalló N, Borrego-Écija S, Ramos-Campoy O, Fernández-Villullas G, Bosch B, Olives J, Tort-Merino A, Antonell A, Castellví M, Allen IE, Sánchez-Valle R, Lladó A. Diagnostic accuracy of MRI visual rating scales in the diagnosis of early onset cognitive impairment. J Alzheimer's Disease. 2020;73:1575–83. https://doi.org/10.3233/JAD-191167.
4. Fazekas F, Chawluk JB, Zimmerma A, June M. MR Signal Abnormalities at 1. 5 T in Alzheimer's Dementia and Normal Aging deficiency; 1987.
5. Fumagalli GG, Basilico P, Arighi A, Bocchetta M, Dick KM, Cash DM, Harding S, Mercurio M, Fenoglio C, Pietroboni AM, Ghezzi L, Van Swieten J, Borroni B, De Mendonça A, Masellis M, Tartaglia MC, Rowe JB, Graff C, Tagliavini F, Frisoni GB, Laforce R, Finger E, Sorbi S, Scarpini E, Rohrer JD, Galimberti D. Distinct patterns of brain atrophy in genetic Frontotemporal Dementia Initiative (GENFI) cohort revealed by visual rating scales. Alzheimer's Res Therapy. 2018;10. https://doi.org/10.1186/s13195-018-0376-9.
6. Fumagalli GG, Basilico P, Arighi A, Mercurio M, Scarioni M, Carandini T, Colombi A, Pietroboni AM, Sacchi L, Conte G, Scola E, Triulzi F, Scarpini E, Galimberti D. Parieto-occipital sulcus widening differentiates posterior cortical atrophy from typical Alzheimer disease. NeuroImage Clin. 2020;28. https://doi.org/10.1016/j.nicl.2020.102453.
7. Gorno-Tempini ML, Dronkers NF, Rankin KP, Ogar JM, Phengrasamy L, Rosen HJ, Johnson JK, Weiner MW, Miller BL. Cognition and anatomy in three variants of primary progressive aphasia. Ann Neurol. 2004;55(3):335–46. https://doi.org/10.1002/ana.10825.
8. Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, Ogar JM, Rohrer JD, Black S, Boeve BF, Manes F, Dronkers NF, Vandenberghe R, Rascovsky K, Patterson K, Miller BL, Knopman DS, Hodges JR, Mesulam MM, Grossman M. Classification of primary progressive aphasia and its variants. Neurology. 2011;76:1006–14. https://doi.org/10.1212/WNL.0b013e31821103e6.
9. Harper L, Fumagalli GG, Barkhof F, Scheltens P, Brien JTO, Bouwman F, Burton EJ, Rohrer JD, Fox NC, Ridgway GR, Schott JM. 2016. MRI visual rating scales in the diagnosis of dementia: evaluation in 184 post-mortem confirmed cases 1–15. https://doi.org/10.1093/brain/aww005.
10. Illán-Gala I, Falgàs N, Friedberg A, Castro-Suárez S, Keret O, Rogers N, Oz D, Nigro S, Quattrone, Andrea, Quattrone, Aldo, Wolf A, Younes K, Santos-Santos M, Borrego-Écija S, Cobigo Y, Dols-Icardo O, Lladó A, Sánchez-Valle R, Clarimon J, Blesa R, Alcolea D, Fortea J, Lleó A, Grinberg LT, Spina S, Kramer JH, Rabinovici GD, Boxer A, Gorno Tempini ML, Miller BL, Seeley WW, Rosen HJ, Perry DC. Diagnostic utility of measuring cerebral atrophy in the Behavioral Variant of Frontotemporal Dementia and Association with Clinical Deterioration. JAMA Netw Open. 2021;4:1–14. https://doi.org/10.1001/jamanetworkopen.2021.1290.
11. Ingala S, van Maurik IS, Altomare D, Wurm R, Dicks E, van Schijndel RA, Zwan M, Bouwman F, Schoonenboom N, Boelaarts L, Roks G, van Marum R, van Harten B, van Uden I, Claus J, Wottschel V, Vrenken H, Wattjes MP, van der Flier WM, Barkhof F. Clinical applicability of quantitative atrophy measures on MRI in patients suspected of Alzheimer's disease. Eur Radiol. 2022;32(11):7789–99. https://doi.org/10.1007/s00330-021-08503-7.
12. Josephs KA, Duffy JR, Strand EA, Whitwell JL, Layton KF, Parisi JE, Hauser MF, Witte RJ, Boeve BF, Knopman DS, Dickson DW, Jack CR Jr, Petersen RC. Clinicopathological and imaging correlates of progressive aphasia and apraxia of speech. Brain. 2006;129(Pt 6):1385–98. https://doi.org/10.1093/brain/awl078.
13. Loreto F, Gontsarova A, Scott G, Patel N, Win Z, Carswell C, Perry R, Malhotra P. Visual atrophy rating scales and amyloid PET status in

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RESEARCH ARTICLE

Extending the phenotypic spectrum assessed by the CDR plus NACC FTLD in genetic frontotemporal dementia

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Abstract

INTRODUCTION: We aimed to expand the range of the frontotemporal dementia (FTD) phenotypes assessed by the Clinical Dementia Rating Dementia Staging Instrument plus National Alzheimer's Coordinating Center Behavior and Language Domains (CDR plus NACC FTLD).

METHODS: Neuropsychiatric and motor domains were added to the standard CDR plus NACC FTLD generating a new CDR plus NACC FTLD-NM scale. This was assessed in 522 mutation carriers and 310 mutation-negative controls from the Genetic Frontotemporal dementia Initiative (GENFI).

RESULTS: The new scale led to higher global severity scores than the CDR plus NACC FTLD: 1.4% of participants were now considered prodromal rather than asymptomatic, while 1.3% were now considered symptomatic rather than asymptomatic or

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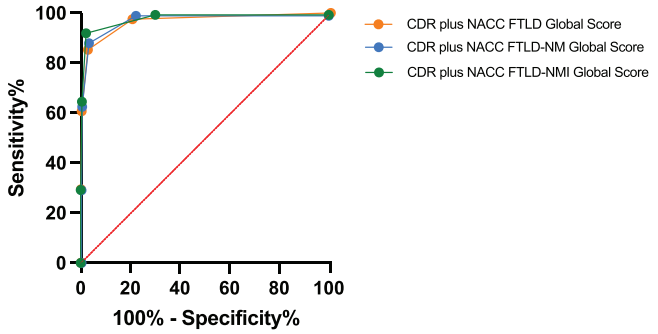

FIGURE 3 Receiver operating characteristic analysis of the utility of the CDR plus NACC FTLD, CDR plus NACC FTLD-NM, and CDR plus NACC FTLD-NMI for detection of clinician-judged symptomatic individuals. The red line represents the line of no discrimination. CDR plus NACC FTLD; Clinical Dementia Rating Dementia Staging Instrument plus National Alzheimer's Coordinating Center Behavior and Language Domains; CDR plus NACC FTLD-NM, Clinical Dementia Rating Dementia Staging Instrument plus National Alzheimer's Coordinating Center Behavior and Language Domains plus Neuropsychiatric and Motor domains; CDR plus NACC FTLD-NMI, Clinical Dementia Rating Dementia Staging Instrument plus National Alzheimer's Coordinating Center Behavior and Language Domains plus Neuropsychiatric and Motor domains by individual symptoms.

4.1 | Limitations

Firstly, although a large genetic FTD cohort was studied there were modest numbers in each group after stratification. Future studies with larger numbers aimed at replicating this work will be helpful. Such studies should also formally assess both intra- and inter-rater variability as well as investigate the longitudinal change in these scales. Further work will be needed to better understand the ability of the scale to detect specific changes in disease stage, for example, to identify phenoconverters. Second, there are a number of limitations of the scales themselves as they are currently set up: the language scale includes a number of individual items that are best assessed by a combination of history and examination, and future versions of the -NMI scale will require a focus on those symptoms assessed best by history; the motor scale is a symptom score only and therefore will not score examination features that are not noted by participants or informants, for example, subtle fasciculations or hyperreflexia that may herald early ALS—future versions of the scale should consider incorporating examination features alongside the history; and finally, although we include functional problems with the hands as an individual item in the motor scale, there are no other measures of the functional impact of motor deficits, which will need to be addressed in future iterations of the scale. Third, the scales have been constructed from the GENFI symptom questionnaires and so future iterations of the -NM and -NMI scales will require fully operationalized instructions on how to derive the global and algorithm-based scores and which symptoms to include within each component. Last, for future versions that might be performed remotely (e.g., by phone or video), there should be some caution over the possibility of missing some features that can only be detected by face-to-face examination (e.g., subtle motor findings).

4.2 | Summary

This study has highlighted the importance of updating the current method of assessing disease severity in FTD to include all symptom domains that can be affected in this disease. Much further work will be needed to be done to ensure this scale is ready for use in clinical trials, including more reliability and validity analyses. However, hopefully this work will be a first crucial step in the development of more appropriate staging and outcome measures in future clinical trials of genetic FTD.

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BRAIN COMMUNICATIONS

Impaired glymphatic system in genetic frontotemporal dementia: a GENFI study

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The glymphatic system is an emerging target in neurodegenerative disorders. Here, we investigated the activity of the glymphatic system in genetic frontotemporal dementia with a diffusion-based technique called diffusion tensor image analysis along the perivascular space. We investigated 291 subjects with symptomatic or presymptomatic frontotemporal dementia (112 with *chromosome 9 open reading frame 72* [*C9orf72*] expansion, 119 with *granulin* [*GRN*] mutations and 60 with *microtubule-associated protein tau* [*MAPT*] mutations) and 83 non-carriers (including 50 young and 33 old non-carriers). We computed the diffusion tensor image analysis along the perivascular space index by calculating diffusivities in the *x*-, *y*- and *z*-axes of the plane of the lateral ventricle body. Clinical stage and blood-based markers were considered. A subset of 180 participants underwent cognitive follow-ups for a total of 640 evaluations. The diffusion tensor image analysis along the perivascular space index was lower in symptomatic frontotemporal dementia (estimated marginal mean ± standard error, 1.21 ± 0.02) than in old non-carriers (1.29 ± 0.03, $P = 0.009$) and presymptomatic mutation carriers (1.30 ± 0.01, $P < 0.001$). In mutation carriers, lower diffusion tensor image analysis along the perivascular space was associated with worse disease severity ($\beta = -1.16$, $P < 0.001$), and a trend towards a significant association between lower diffusion tensor image analysis along the perivascular space and higher plasma neurofilament light chain was reported ($\beta = -0.28$, $P = 0.063$). Analysis of longitudinal data demonstrated that worsening of disease severity was faster in patients with low diffusion tensor image analysis along the perivascular space at baseline than in those with average ($P = 0.009$) or high ($P = 0.006$) diffusion tensor image analysis along the perivascular space index. Using a non-invasive imaging approach as a proxy for glymphatic system function, we demonstrated glymphatic system abnormalities in the symptomatic stages of genetic frontotemporal dementia. Such measures of the glymphatic system may elucidate pathophysiological processes in human frontotemporal dementia and facilitate early phase trials of genetic frontotemporal dementia.

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RESEARCH ARTICLE

Clinical utility of diffusion MRI-derived measures of cortical microstructure in a real-world memory clinic setting

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Abstract

Objective: To investigate cortical microstructural measures from diffusion MRI as "neurodegeneration" markers that could improve prognostic accuracy in mild cognitive impairment (MCI). **Methods**: The prognostic power of Amyloid/Tau/Neurodegeneration (ATN) biomarkers to predict progression from MCI to AD or non-AD dementia was investigated. Ninety patients underwent clinical evaluation (follow-up interval 32 ± 18 months), lumbar puncture, and MRI. Participants were grouped by clinical stage and cerebrospinal fluid Amyloid and Tau status. T1-structural and diffusion MRI scans were analyzed to calculate diffusion metrics related to cortical columnar structure (AngleR, ParlPD, PerpPD^{+}), cortical mean diffusivity, and fractional anisotropy. Statistical tests were corrected for multiple comparisons. Prognostic power was assessed using receiver operating characteristic (ROC) analysis and related indices. **Results**: A progressive increase of whole-brain cortical diffusion values was observed along the AD continuum, with all A+ groups showing significantly higher AngleR than A−T−. Investigating clinical progression to dementia, the AT biomarkers together showed good positive predictive value (with 90.91% of MCI A+T+ converting to dementia) but poor negative predictive value (with 40% of MCI A−T− progressing to a mix of AD and non-AD dementias). Adding whole-brain AngleR as an N marker, produced good differentiation between stable and converting MCI A−T− patients (0.8 area under ROC curve) and substantially improved negative predictive value (+21.25%). **Interpretation**: Results support the clinical utility of cortical microstructure to aid prognosis, especially in A−T− patients. Further work will investigate other complexities of the real-world clinical setting, including A−T+ groups. Diffusion MRI measures of neurodegeneration may complement fluid AT markers to support clinical decision-making.

Introduction

Dementia represents one of the main medical problems and arises from a variety of neuropathological processes and injuries that primarily or secondarily affect the human brain. These brain changes start many years before clinical onset,[1] so a timely and accurate diagnosis plays a key role, enabling therapeutic decisions and support for individuals.

In the last decade, the combination of fluid amyloid beta (Aβ42, Aβ40, Aβ 42/40 ratio), phosphorylated tau (pTau-181, pTau-217, etc.) biomarkers, and magnetic resonance imaging (MRI) have been shown to have predictive value for progression to dementia.[2–4] For Alzheimer's disease (AD), a biological framework for diagnosis, based on the presence of pathology rather than the presence of clinical symptoms, has been proposed[5] and is currently being updated. The Amyloid/Tau/Neurodegeneration

confidence for the clinician to recommend decisions about patient management.

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Author Contributions

The authors confirm contribution to the paper as follows: Study conception and design: MT, GRR, and SAC; acquisition and analysis of data: MT, GF, GRR, VEC, ES, IH, DG, and AA; drafting manuscript: MT, GF, GRR, VEC, ES, DG, SAC, and AA. All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest

S.A. Chance is a co-founder of a company, Oxford Brain Diagnostics, from which he has received funding for the research and preparation of this manuscript; M. Torso, G.R. Ridgway, and I. Hardingham are currently employed at a company, Oxford Brain Diagnostics; S.A. Chance has a patent (WO2016162682A1) related to the diffusion MRI analysis used in the present study; G. Fumagalli, V.E. Contarino, E. Scarpini, D. Galimberti, and A. Arighi report no disclosures relevant to the manuscript.

Data Availability Statement

Anonymized data can be obtained by reasonable request from any qualified investigator.

References

1. Holtzman DM, Morris JC, Goate AM. Alzheimer's disease: the challenge of the second century. Sci Transl Med. 2011;3(77):77sr1. doi:10.1126/scitranslmed.30023
2. van Maurik IS, Zwan MD, Tijms B, et al. Interpreting biomarker results in individual MCI patients—the ABIDE project. JAMA Neurol. 2017;74(12):1481-1491. doi:10.1001/jamaneurol.2017.2712
3. Simrén J, Leuzy A, Karikari TK, et al. The diagnostic and prognostic capabilities of plasma biomarkers in Alzheimer's disease. Alzheimers Dement. 2021;17(7):1145-1156. doi:10.1002/alz.12283
4. Benedet AL, Brum WS, Hansson O, et al. The accuracy and robustness of plasma biomarker models for amyloid PET positivity. Alzheimers Res Ther. 2022;14(1):1-11. doi:10.1186/s13195-021-00942-0
5. Jack CR Jr, Bennett DA, Blennow K, et al. NIA-AA research framework: toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535-562. doi:10.1016/j.jalz.2018.02.018
6. Festari C, Massa F, Cotta Ramusino M, et al. European consensus for the diagnosis of MCI and mild dementia: preparatory phase. Alzheimers Dement. 2023;19(5):1729-1741. doi:10.1002/alz.12798
7. Bocchetta M, Galluzzi S, Kehoe PG, et al. The use of biomarkers for the etiologic diagnosis of MCI in Europe: an EADC survey. Alzheimers Dement. 2015;11(2):195-206. doi:10.1016/j.jalz.2014.06.006
8. Caprioglio C, Garibotto V, Jessen F, et al. The clinical use of Alzheimer's disease biomarkers in patients with mild cognitive impairment: a European Alzheimer's disease consortium survey. J Alzheimers Dis. 2022;89(2):535-551. doi:10.3233/JAD-220333
9. Leuzy A, Mattsson-Carlgren N, Palmqvist S, Janelidze S, Dage JL, Hansson O. Blood-based biomarkers for Alzheimer's disease. EMBO Mol Med. 2022;14(1):e14408. doi:10.15252/emmm.202114408
10. Gleerup HS, Simonsen AH, Høgh P. The added value of cerebrospinal fluid neurofilament light chain to existing diagnostic methods and biomarkers in a mixed memory clinic cohort of consecutive patients. Journal of Alzheimer's Disease Reports. 2022;6(1):121-127. doi:10.3233/ADR-210047
11. Lombardi G, Crescioli G, Cavedo E, et al. Structural magnetic resonance imaging for the early diagnosis of dementia due to Alzheimer's disease in people with mild cognitive impairment. Cochrane Database Syst Rev. 2020;3:3. doi:10.1002/14651858.CD009628.pub2
12. Smailagic N, Vacante M, Hyde C, Martin S, Ukoumunne O, Sachpekidis C. 18 F-FDG PET for the early diagnosis of Alzheimer's disease dementia and other dementias in people with mild cognitive impairment (MCI). Cochrane Database Syst Rev. 2015;1(1):CD010632. doi:10.1002/14651858.CD010632.pub2
13. Torso M, Bozzali M, Zamboni G, Jenkinson M, Chance SA, for Alzheimer's Disease Neuroimage Initiative. Detection of Alzheimer's disease using cortical diffusion tensor imaging. Hum Brain Mapp. 2020;42(4):967-977. doi:10.1002/hbm.25271
14. Torso M, Ridgway GR, Hardingham I, Schwarz AJ. Chance SA for Alzheimer's disease neuroimage initiative. In vivo detection of changes related to cortical columnar organization and neuroinflammation across the AD continuum. J Prev Alzheimers Dis. 2022;9:769-779. doi:10.14283/jpad.2022.59
15. Torso M, Ridgway GR, Valotti M, Hardingham I, Chance SA. In vivo cortical diffusion imaging relates to Alzheimer's disease neuropathology. Alzheimers Res Ther. 2023;15:165. doi:10.1186/s13195-023-01309-3

RESEARCH ARTICLE OPEN ACCESS

Frequency and Longitudinal Course of Behavioral and Neuropsychiatric Symptoms in Participants With Genetic Frontotemporal Dementia

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Abstract

Background and Objectives

Behavioral and neuropsychiatric symptoms are frequent in patients with genetic frontotemporal dementia (FTD). We aimed to describe behavioral and neuropsychiatric phenotypes in genetic FTD, quantify their temporal association, and investigate their regional association with brain atrophy.

Methods

We analyzed data of pathogenic variant carriers in the chromosome 9 open reading frame 72 (*c9orf72*), progranulin (*GRN*), or microtubule-associated protein tau (*MAPT*) gene from the Genetic Frontotemporal dementia Initiative cohort study that enrolls both symptomatic pathogenic variant carriers and first-degree relatives of known carriers. Principal component

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RESEARCH ARTICLE

Frontoparietal network integrity supports cognitive function in pre-symptomatic frontotemporal dementia: Multimodal analysis of brain function, structure, and perfusion

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Abstract

INTRODUCTION: Genetic mutation carriers of frontotemporal dementia can remain cognitively well despite neurodegeneration. A better understanding of brain structural, perfusion, and functional patterns in the pre-symptomatic stage could inform accurate staging and potential mechanisms.

METHODS: We included 207 pre-symptomatic genetic mutation carriers and 188 relatives without mutations. The gray matter volume, cerebral perfusion, and resting-state functional network maps were co-analyzed using linked independent component analysis (LICA). Multiple regression analysis was used to investigate the relationship of LICA components to genetic status and cognition.

RESULTS: Pre-symptomatic mutation carriers showed an age-related decrease in the left frontoparietal network integrity, while non-carriers did not. Executive functions of mutation carriers became dependent on the left frontoparietal network integrity in older age.

DISCUSSION: The frontoparietal network integrity of pre-symptomatic mutation carriers showed a distinctive relationship to age and cognition compared to non-carriers, suggesting a contribution of the network integrity to brain resilience.

non-carriers.[61] Our study provides further evidence suggesting that these cognitive domains are sensitive to alternations at the earlier stage of the disease. Given that CBF and GMV significantly decreased with age regardless of genetic mutations, and the reliance on other functional networks for cognitive performance broke down in genetic mutation carriers, maintaining frontoparietal network integrity might be the key to slowing cognitive decline, particularly executive declines, at the pre-symptomatic stage of FTD.

The atrophy patterns can be different across different genetic mutations. The *GRN* genetic mutation is known for causing asymmetric atrophy while the atrophy patterns of FTD associated with *MAPT* genetic mutation are typically symmetric.[7,55,64] We observed asymmetric relationship between functional network integrity and age in *GRN* mutation carriers, indicating that the asymmetric vulnerability to genetic mutation can be manifested at the pre-symptomatic stage. Specifically, we observed a relationship between age and the left frontoparietal network in *GRN* mutation carriers, although the lack of significance in other genetic groups may be attributed to smaller sample sizes compared to *GRN* mutation carriers. Such finding is consistent with previous studies showing selective vulnerability of the left hemisphere.[55,65,66] Moreover, there is inherent asymmetry in several human cognitive systems, including language and executive functions, which could be significantly impaired in FTD.[67–69] Although the cellular mechanisms of selective vulnerability are not well understood, it would be important to investigate the laterality of changes in future studies, especially considering the dynamical interactions between brain networks which shape cognition.

This study benefits from pathological confidence arising from genetic characterization, and the large sample size of pre-symptomatic mutation carriers through the multi-center GENFI study. This study combines GMV, CBF, and functional networks in pre-symptomatic FTD genetic mutation carriers. Linking neurobiological changes is important given potential synergistic effects. Although, we found no interplay across modalities, relating the frontoparietal network to other unexplored pathologies like tau, amyloid, and neurotransmitters may be informative,[45,58,70,71] given its age- and cognition-related distinctions between genetic mutation carriers and non-carriers observed in our study.

The study also has limitations. First, the variability of MRI acquisition scanners and sequences through the multi-center cohort is higher than in a single-center study. However, we mitigated the effects through the use of normalization, denoising, and statistical adjustment for side effects. We recognize that multi-center and multi-scanner correction for ASL could potentially be improved. A standard approach would be the use of flow phantoms for calibrating a scanner's ASL signal to a ground-truth flow rate.[72] Currently, however, this is not implemented in most ASL studies. Existing methods of pre-model or within-model corrections[73] along with data-driven and model-driven corrections for sites and scanners remain the most pragmatic approach. Second, this study is cross-sectional. This should be noted when interpreting age effects, as dynamic aging effects require longitudinal data. More follow-up visits of the ongoing GENFI cohort will allow a longitudinal examination. Third, only adults were included, thus potentially missing the changes manifested before adulthood caused by genetic mutation. A new cohort within GENFI is starting which aims to study family members below the age of 18. Fourth, there were some pre-symptomatic genetic mutation carriers with a CDR plus NACC FTLD global score of 0.5, indicating that they might have mild clinical symptoms but were not diagnosed as FTD. However, the pre-symptomatic mutation carriers did not differ from non-carriers in their groupwise CDR plus NACC FTLD score, CBI-R, or MMSE. This suggests that the difference in functional networks observed in this study is not likely to be related to mis-assigned early-symptomatic patients carrying mutations. Future studies can implement a more refined and multidimensional classification of the pre-symptomatic stage, such as the mild cognitive and/or behavioral and/or motor impairment (MCBMI) criteria,[74] to distinguish those at different "pre-symptomatic" stages. Finally, our study focused on integrating spatial maps of network activity in relation to atrophy and perfusion. Functional connectivity between networks is another important factor to be considered.[4] The joint consideration of activity and connectivity might better characterize brain dynamics and cognitive performance.[75] Future research could investigate the intercorrelations between functional connectivity and multiple neuroimaging modalities.

In conclusion, we demonstrated that frontoparietal network integrity might support cognitive function in pre-symptomatic FTD. Linking neuroimaging, especially functional network integrity, with other neuropathological changes may be a future study direction for pre-symptomatic genetic FTD. The dissociation of changes in structure, perfusion, and network activity in pre-symptomatic FTD has implications for strategies to prevent or treat cognitive decline in people at high risk of FTD.

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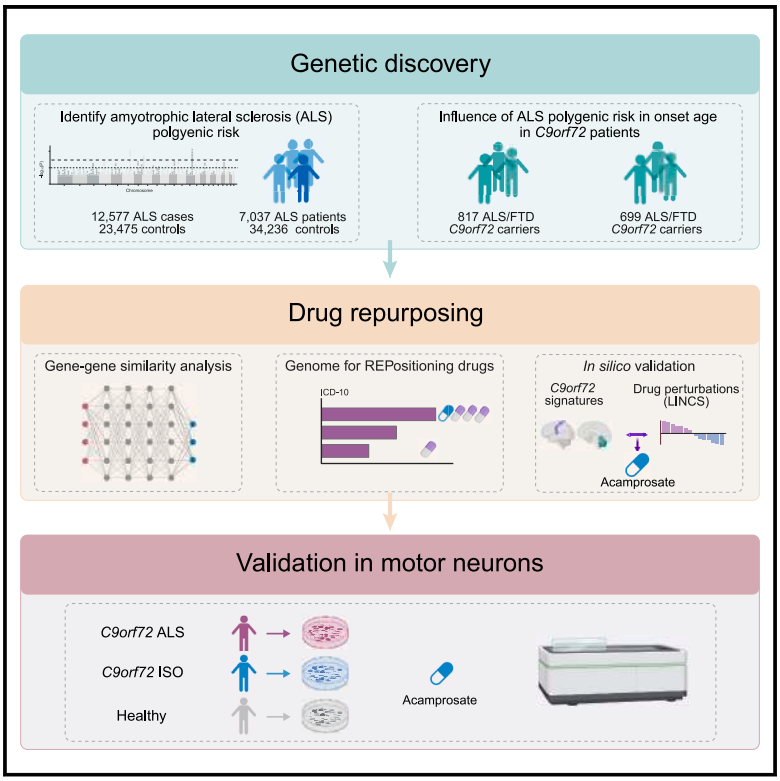
Cell Genomics

Article

Mechanism-free repurposing of drugs for *C9orf72*-related ALS/FTD using large-scale genomic data

Graphical abstract



Highlights

- Repeat expansions in *C9orf72* are the most common genetic cause of ALS and FTD
- The genetic risk of general ALS modifies the age at onset in *C9orf72* cases
- We performed a drug screen based on the genetic variants influencing age at onset
- We identified acamprosate, a GABA analog, as a potential treatment for *C9orf72*

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In brief

Saez-Atienzar et al. identify genetic variants influencing the age at onset among patients carrying *C9orf72* repeat expansions. A drug screen based on these variants revealed acamprosate, a GABA analog, as a potentially repurposable treatment for *C9orf72*-related disease. The work underscores the potential of leveraging large-scale genomic data for drug repurposing.

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Alzheimer's Research & Therapy

RESEARCH

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Clinical validity of the Italian adaptation of the Uniform Data Set Neuropsychological Test Battery (I-UDSNB) in Mild Cognitive Impairment and Alzheimer's Disease

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Abstract

Background The identification and staging of Alzheimer's Disease (AD) represent a challenge, especially in the prodromal stage of Mild Cognitive Impairment (MCI), when cognitive changes can be subtle. Worldwide efforts were dedicated to select and harmonize available neuropsychological instruments. In Italy, the Italian Network of Neuroscience and Neuro-Rehabilitation has promoted the adaptation of the Uniform Data Set Neuropsychological Test Battery (I-UDSNB), collecting normative data from 433 healthy controls (HC).

Here, we aimed to explore the ability of I-UDSNB to differentiate between a) MCI and HC, b) AD and HC, c) MCI and AD.

Methods One hundred thirty-seven patients (65 MCI, 72 AD) diagnosed after clinical-neuropsychological assessment, and 137 HC were included. We compared the I-UDSNB scores between a) MCI and HC, b) AD and HC, c) MCI and AD, with t-tests. To identify the test(s) most capable of differentiating between groups, significant scores were entered in binary logistic and in stepwise regressions, and then in Receiver Operating Characteristic curve analyses.

Results Two episodic memory tests (Craft Story and Five Words test) differentiated MCI from HC subjects; Five Words test, Semantic Fluency (vegetables), and TMT-part B differentiated AD from, respectively, HC and MCI.

Conclusions Our findings indicate that the I-UDSNB is a suitable tool for the harmonized and concise assessment of patients with cognitive decline, showing high sensitivity and specificity for the diagnosis of MCI and AD.

Keywords Neuropsychological tests, UDS, Alzheimer's Disease, Mild Cognitive Impairment, Cognition

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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.

Consent for publication
Not applicable.

Competing interests
V. Silani received compensation for consulting services and/or speaking activities from AveXis, Cytokinetics, Italfarmaco, Liquidweb S.r.l., Novartis Pharma AG and Zambon Biotech SA; he receives or has received research supports form the Italian Ministry of Health, AriSLA, E-Rare Joint Transnational Call, and the ERN Euro-NMD. He is in the Editorial Board of Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, European Neurology, American Journal of Neurodegenerative Diseases, Frontiers in Neurology, and Exploration of Neuroprotective Therapy. E. Canu receives research supports from the Italian Ministry of Health. M. Filippi is Editor-in-Chief of the Journal of Neurology, Associate Editor of Human Brain Mapping, Neurological Sciences, and Radiology; he received compensation for consulting services from Alexion, Almirall, Biogen, Merck, Novartis, Roche, Sanofi and for speaking activities from Bayer, Biogen, Celgene, Chiesi Italia SpA, Eli Lilly, Genzyme, Janssen, Merck-Serono, Neopharmed Gentili, Novartis, Novo Nordisk, Roche, Sanofi, Takeda, and TEVA; participation in Advisory Boards for Alexion, Biogen, Bristol-Myers Squibb, Merck, Novartis, Roche, Sanofi, Sanofi-Aventis, Sanofi-Genzyme, Takeda; scientific direction of educational events for Biogen, Merck, Roche, Celgene, Bristol-Myers Squibb, Lilly, Novartis, Sanofi-Genzyme; he receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, the Italian Ministry of Health, the Italian Ministry of University and Research, and Fondazione Italiana Sclerosi Multipla.

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References

1. Jutten RJ, Thompson L, Sikkes SA, Maruff P, Molinuevo JL, Zetterberg H, Snyder PJ. A neuropsychological perspective on defining cognitive impairment in the clinical study of Alzheimer's disease: Towards a more continuous approach. J Alzheimer's Dis. 2022;86(2):511–24.
2. Frisoni GB, Boccardi M, Barkhof F, Blennow K, Cappa S, Chiotis K, Winblad B. Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. The Lancet Neurology. 2017;16(8):661–76.
3. McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR Jr, Kawas CH, Phelps CH. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011;7(3):263–9.
4. Albert MS, DeKosky ST, Dickson D, Dubois B, Feldman HH, Fox NC, Phelps CH. The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimer's Dement. 2011;7(3):270–9.
5. Gallagher D, Mhaolain AN, Coen R, Walsh C, Kilroy D, Belinski K, Lawlor BA. Detecting prodromal Alzheimer's disease in mild cognitive impairment: utility of the CAMCOG and other neuropsychological predictors. Int J Geriatr Psychiatry. 2010;25(12):1280–7.
6. Tabert MH, Manly JJ, Liu X, Pelton GH, Rosenblum S, Jacobs M, Devanand DP. Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment. Arch Gen Psychiatry. 2006;63(8):916–24.
7. Belleville S, Gauthier S, Lepage É, Kergoat MJ, Gilbert B. Predicting decline in mild cognitive impairment: A prospective cognitive study. Neuropsychology. 2014;28(4):643.
8. Boccardi M, Monsch AU, Ferrari C, Altomare D, Berres M, Bos I, ... & Consortium for the Harmonization of Neuropsychological Assessment for Neurocognitive Disorders. Harmonizing neuropsychological assessment for mild neurocognitive disorders in Europe. Alzheimers Dement. 2022;18(1):29–42
9. Wang H, Fan Z, Shi C, Xiong L, Zhang H, Li T, Yu X. Consensus statement on the neurocognitive outcomes for early detection of mild cognitive impairment and Alzheimer dementia from the Chinese Neuropsychological Normative (CN-NORM) Project. J Glob Health. 2019;9(2):020320.
10. Acevedo A, Krueger KR, Navarro E, Ortiz F, Manly JJ, Padilla-Vélez MM, Mungas D. The Spanish translation and adaptation of the uniform data set of the National Institute on Aging Alzheimer's Disease Centers. Alzheimer Disease and associated disorders. 2009;23(2):102.
11. Benson G, de Felipe J, Sano M. Performance of Spanish-speaking community-dwelling elders in the United States on the Uniform Data Set. Alzheimers Dement. 2014;10:S338–43.
12. Weintraub S, Besser L, Dodge HH, Teylan M, Ferris S, Goldstein FC, Morris JC. Version 3 of the Alzheimer Disease Centers' neuropsychological test battery in the Uniform Data Set (UDS). Alzheimer Dis Assoc Disord. 2018;32(1):10.
13. Weintraub S, Salmon D, Mercaldo N, Ferris S, Graff-Radford NR, Chui H, Morris JC. The Alzheimer's disease centers' uniform data set (UDS): The neuropsychological test battery. Alzheimer Dis Assoc Disord. 2009;23(2):91.
14. Beekly DL, Ramos EM, Lee WW, Deitrich WD, Jacka ME, Wu J, Kukull WA. The National Alzheimer's Coordinating Center (NACC) database: the uniform data set. Alzheimer Dis Assoc Disord. 2007;21(3):249–58.
15. Conca F, Esposito V, Rundo F, Quaranta D, Muscio C, Manenti R, Cappa SF. Italian adaptation of the Uniform Data Set Neuropsychological Test Battery (I-UDSNB 10): development and normative data. Alzheimer's Research & Therapy. 2022;14(1):113.
16. Ivanova I, Salmon DP, Gollan TH. The multilingual naming test in Alzheimer's disease: clues to the origin of naming impairments. J Int Neuropsychol Soc. 2013;19(3):272–83.
17. Stasenko A, Jacobs DM, Salmon DP, Gollan TH. The Multilingual Naming Test (MINT) as a measure of picture naming ability in Alzheimer's disease. J Int Neuropsychol Soc. 2019;25(8):821–33.
18. Staffaroni AM, Asken BM, Casaletto KB, Fonseca C, You M, Rosen HJ, Kramer JH. Development and validation of the Uniform Data Set (v3. 0) executive function composite score (UDS3-EF). Alzheimers Dement. 2021;17(4):574–83.
19. Mathews M, Abner E, Kryscio R, Jicha G, Cooper G, Smith C, Schmitt FA. Diagnostic accuracy and practice effects in the National Alzheimer's Coordinating Center Uniform Data Set neuropsychological battery. Alzheimers Dement. 2014;10(6):675–83.

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Review

Advancing Biomarker Discovery and Therapeutic Targets in Duchenne Muscular Dystrophy: A Comprehensive Review

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Abstract: Mounting evidence underscores the intricate interplay between the immune system and skeletal muscles in Duchenne muscular dystrophy (DMD), as well as during regular muscle regeneration. While immune cell infiltration into skeletal muscles stands out as a prominent feature in the disease pathophysiology, a myriad of secondary defects involving metabolic and inflammatory pathways persist, with the key players yet to be fully elucidated. Steroids, currently the sole effective therapy for delaying onset and symptom control, come with adverse side effects, limiting their widespread use. Preliminary evidence spotlighting the distinctive features of T cell profiling in DMD prompts the immuno-characterization of circulating cells. A molecular analysis of their transcriptome and secretome holds the promise of identifying a subpopulation of cells suitable as disease biomarkers. Furthermore, it provides a gateway to unraveling new pathological pathways and pinpointing potential therapeutic targets. Simultaneously, the last decade has witnessed the emergence of novel approaches. The development and equilibrium of both innate and adaptive immune systems are intricately linked to the gut microbiota. Modulating microbiota-derived metabolites could potentially exacerbate muscle damage through immune system activation. Concurrently, genome sequencing has conferred clinical utility for rare disease diagnosis since innovative methodologies have been deployed to interpret the functional consequences of genomic variations. Despite numerous genes falling short as clinical targets for MD, the exploration of Tdark genes holds promise for unearthing novel and uncharted therapeutic insights. In the quest to expedite the translation of fundamental knowledge into clinical applications, the identification of novel biomarkers and disease targets is paramount. This initiative not only advances our understanding but also paves the way for the design of innovative therapeutic strategies, contributing to enhanced care for individuals grappling with these incapacitating diseases.

Keywords: Duchenne muscular dystrophy; Tdark gene; gut microbiota; immunity; biomarker

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1. Introduction

Muscular dystrophies (MDs) represent a group of disorders characterized by primary skeletal muscle wasting and the subsequent emergence of co-morbidities such as inflammation, mitochondrial dysfunction, and metabolic irregularities. These conditions predominantly result from mutations in proteins that connect the cytoskeleton to the basal lamina [1].

Duchenne muscular dystrophy (DMD), the most prevalent form of muscular dystrophy, is a genetic disorder stemming from mutations in the dystrophin gene. Dystrophin deficiency leads to plasma-membrane instability, causing myofiber necrosis and muscle weakness [2]. The absence of dystrophin disrupts the contraction machinery, and the continuous degeneration/regeneration cycles in dystrophic muscles lead to persistent muscular injury and inhibition of regenerative potential caused by the depletion of satellite cells. This scenario culminates in the disruption of interactions between ion channels and components of the dystrophin glycoprotein complex, resulting in the dysfunction of transient

These findings pave the way for a more nuanced understanding of DMD and the potential development of targeted therapeutic interventions that extend beyond traditional approaches.

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References

1. Cossu, G.; Sampaolesi, M. New therapies for Duchenne muscular dystrophy: Challenges, prospects and clinical trials. *Trends Mol. Med.* **2007**, *13*, 520–526. [CrossRef] [PubMed]
2. Farini, A.; Razini, P.; Erratico, S.; Torrente, Y.; Meregalli, M. Cell based therapy for Duchenne muscular dystrophy. *J. Cell Physiol.* **2009**, *221*, 526–534. [CrossRef] [PubMed]
3. Leyva-Leyva, M.; Sandoval, A.; Felix, R.; Gonzalez-Ramirez, R. Biochemical and Functional Interplay Between Ion Channels and the Components of the Dystrophin-Associated Glycoprotein Complex. *J. Membr. Biol.* **2018**, *251*, 535–550. [CrossRef] [PubMed]
4. Dubinin, M.V.; Belosludtsev, K.N. Ion Channels of the Sarcolemma and Intracellular Organelles in Duchenne Muscular Dystrophy: A Role in the Dysregulation of Ion Homeostasis and a Possible Target for Therapy. *Int. J. Mol. Sci.* **2023**, *24*, 2229. [CrossRef]
5. Spencer, M.J.; Montecino-Rodriguez, E.; Dorshkind, K.; Tidball, J.G. Helper (CD4(+)) and cytotoxic (CD8(+)) T cells promote the pathology of dystrophin-deficient muscle. *Clin. Immunol.* **2001**, *98*, 235–243. [CrossRef] [PubMed]
6. Denis, M.C.; Desjardins, Y.; Furtos, A.; Marcil, V.; Dudonne, S.; Montoudis, A.; Garofalo, C.; Delvin, E.; Marette, A.; Levy, E. Prevention of oxidative stress, inflammation and mitochondrial dysfunction in the intestine by different cranberry phenolic fractions. *Clin. Sci.* **2015**, *128*, 197–212. [CrossRef] [PubMed]
7. Vo, A.H.; McNally, E.M. Modifier genes and their effect on Duchenne muscular dystrophy. *Curr. Opin. Neurol.* **2015**, *28*, 528–534. [CrossRef]
8. Desguerre, I.; Christov, C.; Mayer, M.; Zeller, R.; Becane, H.M.; Bastuji-Garin, S.; Leturcq, F.; Chiron, C.; Chelly, J.; Gherardi, R.K. Clinical heterogeneity of duchenne muscular dystrophy (DMD): Definition of sub-phenotypes and predictive criteria by long-term follow-up. *PLoS ONE* **2009**, *4*, e4347. [CrossRef]
9. Aartsma-Rus, A.; Spitali, P. Circulating Biomarkers for Duchenne Muscular Dystrophy. *J. Neuromuscul. Dis.* **2015**, *2*, S49–S58. [CrossRef]
10. Hathout, Y.; Marathi, R.L.; Rayavarapu, S.; Zhang, A.; Brown, K.J.; Seol, H.; Gordish-Dressman, H.; Cirak, S.; Bello, L.; Nagaraju, K.; et al. Discovery of serum protein biomarkers in the mdx mouse model and cross-species comparison to Duchenne muscular dystrophy patients. *Hum. Mol. Genet.* **2014**, *23*, 6458–6469. [CrossRef]
11. Kotelnikova, E.; Shkrob, M.A.; Pyatnitskiy, M.A.; Ferlini, A.; Daraselia, N. Novel approach to meta-analysis of microarray datasets reveals muscle remodeling-related drug targets and biomarkers in Duchenne muscular dystrophy. *PLoS Comput. Biol.* **2012**, *8*, e1002365. [CrossRef] [PubMed]
12. Meregalli, M.; Farini, A.; Parolini, D.; Maciotta, S.; Torrente, Y. Stem cell therapies to treat muscular dystrophy: Progress to date. *BioDrugs* **2010**, *24*, 237–247. [CrossRef] [PubMed]
13. Goemans, N.; Tulinius, M.; Kroksmark, A.K.; Wilson, R.; van den Hauwe, M.; Campion, G. Comparison of ambulatory capacity and disease progression of Duchenne muscular dystrophy subjects enrolled in the drisapersen DMD114673 study with a matched natural history cohort of subjects on daily corticosteroids. *Neuromuscul. Disord. NMD* **2017**, *27*, 203–213. [CrossRef] [PubMed]
14. Hathout, Y.; Brody, E.; Clemens, P.R.; Cripe, L.; DeLisle, R.K.; Furlong, P.; Gordish-Dressman, H.; Hache, L.; Henricson, E.; Hoffman, E.P.; et al. Large-scale serum protein biomarker discovery in Duchenne muscular dystrophy. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 7153–7158. [CrossRef] [PubMed]
15. Grounds, M.D.; Terrill, J.R.; Al-Mshhdani, B.A.; Duong, M.N.; Radley-Crabb, H.G.; Arthur, P.G. Biomarkers for Duchenne muscular dystrophy: Myonecrosis, inflammation and oxidative stress. *Dis. Models Mech.* **2020**, *13*, dmm043638. [CrossRef] [PubMed]
16. Rodriguez, M.C.; Tarnopolsky, M.A. Patients with dystrophinopathy show evidence of increased oxidative stress. *Free Radic. Biol. Med.* **2003**, *34*, 1217–1220. [CrossRef] [PubMed]
17. Terrill, J.R.; Radley-Crabb, H.G.; Grounds, M.D.; Arthur, P.G. N-Acetylcysteine treatment of dystrophic mdx mice results in protein thiol modifications and inhibition of exercise induced myofibre necrosis. *Neuromuscul. Disord. NMD* **2012**, *22*, 427–434. [CrossRef]

Article

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EMBO Molecular Medicine

Cell-mediated exon skipping normalizes dystrophin expression and muscle function in a new mouse model of Duchenne Muscular Dystrophy

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Abstract

Cell therapy for muscular dystrophy has met with limited success, mainly due to the poor engraftment of donor cells, especially in fibrotic muscle at an advanced stage of the disease. We developed a cell-mediated exon skipping that exploits the multinucleated nature of myofibers to achieve cross-correction of resident, dystrophic nuclei by the U7 small nuclear RNA engineered to skip exon 51 of the dystrophin gene. We observed that co-culture of genetically corrected human DMD myogenic cells (but not of WT cells) with their dystrophic counterparts at a ratio of either 1:10 or 1:30 leads to dystrophin production at a level several folds higher than what predicted by simple dilution. This is due to diffusion of U7 snRNA to neighbouring dystrophic resident nuclei. When transplanted into NSG-mdx-Δ51mice carrying a mutation of exon 51, genetically corrected human myogenic cells produce dystrophin at much higher level than WT cells, well in the therapeutic range, and lead to force recovery even with an engraftment of only 3–5%. This level of dystrophin production is an important step towards clinical efficacy for cell therapy.

Keywords Duchenne Muscular Dystrophy; Cell Therapy; Exon Skipping; Mesoangioblast; Regenerative Medicine
Subject Categories Genetics, Gene Therapy & Genetic Disease; Musculoskeletal System; Stem Cells & Regenerative Medicine
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Introduction

Duchenne Muscular Dystrophy (DMD) is the most common and one of the most severe muscular dystrophies, affecting approximately one in four thousand newly born children (Emery, 2002). It is characterized by progressive wasting of skeletal and cardiac muscle, leading to a variable but progressive muscle weakness that limits the patient's motility and, in later years affects cardiac and respiratory functions (Muntoni et al, 2003). Duchenne Muscular Dystrophy (DMD) is caused by different mutations of the dystrophin gene, located on the X chromosome (Worton et al, 1984; Nallamilli et al, 2014). In 90% of cases, mutations lead to a change in the mRNA reading frame that prevents dystrophin protein production (Bladen et al, 2015). In frame deletions lead to a shorter but partially functional dystrophin, associated with milder Becker Muscular Dystrophy (BMD) (Den Dunnen et al, 1989).

Dystrophin and the dystrophin-associated glycoproteins (e.g., sarcoglycans) have a critical role in muscle cell interaction with the basal lamina and provide elastic resistance to the sarcolemma during contraction. In the absence of dystrophin and associated proteins, the membrane is more easily damaged, leading to calcium influx, hyper contraction, proteolysis and fibre degeneration (Davies and Nowak, 2006). Degeneration is followed by regeneration carried out by satellite cells, resident myogenic stem/progenitor cells and, to a minor extent, interstitial cells such as pericytes (Biressi et al, 2020). In humans, adult myogenic cells have limited self-renewal potency and in DMD the continuous regeneration cycles eventually lead to depletion of the myogenic cell populations. Muscle degeneration is accompanied by chronic inflammation that progressively leads to accumulation of dense connective and adipose tissues that replace muscle fibres (Klingler et al, 2012) making any therapy ineffective at this stage.

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Combinatorial strategies targeting NEAT1 and AURKA as new potential therapeutic options for multiple myeloma

by Noemi Puccio, Gloria Manzotti, Elisabetta Mereu, Federica Torricelli, Domenica Ronchetti, Michela Cumerlato, Ilaria Craparotta, Laura Di Rito, Marco Bolis, Valentina Traini, Veronica Manicardi, Valentina Fragliasso, Yvan Torrente, Nicola Amodio, Niccolo Bolli, Elisa Taiana, Alessia Ciarrocchi, Roberto Piva, and Antonino Neri

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Combinatorial strategies targeting NEAT1 and AURKA as new potential therapeutic options for multiple myeloma.

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Running Title: AURKA and NEAT1 cooperate to support MM pathogenesis.

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DIS3 depletion in multiple myeloma causes extensive perturbation in cell cycle progression and centrosome amplification

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Abstract

DIS3 gene mutations occur in approximately 10% of patients with multiple myeloma (MM); furthermore, *DIS3* expression can be affected by monosomy 13 and del(13q), found in roughly 40% of MM cases. Despite the high incidence of *DIS3* mutations and deletions, the biological significance of *DIS3* and its contribution to MM pathogenesis remain poorly understood. In this study we investigated the functional role of *DIS3* in MM, by exploiting a loss-of-function approach in human MM cell lines. We found that *DIS3* knockdown inhibits proliferation in MM cell lines and largely affects cell cycle progression of MM plasma cells, ultimately inducing a significant increase in the percentage of cells in the G0/G1 phase and a decrease in the S and G2/M phases. *DIS3* plays an important role not only in the control of the MM plasma cell cycle, but also in the centrosome duplication cycle, which are strictly co-regulated in physiological conditions in the G1 phase. Indeed, *DIS3* silencing leads to the formation of supernumerary centrosomes accompanied by the assembly of multipolar spindles during mitosis. In MM, centrosome amplification is present in about a third of patients and may represent a mechanism leading to genomic instability. These findings strongly prompt further studies investigating the relevance of *DIS3* in the centrosome duplication process. Indeed, a combination of *DIS3* defects and deficient spindle-assembly checkpoint can allow cells to progress through the cell cycle without proper chromosome segregation, generating aneuploid cells which ultimately lead to the development of MM.

Introduction

Multiple myeloma (MM) is a hematologic malignancy that is still incurable despite the recent introduction of a large array of innovative therapies.[1] MM is characterized by the abnormal proliferation of plasma cells (PC) in the bone marrow and has different clinical courses and a highly heterogeneous genetic background with both structural chromosomal alterations and specific gene mutations affecting the expression and the activity of both putative oncogenes and tumor suppressor genes.[2]

Among the frequently mutated genes in MM, *DIS3* has been reported to be mutated in roughly 10% of patients and to have a significant impact on clinical outcome.[3-6] Despite the

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Editorial: Inflammation in muscular dystrophies: mediators, mechanisms, and therapeutics

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KEYWORDS

miRNA, DMD, muscular dystrophies, inflammation, GHS (growth hormone secretagogues), NLRP3

Editorial on the Research Topic
Inflammation in muscular dystrophies: mediators, mechanisms, and therapeutics

The search for effective therapies to treat muscular dystrophies, particularly Duchenne muscular dystrophy (DMD), has been a persistent and formidable challenge. This research is motivated by the urgent need to address the complex pathophysiology of these debilitating diseases, which significantly compromise patients' quality of life and lifespan.

DMD, in particular, poses a substantial challenge due to its progressive nature and severe impairment of skeletal muscle function. The genetic etiology of the disease, characterized by mutations in the dystrophin gene, initiates a cascade of pathological events, including chronic inflammation.

This Research Topic provides innovative therapeutic strategies targeting inflammation in muscular dystrophies, including the exploration of innate immunity, the therapeutic potential of growth hormone secretagogues, the underlying mechanisms of inflammation-induced muscle atrophy, and the regenerative capabilities of extracellular vesicle-derived miRNAs.

Petrof et al. present a compelling case for the involvement of trained immunity in the pathogenesis of DMD, emphasizing the role of dysregulated inflammation mediated by innate immune cells. Their findings establish a novel framework wherein epigenetic and metabolic alterations induce a hyper-responsive state in innate immune cells, potentially exacerbating tissue damage in DMD.

Complementing this perspective, Boccanegra et al. provide preclinical evidence supporting the therapeutic potential of growth hormone secretagogues (GHS) in DMD. GHSs have demonstrated efficacy in attenuating key drivers of disease progression such as inflammation and fibrosis, and concomitantly exhibited beneficial effects on muscle function and metabolism, suggesting a multifaceted therapeutic potential for improving the quality of life in DMD patients.

In parallel, Liu et al. explore the intricate relationship between inflammation and skeletal muscle atrophy, particularly in the context of sepsis-induced complications. Their investigation of the NLRP3 inflammasome reveals a critical role in driving catabolic processes, implicating it as a potential target for mitigating muscle wasting and associated comorbidities.

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Review

Exploring the Gut Microbiota–Muscle Axis in Duchenne Muscular Dystrophy

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Abstract: The gut microbiota plays a pivotal role in maintaining the dynamic balance of intestinal epithelial and immune cells, crucial for overall organ homeostasis. Dysfunctions in these intricate relationships can lead to inflammation and contribute to the pathogenesis of various diseases. Recent findings uncovered the existence of a gut–muscle axis, revealing how alterations in the gut microbiota can disrupt regulatory mechanisms in muscular and adipose tissues, triggering immune-mediated inflammation. In the context of Duchenne muscular dystrophy (DMD), alterations in intestinal permeability stand as a potential origin of molecules that could trigger muscle degeneration via various pathways. Metabolites produced by gut bacteria, or fragments of bacteria themselves, may have the ability to migrate from the gut into the bloodstream and ultimately infiltrate distant muscle tissues, exacerbating localized pathologies. These insights highlight alternative pathological pathways in DMD beyond the musculoskeletal system, paving the way for nutraceutical supplementation as a potential adjuvant therapy. Understanding the complex interplay between the gut microbiota, immune system, and muscular health offers new perspectives for therapeutic interventions beyond conventional approaches to efficiently counteract the multifaceted nature of DMD.

Keywords: gut microbiota; muscular inflammation; Duchenne muscular dystrophy; muscle wasting

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1. The Bidirectional Gut–Muscle Axis

Comprising 10^{14} microbial cells within the digestive tract, the gut microbiota is categorized into various species, families, and phyla. This intricate community comprehends not only bacteria, but also eukaryotes and viruses, that establish a synergic communication among themselves and with the host, largely influencing human physiology, homeostasis, and health. Albeit a far more in-depth knowledge about bacterial components is reported in the literature, recent studies have focused on the eukaryotic communities and the consortium of viruses, forming the so-called human virome harbored in the digestive system [1].

Due to this cardinal role in human health and disease, the gut microbiota is sometimes named as our "forgotten organ". The impact of the gut microbiota on human well-being is partially attributable to its co-evolvement with the host to reciprocally satisfy biological and biochemical needs [2]. Indeed, it is implicated in numerous metabolic processes, including energy production and storage, as well as the fermentation and absorption of undigested carbohydrates. These functions probably led to a vigorous evolutionary driving force toward the development of a symbiotic relationship between humans and gut bacteria [1]. Fundamental for host homeostasis, the interactions between the gut microbiota and the host extend beyond the digestive system, reaching organs such as the cardiovascular system, brain, skin, pancreas, and skeletal muscles [2,3]. Many studies focused on the effects of

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Flvcr1a deficiency promotes heme-based energy metabolism dysfunction in skeletal muscle

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SUMMARY

The definition of cell metabolic profile is essential to ensure skeletal muscle fiber heterogeneity and to achieve a proper equilibrium between the self-renewal and commitment of satellite stem cells. Heme sustains several biological functions, including processes profoundly implicated with cell metabolism. The skeletal muscle is a significant heme-producing body compartment, but the consequences of impaired heme homeostasis on this tissue have been poorly investigated. Here, we generate a skeletal-muscle-specific feline leukemia virus subgroup C receptor 1a (FLVCR1a) knockout mouse model and show that, by sustaining heme synthesis, FLVCR1a contributes to determine the energy phenotype in skeletal muscle cells and to modulate satellite cell differentiation and muscle regeneration.

INTRODUCTION

Regulating metabolic pathways essential for muscle functions is emerging as a promising strategy to counteract skeletal muscle disease progression or to potentiate the innate ability of skeletal muscle to regenerate. Skeletal muscles display diverse metabolic compositions, matching energy needs with contractile demands. This diversity extends to satellite cells (SCs), a designated population of muscle stem cells,[1] highlighting the importance of mitochondrial metabolism in balancing their self-renewal and commitment during muscle recovery upon injury.[2]

Feline leukemia virus subgroup C receptor 1a (FLVCR1a) is a ubiquitously expressed membrane transporter. Traditionally recognized as a heme exporter,[3] recent works[4–7] proposed that it may alternatively/additionally import choline. Both heme and choline metabolism play pivotal roles in processes that provide energy to cells. Consequently, the modulation of FLVCR1a's function could potentially impact skeletal muscle energetic metabolism. Furthermore, previous studies have shown that FLVCR1a participates in a common functional axis with δ-aminolevulinic acid synthase 1 (ALAS1),[8–10] the rate-limiting enzyme for heme synthesis, thereby affecting metabolic pathways dependent on heme and/or interconnected with ALAS1, including the tricarboxylic acid (TCA) cycle.[8]

Here, by using skeletal-muscle-specific *Flvcr1a*-null mice, we demonstrate that FLVCR1a is a critical determinant of skeletal muscle metabolism required for proper muscle function and regeneration.

RESULTS

FLVCR1a loss in skeletal muscle reduces heme biosynthesis

To evaluate the impact of FLVCR1a loss in skeletal muscles, we generated skeletal-muscle-specific *Flvcr1a*-knockout (*Flvcr1a*^{KO}) animals (Figure S1A). As *Flvcr1a* is equally expressed in

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Magnetic-field-driven targeting of exosomes modulates immune and metabolic changes in dystrophic muscle

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Exosomes are promising therapeutics for tissue repair and regeneration to induce and guide appropriate immune responses in dystrophic pathologies. However, manipulating exosomes to control their biodistribution and targeting them in vivo to achieve adequate therapeutic benefits still poses a major challenge. Here we overcome this limitation by developing an externally controlled delivery system for primed annexin A1 myo-exosomes (Exo^{myo}). Effective nanocarriers are realized by immobilizing the Exo^{myo} onto ferromagnetic nanotubes to achieve controlled delivery and localization of Exo^{myo} to skeletal muscles by systemic injection using an external magnetic field. Quantitative muscle-level analyses revealed that macrophages dominate the uptake of Exo^{myo} from these ferromagnetic nanotubes in vivo to synergistically promote beneficial muscle responses in a murine animal model of Duchenne muscular dystrophy. Our findings provide insights into the development of exosome-based therapies for muscle diseases and, in general, highlight the formulation of effective functional nanocarriers aimed at optimizing exosome biodistribution.

Timely resolution of inflammation is necessary to restore muscle homeostasis following infection or damage and avoid chronic inflammatory pathologies, including muscular dystrophies. Macrophages are key players in this process due to their capacity to transition from a generally proinflammatory state to an anti-inflammatory phenotype. Engineered exosomes, extracellular vesicles <200 nm in size[1,2], can carry multiple signalling biomolecules, including pro-resolving immune mediators, such as annexin A1 (ANXA1), which has emerged as a key regulator of macrophage polarization[2,3]. Previous studies of exosome delivery for treatment of different forms of muscular dystrophy have had promising results[4–6], suggesting that accurate manipulation of exosomes can facilitate tissue repair during pathological processes. However, systemic biodistribution is challenging due to the influence of exosomal composition, in particular the lipid and protein content, on pharmacokinetics and bioavailability[2,7–9].

A potential solution to this issue is the use of exosome carriers that can be controlled by external stimuli for accurate targeting and delivery, allowing on-demand manipulation of the biodistribution of therapeutic exosomes[10]. We propose using ferromagnetic nanotubes (NT-MAGs) for magnetic-field-controlled exosome delivery and localization in vivo. The NT-MAGs are primed with surface-anchored ANXA1 myo-exosomes (Exo^{myo}) by exploiting a Coulombic interaction.

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RNA isolation and sequencing analysis

Macrophages and single muscle fibres were frozen immediately after sorting in liquid nitrogen and RNA extracted using an RNeasy Micro kit. RNAs were qualified and quantified on an Agilent Tapestation 2200 using a high-sensitivity RNA chip. To prepare the library, 150–300 ng of total RNA was reverse-transcribed using Illumina's TruSeq stranded mRNA library preparation kit. Each sample was fitted with one of 96 adapters containing a different 8 base molecular barcode for high-level multiplexing.

Libraries were sequenced on an Illumina NovaSeq 6000. To ensure quality, the FASTQ files were checked with FastQC. We determined transcript/gene abundance using salmon v.1.10.2 and a specific transcriptome index (GRCm38). Normalization using edgeR v.3.42.0 was based on the read count matrix. Genes were considered expressed if their raw counts were >20 in at least two out of three replicates per condition. We used ggplot2 v.3.4.4 for volcano plot analysis of the RNA-seq expression data. Fold-changes between groups were calculated using the Bioconductor package EdgeR with the likelihood ratio test (http://www.bioconductor.org/packages/release/bioc/html/edgeR.html). Genes were considered differentially expressed if |log(fold change)| ≥ 1.5 (ref. 60). Differentially expressed genes were submitted to clusterProfiler v.4.8.2 for GO analysis[61] in the GO Ontology database (v.2021-05-01). We used all genes expressed in the experiment and the GO terms 'biological processes', 'cellular components', 'molecular functions' as the annotation dataset. Significantly enriched GO terms were identified by an adjusted $P < 0.05$. GSEA was performed by dedicated software (release 4.2.3) in the Molecular Signatures Database (MsigDB). The 'Hallmark' annotated gene set collection was used for analysis of ranked gene lists. Data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE263457.

Statistical analysis and reproducibility

No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications[6,10]. The sample sizes and the specific statistical tests for each experiment are detailed in each figure caption. Statistical analysis was performed using Excel and GraphPad Prism. Statistical significance was set at $P < 0.05$.

Data availability

The MS data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier ftp://MSV000094590@massive.ucsd.edu. Transcriptomic data have been deposited in NCBI's Gene Expression Omnibus and are accessible via GEO Series accession number GSE263457. Source data are provided with this paper.

References

45. Soo, C. Y. et al. Nanoparticle tracking analysis monitors microvesicle and exosome secretion from immune cells. *Immunology* **136**, 192–197 (2012).
46. van der Pol, E. et al. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. *J. Thromb. Haemost.* **12**, 1182–1192 (2014).
47. Thery, C., Amigorena, S., Raposo, G. & Clayton, A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell Biol.* https://doi.org/10.1002/0471143030.cb0322s30 (2006).
48. Bari, E. et al. Pilot production of mesenchymal stem/stromal freeze-dried secretome for cell-free regenerative nanomedicine: a validated GMP-compliant process. *Cells* https://doi.org/10.3390/cells7110190 (2018).
49. Gessulat, S. et al. Prosit: proteome-wide prediction of peptide tandem mass spectra by deep learning. *Nat. Methods* **16**, 509–518 (2019).
50. Vizcaino, J. A. et al. 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res.* **44**, D447–D456 (2016).
51. Secchi, V. et al. Energy partitioning in multicomponent nanoscintillators for enhanced localized radiotherapy. *ACS Appl. Mater. Interfaces* **15**, 24693–24700 (2023).
52. De Luca, G. et al. Self-organizing functional materials via ionic self assembly: porphyrins H- and J-aggregates on synthetic chrysotile nanotubes. *J. Am. Chem. Soc.* **131**, 6920–6921 (2009).
53. Sun, S. & Zeng, H. Size-controlled synthesis of magnetite nanoparticles. *J. Am. Chem. Soc.* **124**, 8204–8205 (2002).
54. Dey, S., Mohanta, K. & Pal, A. J. Magnetic-field-assisted layer-by-layer electrostatic assembly of ferromagnetic nanoparticles. *Langmuir* **26**, 9627–9631 (2010).
55. Mauri, M. et al. Time domain nuclear magnetic resonance: a key complementary technique for the forensic differentiation of foam traces. *Anal. Methods* **5**, 4336–4344 (2013).
56. Mauri, M., Thomann, Y., Schneider, H. & Saalwächter, K. Spin-diffusion NMR at low field for the study of multiphase solids. *Solid State Nucl. Mag.* **34**, 125–141 (2008).
57. Farini, A. et al. Therapeutic potential of immunoproteasome inhibition in Duchenne muscular dystrophy. *Mol. Ther.* **24**, 1898–1912 (2016).
58. Graham, K. M. et al. Excessive collagen accumulation in dystrophic (mdx) respiratory musculature is independent of enhanced activation of the NF-κB pathway. *J. Neurol. Sci.* **294**, 43–50 (2010).
59. Pegoli, G., Lucini, F., Mozzetta, C. & Lanzuolo, C. Single myofiber isolation and culture from a murine model of Emery–Dreifuss muscular dystrophy in early post-natal development. *J. Vis. Exp.* https://doi.org/10.3791/61516 (2020).
60. Metsalu, T. & Vilo, J. ClustVis: a web tool for visualizing clustering of multivariate data using principal component analysis and heatmap. *Nucleic Acids Res.* **43**, W566–W570 (2015).
61. Gene Ontology Consortium. Gene Ontology Consortium: going forward. *Nucleic Acids Res.* **43**, D1049–D1056 (2015).

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Porphyromonas gingivalis fuels colorectal cancer through CHI3L1-mediated iNKT cell-driven immune evasion

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ABSTRACT

The interaction between the gut microbiota and invariant Natural Killer T (iNKT) cells plays a pivotal role in colorectal cancer (CRC). The pathobiont *Fusobacterium nucleatum* influences the anti-tumor functions of CRC-infiltrating iNKT cells. However, the impact of other bacteria associated with CRC, like *Porphyromonas gingivalis*, on their activation status remains unexplored. In this study, we demonstrate that mucosa-associated *P. gingivalis* induces a protumour phenotype in iNKT cells, subsequently influencing the composition of mononuclear-phagocyte cells within the tumor microenvironment. Mechanistically, *in vivo* and *in vitro* experiments showed that *P. gingivalis* reduces the cytotoxic functions of iNKT cells, hampering the iNKT cell lytic machinery through increased expression of chitinase 3-like-1 protein (CHI3L1). Neutralization of CHI3L1 effectively restores iNKT cell cytotoxic functions suggesting a therapeutic potential to reactivate iNKT cell-mediated antitumour immunity. In conclusion, our data demonstrate how *P. gingivalis* accelerates CRC progression by inducing the upregulation of CHI3L1 in iNKT cells, thus impairing their cytotoxic functions and promoting host tumor immune evasion.

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KEYWORDS
iNKT cells; CRC; *Porphyromonas gingivalis*; CHI3L1

Introduction

Colorectal cancer (CRC) is the third most prevalent cancer worldwide and the second leading cause of cancer-related death.[1] The mutational landscape and the mechanisms of tumor initiation in CRC have been widely described, but colon carcinogenesis also depends on the interaction between cancer cells and the tumor microenvironment (TME).[2] Indeed, the polarization and activation profiles of immune cells within the TME are highly informative to predict CRC patient survival or their response to therapy, highlighting the importance of the inflammatory microenvironment for CRC tumorigenesis.[2] Microbiota-elicited inflammation is an important contributor to CRC pathogenesis regardless of pre-cancer inflammatory history.[3] Pro-carcinogenic bacteria are able to initiate and promote colon cancer, partly through mechanisms that are not fully understood.[4] *Porphyromonas gingivalis* is an opportunistic oral pathogen associated with different inflammatory diseases and cancers[5,6] and specifically enriched in CRC patients.[7,8] *P. gingivalis* accelerates epithelial cell proliferation through the MAPK/ERK signaling pathway[9] and upregulates the expression of senescence and proinflammatory genes through the local production of butyrate.[10] Moreover, *P. gingivalis* promotes CRC immune subversion through activation of the hematopoietic NOD-like receptor protein 3 inflammasome in tumor-infiltrating myeloid cells.[11] Recently, we demonstrated that tumor-infiltrating invariant Natural Killer T (iNKT) cells favor a

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BRAIN COMMUNICATIONS

Investigating the prevalence of *MFN2* mutations in amyotrophic lateral sclerosis: insights from an Italian cohort

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The *MFN2* gene encodes mitofusin 2, a key protein for mitochondrial fusion, transport, maintenance and cell communication. *MFN2* mutations are primarily linked to Charcot–Marie–Tooth disease type 2A. However, a few cases of amyotrophic lateral sclerosis and amyotrophic lateral sclerosis/frontotemporal dementia phenotypes with concomitant *MFN2* mutations have been previously reported. This study examines the clinical and genetic characteristics of an Italian cohort of amyotrophic lateral sclerosis patients with rare, non-synonymous *MFN2* mutations. A group of patients ($n = 385$) diagnosed with amyotrophic lateral sclerosis at our Neurology Units between 2008 and 2023 underwent comprehensive molecular testing, including *MFN2*. After excluding pathogenic mutations in the main amyotrophic lateral sclerosis–related genes (i.e. *C9orf72*, *SOD1*, *FUS* and *TARDBP*), *MFN2* variants were classified based on the American College of Medical Genetics and Genomics guidelines, and demographic and clinical data of *MFN2*-mutated patients were retrieved. We identified 12 rare, heterozygous, non-synonymous *MFN2* variants in 19 individuals (4.9%). Eight of these variants, carried by nine patients (2.3%), were either pathogenic, likely pathogenic or variants of unknown significance according to the American College of Medical Genetics and Genomics guidelines. Among these patients, four exhibited a familial pattern of inheritance. The observed phenotypes included classic and bulbar amyotrophic lateral sclerosis, amyotrophic lateral sclerosis/frontotemporal dementia, flail arm, flail leg and progressive muscular atrophy. Median survival after disease onset was extremely variable, ranging from less than 1 to 13 years. This study investigates the prevalence of rare, non-synonymous *MFN2* variants within an Italian cohort of amyotrophic lateral sclerosis patients, who have been extensively investigated, enhancing our knowledge of the underlying phenotypic spectrum. Further research is needed to understand whether *MFN2* mutations contribute to motor neuron disease and to what extent. Improving our knowledge regarding the genetic basis of amyotrophic lateral sclerosis is crucial both in a diagnostic and therapeutic perspective.

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Modelling pathological spread through the structural connectome in the frontotemporal dementia clinical spectrum

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Abstract

The ability to predict the pathology spreading in patients with frontotemporal dementia (FTD) is crucial for early diagnosis and targeted interventions.

This study examined the relationship between network vulnerability and longitudinal atrophy progression in FTD patients, using Network Diffusion Model (NDM) of pathology spread.

Thirty behavioural-variant FTD (bvFTD), 13 semantic-variant primary progressive aphasia (svPPA), 14 nonfluent-variant PPA (nfvPPA) and 12 semantic behavioral variant FTD (sbvFTD) patients underwent longitudinal T1-weighted MRI. Fifty young controls (YC) (20-31 years) underwent multi-shell diffusion MRI scan. NDM was developed to model FTD pathology progression as a spreading process from a seed through the healthy structural connectome, using connectivity measures from fractional anisotropy (FA) and intra-cellular volume fraction (ICVF) in YC. Four disease epicenters were initially identified from the peaks of atrophy of each FTD variant: left insula (bvFTD), left temporal pole (svPPA), right temporal pole (sbvFTD) and left supplementary motor area (nfvPPA). Pearson's correlations were calculated between NDM-predicted atrophy in YC and the observed longitudinal atrophy in FTD patients over a follow-up of 24 months. The NDM was then run for all the 220 brain seeds to verify whether the four epicenters were among those that yielded the highest correlation.

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Running title: Network spread models of FTD spectrum

Keywords: Connectomics; frontotemporal dementia; network spreading

Abbreviations: behavioural-variant frontotemporal dementia (bvFTD); Clinical Dementia Rating (CDR); diffusion tensor (DT); fractional anisotropy (FA); frontotemporal lobar degeneration (FTLD); Grey matter (GM); intra-cellular volume fraction (ICVF); neurite orientation dispersion and density imaging (NODDI); Network Diffusion Model (NDM); nonfluent-variant primary progressive aphasia (nfvPPA); semantic-variant primary progressive aphasia (svPPA); semantic behavioral variant Frontotemporal Dementia (sbvFTD); regions of interest (ROIs).

Introduction

The most common neurodegenerative conditions are characterized by a pathological deposition of misfolded proteins throughout the central nervous system. This process is believed to proceed in mostly stereotyped patterns, as described by histopathological staging systems of Alzheimer's disease (AD),[1] Parkinson's disease,[2] frontotemporal dementia (FTD)[3] and amyotrophic lateral

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ORIGINAL ARTICLE

Psychometrics and diagnostics of the Italian version of the Alternate Verbal Fluency Battery (AVFB) in non-demented Parkinson's disease patients

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Abstract

Background Verbal fluency (VF) tasks are known as suitable for detecting cognitive impairment (CI) in Parkinson's disease (PD). This study thus aimed to evaluate the psychometrics and diagnostics of the Alternate Verbal Fluency Battery (AVFB) by Costa et al. (2014) in an Italian cohort of non-demented PD patients, as well as to derive disease-specific cut-offs for it.

Methods $N = 192$ non-demented PD patients were screened with the Montreal Cognitive Assessment (MoCA) and underwent the AVFB—which includes phonemic, semantic and alternate VF tests (PVF; SVF; AVF), as well as a Composite Shifting Index (CSI) reflecting the "cost" of shifting from a single- to a double-cued VF task. Construct validity and diagnostics were assessed for each AVFB measure against the MoCA. Internal reliability and factorial validity were also tested.

Results The MoCA proved to be strongly associated with PVF, SVF and AVF scores, whilst moderately with the CSI. The AVFB was internally consistent and underpinned by a single component; however, an improvement in both internal reliability and fit to its factorial structure was observed when dropping the CSI. Demographically adjusted scores on PVF, SVF and AVF tests were diagnostically sound in detecting MoCA-defined cognitive impairment, whilst this was not true for the CSI. Disease-specific cut-offs for PVF, SVF and AVF tests were derived.

Discussion In conclusion, PVF, SVF and AVF tests are reliable, valid and diagnostically sound instruments to detect cognitive impairment in non-demented PD patients and are therefore recommended for use in clinical practice and research.

Keywords Verbal fluency · Parkinson's disease · Language; Executive · Neuropsychology · Cognitive impairment

Background

Up to 40% of non-demented patients with Parkinson's disease (PD) present with dysexecutive-like, widespread cognitive impairment (CI) [1], which adversely affects their functional outcomes [2], prognosis [3, 4] and survival [5]. Therefore, the early detection of CI via clinimetrically sound tests is clinically crucial in this population [6].

Verbal fluency (VF) tests have been systematically found to be appropriate for this goal [7], as they capture both dysexecutive-inattentive features and lexical-semantic deficits that characterize PD [8] also in the early stages [9–11]. Indeed, in this population, VF measures have been successfully linked to those brain networks supporting both executive functions and language both in vivo [12–17] and at a neuropathological level [18]. Consistently, their utility has been proven either as individual screeners [9] or when included within second-level cognitive batteries [6]. Remarkably, VF measures have been also shown to be associated with patients' motor and functional outcomes [19–22] and are acknowledged as sensitive indices of post-deep brain stimulation CI [23]. In addition, since VF tests are brief and require only verbal responses, they are suitable for fatigable patients and they are not affected by upper-limb disabilities, making them highly feasible in PD [24].

As highlighted by the Movement Disorders Society (MDS) [25, 26], there is a need for disease-specific clinimetric studies that address those tests that have been historically shown to be appropriate for detecting CI in PD, as is the case for VF. Such investigations would increase their level of recommendation for use in clinical practice and research [27]. Indeed,

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BMC Neurology

RESEARCH Open Access

Reliable change indices for the Italian version of the Montreal Cognitive Assessment (MoCA) in non-demented Parkinson's disease patients

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Abstract

Background . The present study aimed at deriving regression-based reliable change indices (RCIs) for the Montreal Cognitive Assessment (MoCA) in an Italian cohort of non-demented Parkinson's disease (PD) patients.

Methods $N = 33$ consecutive, non-demented PD patients were followed-up at a 5-to-8-month interval ($M = 6.6$; $SD = 0.6$) with the MoCA. Practice effects and test-retest reliability were assessed *via* dependent-sample *t*-tests and intra-class correlation (ICC) coefficients, respectively. RCIs were derived separately for raw and demographically adjusted MoCA scores according to a standardized regression-based approach by accounting for both baseline confounders (i.e., demographics, disease duration and Unified Parkinson's Disease Rating Scale scores) and retest interval.

Results No practice effects were found ($t(32) = 0.29$; $p = .778$), with acceptable test-retest reliability being detected (ICC = 0.67). MoCA scores at T0 proved to be the only significant predictor of T1 MoCA performances within both the model addressing raw scores and that addressing adjusted scores ($ps < 0.001$).

Conclusions The present study provides Italian practitioners and researchers with regression-based RCIs for the MoCA in non-demented PD patients, which can be reliably adopted for retest interval ≥ 5 and ≤ 8 months without encountering any practice effect.

Keywords Reliable change index, Cognitive screening, Parkinson's disease, Montreal Cognitive Assessment, Psychometrics, Neuropsychology

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non-demented PD patients present with clinically meaningful changes on the MoCA or not at a 5-to-8 retest interval.

Finally, it should be borne in mind that this study is not devoid of limitation. First and foremost, the retest interval herewith addressed is much shorter than the ones typically encountered within the concerning literature (i.e., ~12 months) [8]. This of course does not allow for the application of the current RCIs to longer retest intervals and raises the need of further studies embracing different time spans. Second, the current sample is relatively restricted in size only include patients without dementia. Hence, further investigations are needed that address larger cohorts of PD patients stratified according to their cognitive phenotypes (i.e., normal cognition vs. mild cognitive impairment vs. dementia). Third, this study did not explore the longitudinal feasibility of each MoCA subscales/items: it is advisable that future reports aim at testing whether individual MoCA subscales/items are featured by different measurement properties over time in this population.

Future studies are then advisable that test the feasibility of currently available alternate forms of the Italian MoCA [27] for the longitudinal assessment of cognition in this population.

Conclusions

The present study provides Italian practitioners and researchers with SRB RCIs for the MoCA in non-demented PD patients, which can be reliably adopted for retest interval≥5 and ≤8 months without encountering any practice effect.

Abbreviations

H-Y	Modified Hoehn-Yahr's staging system
ICC	Intra-class correlation
MDS	Movement Disorders Society
MoCA	Montreal Cognitive Assessment
PD	Parkinson's disease
RCI	Reliable change index
SRB	Standardized regression-based
UPDRS	Unified Parkinson's Disease Rating Scale

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-024-03920-9.

Supplementary Material 1

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ENA: conceptualization, analyses, drafting, revision; FeSo, ST, RB, FrSc, EC, AM, MO: data collection, revision; GDL, BC: analyses, revision; CM, AD, LM, FV: resources, revision; RF, SB, FR, DM, ADS, AnMa, AP, GP: revision; VS, NT, AC: resources, revision; BP: resources, drafting, revision.

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Data availability

Datasets associated with the present study cannot be made publicly available as including sensitive information, but have been stored on an online repository (https://doi.org/10.5281/zenodo.13955544) and can be made available upon reasonable request of interested researchers to the Corresponding Author that will forward a data transfer agreement request to the relevant Ethical Committees.

Declarations

Competing interests

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References

1. Baiano C, Barone P, Trojano L, Santangelo G. Prevalence and clinical aspects of mild cognitive impairment in Parkinson's disease: a meta-analysis. Mov Disord. 2020;35(1):45–54.
2. Leroi I, McDonald K, Pantula H, Harbishettar V. Cognitive impairment in Parkinson disease: impact on quality of life, disability, and caregiver burden. J Geriatr Psychiatr Neurol. 2012;25(4):208–14.
3. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc. 2005;53(4):695–9.
4. Skorvanek M, Goldman JG, Jahanshahi M, Marras C, Rektorova I, Schmand B, et al. Global scales for cognitive screening in Parkinson's disease: Critique and recommendations. Mov Disord. 2018;33(2):208–18.
5. Biundo R, Weis L, Bostantjopoulou S, Stefanova E, Falup-Pecurariu C, Kramberger MG, et al. MMSE and MoCA in Parkinson's disease and dementia

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QSM-detected iron accumulation in the cerebellar gray matter is selectively associated with executive dysfunction in non-demented ALS patients

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Background: This study aimed to assess whether quantitative susceptibility imaging (QSM)-based measures of iron accumulation in the cerebellum predict cognitive and behavioral features in non-demented amyotrophic lateral sclerosis (ALS) patients.

Methods: A total of ALS patients underwent 3-T MRI and a clinical assessment using the ALS Functional Rating Scale-Revised (ALSFRS-R) and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS). Regression models were applied to each subscale of the cognitive section of the ECAS and the ECAS-Carer Interview to examine the effect of QSM-based measures in white and gray matter (WM; GM) of the cerebellum, separately for right, left, and bilateral cerebellar regions of interest (ROIs). These effects were compared to those of cerebellar volumetrics in WM/GM, right and left hemispheres while controlling for demographics, disease status, and total intracranial volume.

Results: Higher QSM measures of the cerebellar GM on the left, right, and bilateral sides significantly predicted ($ps \leq 0.003$) a greater number of errors on the executive functioning (EF) subscale of the ECAS (ECAS-EF). Moreover, higher GM-related, QSM measures of the cerebellum were associated with an increased probability of a below-cut-off performance on the ECAS-EF ($ps \leq 0.024$). No significant effects were observed for QSM measures of the cerebellar WM or for volumetric measures on the ECAS-EF. Other ECAS measures showed no significant effects. Bilateral QSM measures of the cerebellar GM also selectively predicted performance on backward digit span and social cognition tasks.

Discussion: Iron accumulation within the cerebellar GM, particularly in the cerebellar cortices, may be associated with executive functioning deficits in non-demented ALS patients. Therefore, QSM-based measures could be useful for identifying the neural correlates of extra-motor cognitive deficits in ALS patients.

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Longitudinal Feasibility of the Montreal Cognitive Assessment (MoCA) in Non-Demented ALS Patients

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Keywords
Reliable change index · Cognitive screening · Amyotrophic lateral sclerosis · Montreal cognitive assessment · Frontotemporal degeneration · Neuropsychology

Abstract
Introduction: The present study aimed at testing the longitudinal feasibility of the Montreal Cognitive Assessment (MoCA) in an Italian cohort of non-demented amyotrophic lateral sclerosis (ALS) patients. ***Methods:*** N = 39 non-demented ALS patients were followed-up at a 5-to-10-month interval (M = 6.8; SD = 1.4) with the MoCA and the Edinburgh Cognitive and Behavioral ALS Screen (ECAS). Practice effects, test-retest reliability, and predictive validity (against follow-up ECAS scores) were assessed. Reliable change indices (RCIs) were derived via a regression-based approach by accounting for retest interval and baseline confounders (i.e., demographics, disease duration, and severity and progression rate). ***Results:*** At retest, 100% and 69.2% of patients completed the ECAS and the MoCA, respectively. Patients who could not complete the MoCA showed a slightly more severe and fast-progressing disease. The MoCA was not subject to practice effects (t[32] = −0.80; p = 0.429) and was reliable at retest (intra-class correlation = 0.82). Moreover, baseline MoCA scores predicted the ECAS at retest. RCIs were successfully derived – with baseline MoCA scores being the only significant predictor of retest performances (ps < 0.001). ***Conclusions:*** As long as motor disabilities do not undermine its applicability, the MoCA appears to be longitudinally feasible at a 5-to-10-month

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ORIGINAL ARTICLE

Validity, diagnostics and feasibility of the Italian version of the Montreal Cognitive Assessment (MoCA) in Huntington's disease

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Abstract

Background This study is aimed at assessing the clinimetric properties and feasibility of the Italian version of the Montreal Cognitive Assessment (MoCA) in patients with Huntington's disease (HD).

Methods $N = 39$ motor-manifest HD patients, $N = 74$ Parkinson's disease (PD) patients and $N = 92$ matched HCs were administered the MoCA. HD patients further underwent the Unified Huntington's Disease Rating Scale (UHDRS), self-report questionnaires for anxiety and depression and a battery of first- and second-level cognitive tests. Construct validity was tested against cognitive and behavioural/psychiatric measures, whereas ecological validity against motor-functional subscales of the UHDRS. Sensitivity to disease severity was tested, via a logistic regression, by exploring whether the MoCA discriminated between patients in Shoulson-Fahn stage ≤ 2 *vs.* > 2. The same analysis was employed to test its ability to discriminate HD patients from HCs and PD patients.

Results The MoCA converged towards cognitive and behavioural measures but diverged from psychiatric ones, being also associated with motor/functional measures from the UHDRS. In identifying patients with cognitive impairment, adjusted MoCA scores were highly accurate (AUC = .92), yielding optimal diagnostics at the cut-off of < 19.945 ($J = .78$). The MoCA was able to discriminate patients in the middle-to-advanced from those in the early-to-middle stages of the disease ($p = .037$), as well as to differentiate HD patients from both HCs ($p < .001$) and PD patients ($p < .001$).

Conclusions The MoCA is a valid, diagnostically sound and feasible cognitive screener in motor-manifest HD patients, whose adoption is thus encouraged in clinical practice and research.

Keywords Montreal Cognitive Assessment · Huntington's disease · Cognitive screening · Dysexecutive · Diagnostics · Psychometrics

Introduction

Screening for cognitive dysfunctions in Huntington's disease (HD) patients is pivotal at both prognostic and interventional levels [1]. Moreover, cognitive screening measures are routinely employed as primary/secondary endpoints within epidemiological studies and clinical trials addressing this disorder [2, 3]. To such an aim, the Montreal Cognitive Assessment (MoCA) [4] has been listed amongst the "suggested" screeners by the Movement Disorders Society (MDS) [5], with recent meta-analytic evidence further availing its suitability for use in this population [6].

Nevertheless, it has been highlighted that disease-specific evidence on the diagnostic value of the MoCA in HD patients is seldom delivered—this similarly applying, albeit to a lesser extent, to its psychometrics (*e.g.* validity) and feasibility (*e.g.* its sensitivity to disease severity and its ability to discriminate this population from both normotypical individuals and patients with other brain disorders involving frontostriatal networks) [4–7]. Relevantly, the MDS itself has stressed out that such an unfortunate occurrence does lower the level of recommendation for a given cognitive

Edoardo Nicolò Aiello and Federica Solca contributed equally; Andrea Ciammola and Barbara Poletti contributed equally as well.

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ORIGINAL ARTICLE

Clinical usefulness of the Verbal Fluency Index (VFI) in amyotrophic lateral sclerosis

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Abstract

Background This study aimed at assessing the clinical utility of the Verbal Fluency Index (VFI) over a classical phonemic verbal fluency test in Italian-speaking amyotrophic lateral sclerosis (ALS) patients.

Methods $N = 343$ non-demented ALS patients and $N = 226$ healthy controls (HCs) were administered the *Verbal fluency – S* task from the Edinburgh Cognitive and Behavioural ALS Screen (ECAS). The associations between the number of words produced (NoW), the time to read words aloud (TRW) and the VFI (computed as [(60"-TRW)/NoW]) on one hand and both bulbar/respiratory scores from the ALS Functional Rating Scale – Revised (ALSFRS-R) and the ECAS-Executive on the other were tested. Italian norms for the NoW and the VFI were derived in HCs *via* the Equivalent Score method. Patients were classified based on their impaired/unimpaired performances on the NoW and the VFI (NoW-VFI-; NoW-VFI+; NoW + VFI-; NoW + VFI+), with these groups being compared on ECAS-Executive scores.

Results The VFI, but neither the NoW nor the TRW, were related to ALSFRS-Bulbar/-Respiratory scores; VFI and NoW measures, but not the TRW, were related to the ECAS-Executive ($p < .001$). The NoW slightly overestimated the number of executively impaired patients when compared to the VFI (31.1% vs. 26.8%, respectively). Patients with a defective VFI score – regardless of whether they presented or not with a below-cutoff NoW – reported worse ECAS-Executive scores than NoW + VFI + ones.

Conclusions The present reports support the use of the Italian VFI as a mean to validly assess ALS patients' executive status by limiting the effect of motor disabilities that might undermine their speech rate.

Keywords Amyotrophic lateral sclerosis · Frontotemporal degeneration · Neuropsychology · Verbal fluency · Executive functions

Background

Phonemic verbal fluency (PVF) tests have been historically acknowledged as sensitive markers of executive dysfunctions in amyotrophic lateral sclerosis (ALS) [1]. However, as requiring timed verbal responses, disease-specific versions of PVF tests had to be developed in order for them to be validly administered to this population net of their dysarthric features – which might slow down patients' speech rate and thus alter test results [2–4].

Such a goal has been pursued by developing the so-called Verbal Fluency Index (VFI) [1–4], computed by weighting on the number of words produced the difference between the time limit set by the test – usually 60" – and the time taken to read aloud the words spoken. This procedure is believed to yield a "pure" measure of PVF – i.e., the average "thinking time" *per* word – net of dysarthric features, which are believed to be covaried for *via* a "control condition" (i.e., reading aloud the words) measuring the extent to which patients' speech rate is slowed down [1–4].

However, whilst the use of the VFI to assess executive deficits in ALS is currently widespread [5], no large-scaled study to date has provided objective evidence on the actual need for the abovementioned control condition – and, thus,

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17. Aiello EN, Depaoli EG (2022) Norms and standardizations in neuropsychology via equivalent scores: software solutions and practical guides. Neurol Sci 43:961–966
18. Poletti B, Aiello EN, La Tona A, Solca F, Torre S, Colombo E et al (2023) Single task-level, 2SD-based cutoffs for the Italian version of the Edinburgh cognitive and behavioral ALS screen (ECAS). Amyotroph Lateral Scler Frontotemporal Degener 24(7–8):709–712
19. Siciliano M, Trojano L, Trojsi F, Greco R, Santoro M, Basile G et al (2017) Edinburgh cognitive and behavioural ALS screen (ECAS)-Italian version: regression based norms and equivalent scores. Neurol Sci 38:1059–1068
20. Poletti B, Carelli L, Solca F, Lafronza A, Pedroli E, Faini A et al (2017) An eye-tracking controlled neuropsychological battery for cognitive assessment in neurological diseases. Neurol Sci 38:595–603
21. Poletti B, Carelli L, Faini A, Solca F, Meriggi P, Lafronza A et al (2018) The Arrows and colors cognitive test (ACCT): a new verbal-motor free cognitive measure for executive functions in ALS. PLoS ONE 13(8):e0200953
22. Carelli L, Solca F, Migliore S, Torre S, Brugnera A, Mancini F et al (2021) Compensating for verbal-motor deficits in neuropsychological assessment in movement disorders: sensitivity and specificity of the ECAS in Parkinson's and Huntington's diseases. Neurol Sci 42:4997–5006

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ORIGINAL ARTICLE

Frontotemporal-spectrum disorders and functional independence in non-demented ALS patients

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Abstract

Background The present study aimed at determining whether, net of motor confounders, neuropsychological features affect functional independence (FI) in activities of daily living (ADLs) in non-demented amyotrophic lateral sclerosis (ALS) patients.

Methods $N = 88$ ALS patients without frontotemporal dementia were assessed for FI—Katz's Basic ADL Scale (BADL) and Lawton-Brody's Instrumental ADL Scale (IADL)—, cognition—Edinburgh Cognitive and Behavioural ALS Screen (ECAS)—and behaviour—Beaumont Behavioural Inventory and Dimensional Apathy Scale. The association between cognitive and behavioural measures and BADL/IADL scores was assessed by covarying for demographics, anxiety and depression levels, disease duration and motor confounders—i.e. ALS Functional Rating Scale-Revised (ALSFRS-R) scores, progression rate and both King's and Milano-Torino stages.

Results Higher scores on the ECAS-Language were associated with higher IADL scores ($p = 0.005$), whilst higher apathetic features—as measured by the Dimensional Apathy Scale (DAS)—were inversely related to the BADL ($p = 0.003$). Whilst IADL scores were related to all ECAS-Language tasks, the DAS-Initiation was the only subscale associated with BADL scores. Patients with abnormal ECAS-Language ($p = 0.023$) and DAS ($p = 0.008$) scores were more functionally dependent than those without.

Discussion Among non-motor features, language changes and apathetic features detrimentally affect FI in non-demented ALS patients.

Keywords Amyotrophic lateral sclerosis · Activities of daily living · Neuropsychology · Functional independence · Frontotemporal degeneration

Background

Frontotemporal-spectrum disorders (FTSDs) are acknowledged to detrimentally affect survival in non-demented amyotrophic lateral sclerosis (ALS) patients [1] by interfering with decision-making and adherence within care settings [2, 3].

However, little is known on the extent to which neuropsychological features impact on patients' functional independence (FI) in daily living—likely due to their physical disabilities representing a major confounder to the study of such a matter [4, 5]. Only two reports have indeed to this day addressed this topic—the first, by Mioshi et al. [4], showing that FI was dependent on both motor and behavioural features, and the second, by Kapustin et al. [5], failing to detect an association between cognitive/behavioural features and FI net of ALS severity. However, these studies either preceded the availability of [4], or did not employ [5], ALS-specific cognitive/behavioural measures [6]. Moreover, the only study [5] having explored the association between FI and a performance-based measure of cognition did not provide single domain-level information.

The above being said, assessing how neuropsychological features impact FI in both basic and instrumental activities of daily living (ADL) in this population is prognostically pivotal, as it would shed further light on the ecological relevance of FTSDs in ALS besides their already acknowledged impact on survival [1, 2]. Hence, by employing a

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IADL Questionnaire©, a new tool to measure instrumental activities of daily living in dementia. Neuroepidemiology 41:35–41
59. Sikkes SA, Pijnenburg YA, Knol DL, de Lange-de Klerk ES, Scheltens P, Uitdehaag BM (2013) Assessment of instrumental activities of daily living in dementia: diagnostic value of the Amsterdam Instrumental Activities of Daily Living Questionnaire. J Geriatr Psychiatry Neurol 26:244–250
60. Koster N, Knol DL, Uitdehaag BM, Scheltens P, Sikkes SA (2015) The sensitivity to change over time of the Amsterdam IADL Questionnaire©. Alzheimers Dement 11:1231–1240
61. Jutten RJ, Peeters CF, Leijdesdorff SM et al (2017) Detecting functional decline from normal aging to dementia: development and validation of a short version of the Amsterdam IADL Questionnaire. Alzheimers Dement 8:26–35
62. McMillan CT, Wuu J, Rascovsky K et al (2022) Defining cognitive impairment in amyotrophic lateral sclerosis: an evaluation of empirical approaches. Amyotroph Lateral Scler Frontotemporal Degener 23:517–526
63. Aiello EN, Iazzolino B, Pain D et al (2022) The diagnostic value of the Italian version of the Edinburgh cognitive and behavioral ALS screen (ECAS). Amyotroph Lateral Scler Frontotemporal Degener 23:527–531
64. Aiello EN, Solca F, Torre S et al (2023) Reliable change indices for the Italian Edinburgh cognitive and behavioral ALS screen (ECAS). Amyotroph Lateral Scler Frontotemporal Degener 24:339–342

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Article

Behavioral Disorders of Spatial Cognition in Patients with Mild Cognitive Impairment Due to Alzheimer's Disease (The BDSC-MCI Project): Ecological Validity of the Corsi Learning Suvra-Span Test

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Abstract: Background: Spatial navigation deficits are reported as early symptoms of Alzheimer's disease (AD) alongside episodic memory ones. The aim of the present study was to ascertain whether neuropsychological deficits of visuospatial long-term memory can predict behavioral alterations during the navigation of older adults in novel urban environments along the normal aging–dementia continuum of the Alzheimer's type. Methods: A total of 24 community-dwelling patients with Mild Cognitive Impairment (MCI) due to AD, 27 individuals with subjective cognitive decline (SCD), and 21 healthy controls were assessed in terms of their sequential egocentric and allocentric navigation abilities by using a modified version of the Detour Navigation Test, and neuropsychologically tested by the Corsi learning suvra-span (CLSS) test. Generalized linear models were adopted to verify whether the scores obtained by the three groups in the CLSS test predicted wrong turns and moments of hesitation during the navigation task, with the results presented as topographical disorientation scores. Results: Higher scores in the CLSS test predicted fewer wrong turns ($b = -0.05$; $z = -2.91$; $p = 0.004$; net of between-groups differences) and moments of hesitation for patients with MCI due to AD ($b = -0.14$; $z = -2.43$; $p = 0.015$), and individuals with SCD ($b = -0.17$; $z = -3.85$; $p < 0.001$). Conclusions: Since the CLSS test has been reported to be a reliable measure of ecological navigational abilities in the progression towards AD dementia, we recommend its use in clinical practice and highlight implications for future research.

Keywords: Alzheimer's disease; MCI due to AD; spatial cognition; Corsi suvra-span learning

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Article

Behavioral Disorders of Spatial Cognition in Patients with Mild Cognitive Impairment due to Alzheimer's Disease: Preliminary Findings from the BDSC-MCI Project

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Abstract: (1) Background: Spatial cognition (SC) is one of the earliest cognitive domains to be impaired in the course of Alzheimer's disease (AD), resulting in spatial disorientation and becoming lost even in familiar surroundings as later dementia symptoms. To date, few studies have identified initial alterations of spatial navigation (SN) in the premorbid AD phase by real-world paradigms, and none have adopted an innovative technological apparatus to better detect gait alterations as well as physiological aspects correlated to spatial disorientation (SD). The present study aimed at exploring initial SN defects in patients with prodromal AD via a naturalistic task by using a sensory garment. (2) Methods: 20 community-dwelling patients with Mild Cognitive Impairment (MCI) due to AD and 20 age/education controls were assessed on their sequential egocentric and allocentric navigation abilities by using a modified version of the Detour Navigation Test (DNT-mv). (3) Results: When compared to controls, patients with MCI due to AD exhibited higher wrong turns (WT) and moments of hesitation (MsH) in the DNT-mv, reflecting difficulties both in sequential egocentric and allocentric navigation, depending on hippocampal deterioration. Moreover, they reported more complaints about their SN competencies and lower long-term visuospatial memory abilities than controls. Remarkably, WTs and MsH manifested in the allocentric naturalistic task of the DNT-mv were associated with autonomic nervous system alteration pertaining to cardiac functioning in the whole sample. (4) Conclusions: Naturalistic navigation tests of hippocampal function using a continuous non-invasive monitoring device can provide early markers of spatial disorientation in patients with MCI due to AD. Future studies should develop cognitive remediation techniques able to enhance SC residual abilities in patients at high risk of conversion into dementia and ecological paradigms to be replicated on a large scale.

Keywords: spatial disorientation; mild cognitive impairment; Alzheimer's disease; wearable technology; hippocampus

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Article

Behavioral Alterations of Spatial Cognition and Role of the Apolipoprotein E-ε4 in Patients with MCI Due to Alzheimer's Disease: Results from the BDSC-MCI Project

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Abstract: Background: Beyond memory deterioration, spatial disorientation may occur along the continuum of normal aging—dementia of Alzheimer's type. The present study aims at detecting behavioral disorders of spatial cognition in prodromal Alzheimer's disease (AD) and verifying the association between Apolipoprotein E-ε4 (ApoE-ε4) genotype and gait patterns during a real-world naturalistic task. **Methods**: A sample of 58 elderly participants, of which 20 patients with mild cognitive impairment with CFS biomarker evidence of AD, 23 individuals with subjective cognitive decline (SCD), and 15 healthy controls (HCs), was tested by a modified version of the Detour Navigation Test (DNT-mv). Generalized linear models were run to explore the association between group belonging and wrong turns (WTs)/moments of hesitation (MsH) as behavioral disorientation scores of the DNT-mv as well as the effect of ApoE-ε4 genotype on time and walking speed registered by a smartphone app providing GPS tracking of body movement around urban environments. **Results**: Patients with MCI due to AD reported more WTs than individuals with SCD and HCs. Further, the ApoE-ε4 genotype determined a lower capacity in spatial information processing, influencing gait during naturalistic spatial navigation tasks. **Conclusions**: Behavior alterations of spatial cognition can be detected ecologically in prodromal AD. The use of technological solutions supporting gait analysis may help in corroborating the experimental observation.

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Research paper

Modeling of TDP-43 proteinopathy by chronic oxidative stress identifies rapamycin as beneficial in ALS patient-derived 2D and 3D iPSC models

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterized neuropathologically by TDP-43 proteinopathy with loss of TDP-43 nuclear splicing activity and formation of cytoplasmic TDP-43 aggregates. The lack of suitable experimental models of TDP-43 proteinopathy has hampered the discovery of effective therapies. We already showed that chronic and mild oxidative insult by sodium arsenite (ARS) triggered TDP-43 cytoplasmic aggregation and stress granules (SGs) formation in ALS patient-derived fibroblasts and motor neurons differentiated from induced pluripotent stem cells (iPSC-MNs). However, whether this insult induces a reduction of TDP-43 splicing activity in the nucleus, thus recapitulating both gain and loss of function pathomechanisms, still remains to be determined.

In this study we first showed that chronic ARS in human neuroblastoma cells triggered TDP-43 cytoplasmic mislocalization, SGs formation and defective splicing of TDP-43 target genes *UNC13A* and *POLDIP3* as functional readouts of TDP-43 proteinopathy. Additionally, a dysregulation of autophagy and senescence markers was observed in this condition. In a preliminary drug screening approach with autophagy-promoting drugs, namely rapamycin, lithium carbonate and metformin, only rapamycin prevented ARS-induced loss of TDP-43 splicing activity. We then demonstrated that, in addition to TDP-43 cytoplasmic aggregation, chronic ARS triggered TDP-43 loss of splicing activity also in ALS patient-derived primary fibroblasts and iPSC-MNs and that rapamycin was beneficial to reduce these TDP-43 pathological features. By switching to a neuro-glial 3D *in vitro* model, we observed that treatment of ALS iPSC-brain organoids with chronic ARS also induced a defective TDP-43 splicing activity which was prevented by rapamycin.

Collectively, we established different human cell models of TDP-43 proteinopathy which recapitulate TDP-43 gain and loss of function, prevented by rapamycin administration. Human neuroblastoma cells and patient-derived fibroblasts and 2D- and 3D-iPSC models exposed to chronic oxidative stress represent therefore suitable *in vitro* platforms for future drug screening approaches in ALS.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a fatal adult-onset neurodegenerative disorder affecting upper and/or lower motor neurons (MNs) and leading to progressive muscular atrophy and death by respiratory arrest within 2-3 years from disease onset, with no effective

Abbreviations: ALS, Amyotrophic Lateral Sclerosis; ARS, Sodium arsenite; BSA, Bovine serum albumin; CE, Cryptic exon; DAPI, 4′6-diamidino-2-phenylindole; DMSO, Dimethyl sulfoxide; EBs, Embryoid bodies; fALS, Familial amyotrophic lateral sclerosis; FBS, Fetal bovine serum; iPSCs, Induced Pluripotent Stem Cells; mTOR, Mammalian target of rapamycin; MNs, Motor neurons; m*C9orf72*, Mutated *C9orf72*; NGS, Normal goat serum; O/N, Overnight; P-TDP-43, Phosphorylated TDP-43; PBS, Phosphate Buffered Saline; RT, Room Temperature; sALS, Sporadic amyotrophic lateral sclerosis; SGs, Stress granules; TBS, Tris Buffered Saline; TDP-43, TAR DNA-binding protein 43; WB, Western Blot.

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Quantification of serum TDP-43 and neurofilament light chain in patients with amyotrophic lateral sclerosis stratified by *UNC13A* genotype

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative condition affecting upper and/or lower motor neurons and characterized neuropathologically by TDP-43 proteinopathy. Given its role in ALS pathobiology, it is currently under debate whether TDP-43 might represent a suitable ALS biomarker to be measured in patients' biofluids. The rs12608932 A > C single nucleotide polymorphism in the *UNC13A* gene is a risk factor for ALS and patients homozygous for the high-risk C allele display a higher burden of TDP-43 neuropathology than homozygotes for the low-risk A allele, although the association with TDP-43 levels in biofluids has never been evaluated.

In this study, we measured serum levels of TDP-43 and neurofilament light chain (NFL) by Simoa technology in a cohort of 69 ALS patients stratified according to the *UNC13A* rs12608932 genotype compared to 43 neurologically healthy controls.

By multiple linear regression analysis, serum TDP-43 was significantly elevated in ALS patients compared to controls, with *UNC13A* AA and AC, but not CC, ALS patients showing higher serum TDP-43 levels than controls. We also confirmed that serum NFL concentration was increased in ALS patients, without any correlation with the *UNC13A* genotype.

Our results indicate that serum TDP-43 is higher in ALS patients compared to controls and that, in contrast to NFL, this increase is specifically associated with the *UNC13A* rs12608932 AA and AC genotypes, but not with the high-risk CC genotype. Studies in larger cohorts will be needed to confirm these findings and to elucidate the biological link between serum TDP-43 levels and *UNC13A* genotype.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a rare and fatal neurodegenerative disease selectively affecting upper and/or lower motor neurons, causing progressive muscular paralysis and death within 3-5 years from symptom onset, usually due to respiratory failure [1]. Most ALS cases (90 %) are classified as sporadic (sALS), while in 10 % of cases the etiology is familial (fALS). Sub-clinical cognitive and behavioural alterations are recognized in 40 % of patients and 10 % of them also develop frontotemporal dementia (FTD), which shares clinical, genetic and neuropathological features with ALS, thus forming a disease *continuum* [2]. Neuropathologically, ALS and FTD are TDP-43 proteinopathies where the main hallmark, observed in 97 % of ALS and in 45 % of FTD cases regardless of their sporadic or familial etiology, consists in the accumulation of the ubiquitinated and phosphorylated RNA-binding protein TDP-43 in the cytoplasm of affected neurons, accompanied by a

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ORIGINAL COMMUNICATION

How to detect affect recognition alterations in amyotrophic lateral sclerosis

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Abstract

Objective To define the clinical usability of an affect recognition (AR) battery—the Comprehensive Affect Testing System (CATS)—in an Italian sample of patients with amyotrophic lateral sclerosis (ALS).

Methods 96 ALS patients and 116 healthy controls underwent a neuropsychological assessment including the AR subtests of the abbreviated version of the CATS (CATS-A). CATS-A AR subtests and their global score (CATS-A AR Quotient, ARQ) were assessed for their factorial, convergent, and divergent validity. The diagnostic accuracy of each CATS-A AR measure in discriminating ALS patients with cognitive impairment from cognitively normal controls and patients was tested via receiver-operating characteristics analyses. Optimal cut-offs were identified for CATS-A AR measures yielding an acceptable AUC value (≥ .70). The ability of CATS-A ARQ to discriminate between different ALS cognitive phenotypes was also tested. Gray-matter (GM) volumes of controls, ALS with normal (ALS-nARQ), and impaired ARQ score (ALS-iARQ) were compared using ANCOVA models.

Results CATS-A AR subtests and ARQ proved to have moderate-to-strong convergent and divergent validity. Almost all considered CATS-A measures reached acceptable accuracy and diagnostic power (AUC range = .79–.83). ARQ showed to be the best diagnostic measure (sensitivity = .80; specificity = .75) and discriminated between different ALS cognitive phenotypes. Compared to ALS-nARQ, ALS-iARQ patients showed reduced GM volumes in the right anterior cingulate, right middle frontal, left inferior temporal, and superior occipital regions.

Conclusions The AR subtests of the CATS-A, and in particular the CATS-A ARQ, are sound measures of AR in ALS. AR deficits may be a valid marker of frontotemporal involvement in these patients.

Keywords Amyotrophic lateral sclerosis · Comprehensive Affect Testing System · Emotion recognition · Social cognition · Gray matter volumes

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Medical Information Extraction with NLP-Powered QABots: a Real-World Scenario

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Transcranial static magnetic stimulation for amyotrophic lateral sclerosis: a bicentric, randomised, double-blind placebo-controlled phase 2 trial

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Summary

Background Enhanced glutamatergic transmission leading to motor neuron death is considered the major pathophysiological mechanism of amyotrophic lateral sclerosis (ALS). Motor cortex excitability can be suppressed by transcranial static magnetic stimulation (tSMS), thus tSMS can be evaluated as a potential treatment for ALS. The aim of present study was to investigate the efficacy and safety of tSMS in ALS.

Methods In this phase 2 trial, we randomly assigned ALS patients to receive daily tSMS or placebo stimulation over a period of 6 months. For each participant we calculated mean disease monthly progression rate (MPR) as the variation of the total ALS Functional Rating Scale-Revised (ALSRFS-R) score, before the beginning of the treatment (over a period of at least three months) and over the six-month treatment period. The primary efficacy outcome was the difference in MPR before and after the beginning of treatment. Secondary outcomes included safety and tolerability, compliance, and changes in corticospinal output. A long-term follow-up of 18 months was performed in all patients who completed the six-month treatment considering a composite endpoint event (tracheostomy or death). Trial registered at ClinicalTrials.gov, ID: NCT04393467, status: closed.

Findings Forty participants were randomly assigned to real (n = 21) or placebo stimulation (n = 19). Thirty-two participants (18 real and 14 placebo) completed the 6-month treatment. The MPR did not show statistically significant differences between the two arms during the pre-treatment (mean ± Standard deviation; Real: 1.02 ± 0.62, Sham: 1.02 ± 0.57, p-value = 1.00) and treatment period (Real: 0.90 ± 0.55, Sham: 0.94 ± 0.55, p-value = 0.83). Results for secondary clinical endpoints showed that the treatment is feasible and safe, being compliance with tSMS high. The change in corticospinal output did not differ significantly between the two groups. At the end of the long-term follow-up of 18 months, patients of real group had a statistically significant higher tracheostomy-free survival compared with patients of placebo group (Hazard Ratio = 0.27 95% Confidence interval 0.09–0.80, p-value = 0.019).

Interpretation tSMS did not modify disease progression during the 6 months of treatment. However, long-term follow-up revealed a substantial increase in tracheostomy free survival in patients treated with real stimulation supporting the evaluation of tSMS in larger and more prolonged studies.

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Original Empirical Article

Early Detection of Depression in Parkinson's Disease: Psychometrics and Diagnostics of the Spanish Version of the Beck Depression Inventory

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ABSTRACT

Objective: Depression is one of the most disabling non-motor symptoms in Parkinson's disease (PD) and requires proper diagnosis as it negatively impacts patients' and their relatives quality of life. The present study aimed to examine the psychometric and diagnostic properties of the Beck Depression Inventory-I (BDI-I) in a Spanish PD cohort.

Method: Consecutive PD outpatients completed the Spanish version of the BDI-I and other questionnaires assessing anxiety and apathy. Patients' caregivers completed the depression/dysphoria domain of the Neuropsychiatric Inventory (NPI-D). The internal consistency, convergent and divergent validity and the factorial structure of BDI-I were evaluated, and an optimal cut-off was defined by means of the Youden index.

Results: The BDI-I proved to have a good internal consistency and was underpinned by a mono-component structure. Regarding construct validity, the BDI-I was substantially related to anxiety and apathy measures in PD. Furthermore, the BDI-I overall showed good accuracy with adequate sensitivity and specificity. The optimal cut-off point was defined at 10.

Conclusions: We provided evidence of the psychometric and diagnostic properties of the Spanish version of the BDI-I as a screening tool for depression in Spanish speaking PD patients, suggesting its usefulness in clinical research and practice.

Keywords: Depression; Parkinson's disease; Assessment; Norms/normative studies

INTRODUCTION

Depression is one of the most common non-motor symptoms in Parkinson's disease (PD), with an average prevalence of 22.9% (Goodarzi et al., 2016), with great impact on quality of life (QoL) (Balestrino & Martinez-Martin, 2017). In recent years, the occurrence of depression in Spain has become an important problem of public health, which is the cause of heavy government healthcare spending. A review by Cardila and colleagues (2015) estimated that the prevalence rate for depression in the general population in Spain was 8.56% while, for Spanish PD patients, the prevalence of depression was 32.63% (Chuquilín-Arista et al., 2020). Accordingly, depressive symptoms should be identified early in PD patients to provide timely interventions (e.g., medication changes or psychotherapeutic support).

The Beck Depression Inventory-I (BDI-I) (Beck & Steer, 1987) is among the most widely used questionnaires to assess the occurrence and the severity of self-reported depressive symptoms in both research and clinical settings. The BDI-I, like other depression scales, includes somatic or movement-related and sexual activities often diminished or reduced by the disease itself, which could decrease the psychometric properties of the

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BRIEF COMMUNICATION

Emotional awareness in patients with amyotrophic lateral sclerosis

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Abstract

Introduction It has been recently acknowledged that deficits in experiencing and processing one's own emotions, also termed alexithymia, may possibly feature the frontotemporal-spectrum disorders. This study aims to determine whether alexithymia could be included within the frontotemporal syndromes of amyotrophic lateral sclerosis (ALS).

Methods Alexithymic traits were estimated in a cohort of 68 non-demented ALS patients with the 20-item Toronto Alexithymia Scale (TAS-20). Patients were assessed for the identification of motor-phenotypes and frontotemporal syndromes based on current classification criteria. Spearman's coefficients explored the correlates of TAS-20 measures with motor-functional profiles, global cognitive, social-cognitive (emotion recognition and empathy) and behavioral status.

Results Abnormal TAS-20 scores were found in 13% of patients, and their distribution did not vary within motor and frontotemporal phenotypes. Significant associations were detected between TAS-20 and executive ($p \leq .011$), memory ($p = .006$), state-anxiety ($p \leq .013$) and depression measures ($p \leq .010$). By contrast, TAS-20 scores were unrelated to social-cognitive performances, dysexecutive and apathetic profiles. Disease duration was the only motor-functional feature being related to the TAS-20 ($p \leq .008$).

Conclusions Alexithymia of potential clinical relevance occur in a minority of ALS patients, and its neuropsychological correlates mostly resemble those featuring the general population. Hence, it is unlikely that alexithymia is a specific feature of frontotemporal-*spectrum* characterizing ALS, rather it could be an expression of psychogenic factors as a reaction to the disease.

Keywords Alexithymia · Amyotrophic lateral sclerosis · Frontotemporal degeneration · Neuropsychology · Social cognition

Introduction

It has been recently acknowledged that deficits in experiencing and processing one's own emotions, also termed alexithymia, may possibly feature the frontotemporal-spectrum disorders of amyotrophic lateral sclerosis (ALS) patients [1, 2]. Current evidence suggests that alexithymic traits in ALS are linked to cortical alterations within frontotemporal networks

Barbara Poletti and Edoardo Nicolò Aiello contributed equally to this work.

The submitted work is original and it hasn't been published elsewhere in any form or language.

The data that support the findings of this study are available in a repository of the IRCCS Fondazione Istituto Neurologico "Carlo Besta" at https://doi.org/10.5281/zenodo.10040815.

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ORIGINAL COMMUNICATION

Exploiting the role of CSF NfL, CHIT1, and miR-181b as potential diagnostic and prognostic biomarkers for ALS

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Abstract

Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder characterized by relentless and progressive loss of motor neurons. A molecular diagnosis, supported by the identification of specific biomarkers, might promote the definition of multiple biological subtypes of ALS, improving patient stratification and providing prognostic information. Here, we investigated the levels of neurofilament light chain (NfL), chitotriosidase (CHIT1) and microRNA-181b (miR-181b) in the cerebrospinal fluid (CSF) of ALS subjects ($N=210$) as well as neurologically healthy and neurological disease controls ($N=218$, including $N=74$ with other neurodegenerative diseases) from a large European multicentric cohort, evaluating their specific or combined utility as diagnostic and prognostic biomarkers. NfL, CHIT1 and miR-181b all showed significantly higher levels in ALS subjects compared to controls, with NfL showing the most effective diagnostic performance. Importantly, all three biomarkers were increased compared to neurodegenerative disease controls and, specifically, to patients with Alzheimer's disease (AD; $N=44$), with NfL and CHIT1 being also higher in ALS than in alpha-synucleinopathies ($N=22$). Notably, ALS patients displayed increased CHIT1 levels despite having, compared to controls, a higher prevalence of a polymorphism lowering CHIT1 expression. While no relationship was found between CSF miR-181b and clinical measures in ALS (disease duration, functional disability, and disease progression rate), CSF NfL was the best independent predictor of disease progression and survival. This study deepens our knowledge of ALS biomarkers, highlighting the relative specificity of CHIT1 for ALS among neurodegenerative diseases and appraising the potential diagnostic utility of CSF miR-181b.

Keywords ALS · CSF · Biomarker · NfL · CHIT1 · MiR-181b

Delia Gagliardi and Mafalda Rizzuti have shared Co-first authorship.

Stefania Corti and Federico Verde have shared Co-last authorship.

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ORIGINAL COMMUNICATION

The value of routine blood work-up in clinical stratification and prognosis of patients with amyotrophic lateral sclerosis

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Abstract

Background There is an unmet need in amyotrophic lateral sclerosis (ALS) to provide specific biomarkers for the disease. Due to their easy availability, we aimed to investigate whether routine blood parameters provide useful clues for phenotypic classification and disease prognosis.

Methods We analyzed a large inpatient cohort of 836 ALS patients who underwent deep phenotyping with evaluation of the clinical and neurophysiological burden of upper (UMN) and lower (LMN) motor neuron signs. Disability and progression rate were measured through the revised ALS Functional Rating Scale (ALSFRS-R) and its changes during time. Cox regression analysis was performed to assess survival associations.

Results Creatinine significantly correlated with LMN damage ($r=0.38$), active ($r=0.18$) and chronic ($r=0.24$) denervation and baseline ALSFRS-R ($r=0.33$). Creatine kinase (CK), alanine (ALT) and aspartate (AST) transaminases correlated with active ($r=0.35$, $r=0.27$, $r=0.24$) and chronic ($r=0.37$, $r=0.20$, $r=0.19$) denervation, while albumin and C-reactive protein significantly correlated with LMN score ($r=0.20$ and $r=0.17$). Disease progression rate showed correlations with chloride ($r=-0.19$) and potassium levels ($r=-0.16$). After adjustment for known prognostic factors, total protein [HR 0.70 (95% CI 0.57–0.86)], creatinine [HR 0.86 (95% CI 0.81–0.92)], chloride [HR 0.95 (95% CI 0.92–0.99)], lactate dehydrogenase [HR 0.99 (95% CI 0.99–0.99)], and AST [HR 1.02 (95% CI 1.01–1.02)] were independently associated with survival.

Conclusions Creatinine is a reliable biomarker for ALS, associated with clinical features, disability and survival. Markers of nutrition/inflammation may offer additional prognostic information and partially correlate with clinical features. AST and chloride could further assist in predicting progression rate and survival.

Keywords Amyotrophic lateral sclerosis · Blood · Biomarkers · Survival

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ORIGINAL COMMUNICATION

Clinical and neuroanatomical characterization of the semantic behavioral variant of frontotemporal dementia in a multicenter Italian cohort

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Abstract

Background Semantic behavioral variant frontotemporal dementia (sbvFTD) is a neurodegenerative condition presenting with specific behavioral and semantic derangements and predominant atrophy of the right anterior temporal lobe (ATL). The objective was to evaluate clinical, neuropsychological, neuroimaging, and genetic features of an Italian sbvFTD cohort, defined according to recently proposed guidelines, compared to semantic variant primary progressive aphasia (svPPA) and behavioral variant FTD (bvFTD) patients.

Methods Fifteen sbvFTD, sixty-three bvFTD, and twenty-five svPPA patients and forty controls were enrolled. Patients underwent clinical, cognitive evaluations, and brain MRI. Symptoms of bvFTD patients between onset and first visit were retrospectively recorded and classified as early and late. Grey matter atrophy was investigated using voxel-based morphometry.

Results sbvFTD experienced early criteria-specific symptoms: world, object and person-specific semantic loss (67%), complex compulsions and rigid thought (60%). Sequentially, more behavioral symptoms emerged (apathy/inertia, loss of empathy) along with non-criteria-specific symptoms (anxiety, suspiciousness). sbvFTD showed sparing of attentive/executive functions, especially compared to bvFTD and better language functions compared to svPPA. All sbvFTD patients failed at the famous face recognition test and more than 80% failed in understanding written metaphors and humor. At MRI, sbvFTD had predominant right ATL atrophy, almost specular to svPPA. Three sbvFTD patients presented pathogenic genetic variants.

Conclusion We replicated the application of sbvFTD diagnostic guidelines in an independent Italian cohort, demonstrating that the presence of person-specific semantic knowledge loss and mental rigidity, along with preserved executive functions and a predominant right ATL atrophy with sparing of frontal lobes, should prompt a diagnosis of sbvFTD.

Keywords sbvFTD · FTD · MRI · Voxel-based morphometry · rtvFTD

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Distribution of the *C9orf72* hexanucleotide repeat expansion in healthy subjects: a multicenter study promoted by the Italian IRCCS network of neuroscience and neurorehabilitation

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BRAIN COMMUNICATIONS

CLINICAL TRIAL

Colchicine treatment in amyotrophic lateral sclerosis: safety, biological and clinical effects in a randomized clinical trial

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In preclinical studies, the anti-inflammatory drug colchicine, which has never been tested in amyotrophic lateral sclerosis, enhanced the expression of autophagy factors and inhibited accumulation of transactive response DNA-binding protein 43 kDa, a known histopathological marker of amyotrophic lateral sclerosis. This multicentre, randomized, double-blind trial enrolled patients with probable or definite amyotrophic lateral sclerosis who experienced symptom onset within the past 18 months. Patients were randomly assigned in a 1:1:1 ratio to receive colchicine at a dose of 0.005 mg/kg/day, 0.01 mg/kg/day or placebo for a treatment period of 30 weeks. The number of positive responders, defined as patients with a decrease lesser than 4 points in the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised total score during the 30-week treatment period, was the primary outcome. Disease progression, survival, safety and quality of life at the end of treatment were the secondary clinical outcomes. Secondary biological outcomes included changes from baseline to treatment end of stress granule and autophagy responses, transactive response DNA-binding protein 43 kDa, neurofilament accumulation and extracellular vesicle secretion, between the colchicine and placebo groups. Fifty-four patients were randomized to receive colchicine ($n = 18$ for each colchicine arm) or placebo ($n = 18$). The number of positive responders did not differ between the placebo and colchicine groups: 2 out of 18 patients (11.1%) in the placebo group, 5 out of 18 patients (27.8%) in the colchicine 0.005 mg/kg/day group (odds ratio = 3.1, 97.5% confidence interval 0.4–37.2, $P = 0.22$) and 1 out of 18 patients (5.6%) in the colchicine 0.01 mg/kg/day group (odds ratio = 0.5, 97.5% confidence interval 0.01–10.2, $P = 0.55$). During treatment, a slower Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised decline was detected in patients receiving colchicine 0.005 mg/kg/day (mean difference = 0.53, 97.5% confidence interval 0.07–0.99, $P = 0.011$). Eight patients experienced adverse events in placebo arm (44.4%), three in colchicine 0.005 mg/kg/day (16.7%) and seven in colchicine 0.01 mg/kg/day arm (35.9%). The differences in adverse events were not statistically significant. In conclusion, colchicine treatment was safe for amyotrophic lateral sclerosis patients. Further studies are required to better understand mechanisms of action and clinical effects of colchicine in this condition.

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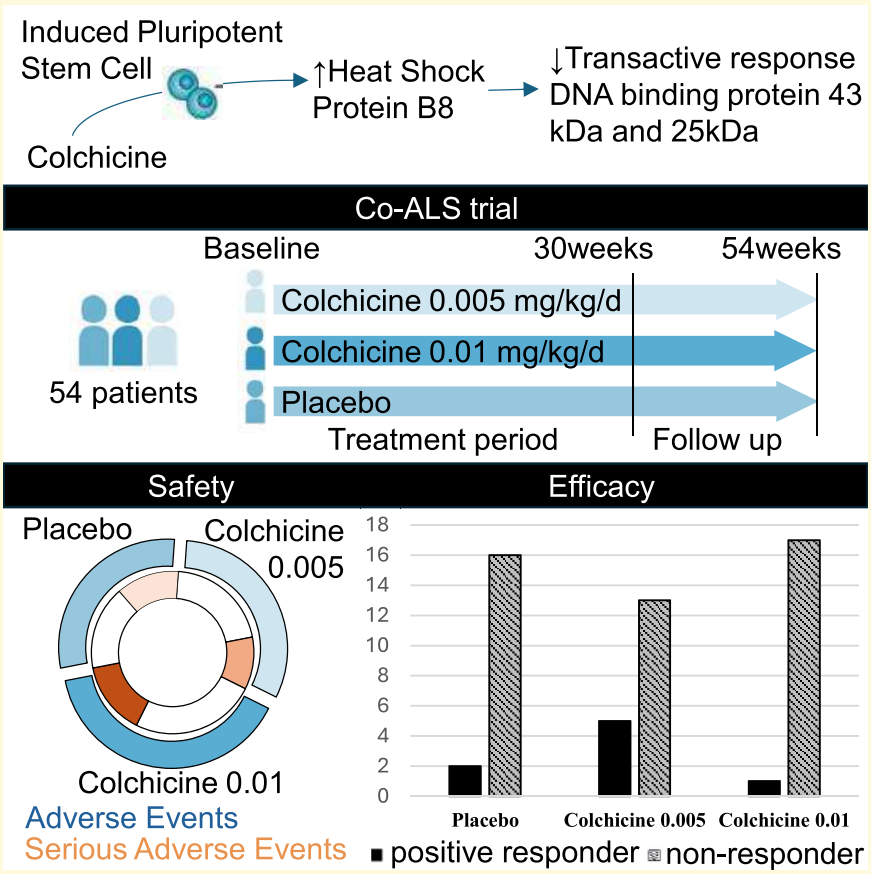
Keywords: amyotrophic lateral sclerosis; colchicine; randomized clinical trial; protein quality control; neuroinflammation

Graphical Abstract



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Letter

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Systematic rare variant analyses identify *RAB32* as a susceptibility gene for familial Parkinson's disease

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Despite substantial progress, causal variants are identified only for a minority of familial Parkinson's disease (PD) cases, leaving high-risk pathogenic variants unidentified[1,2]. To identify such variants, we uniformly processed exome sequencing data of 2,184 index familial PD cases and 69,775 controls. Exome-wide analyses converged on *RAB32* as a novel PD gene identifying c.213C > G/p.S71R as a high-risk variant presenting in ~0.7% of familial PD cases while observed in only 0.004% of controls (odds ratio of 65.5). This variant was confirmed in all cases via Sanger sequencing and segregated with PD in three families. *RAB32* encodes a small GTPase known to interact with LRRK2 (refs. 3,4). Functional analyses showed that RAB32 S71R increases LRRK2 kinase activity, as indicated by increased autophosphorylation of LRRK2 S1292. Here our results implicate mutant RAB32 in a key pathological mechanism in PD—LRRK2 kinase activity[5–7]—and thus provide novel insights into the mechanistic connections between RAB family biology, LRRK2 and PD risk.

Approximately 10–15% of patients with Parkinson's disease (PD) are classified as 'familial cases', a designation conventionally restricted to patients known to have a first degree relative also affected by the disorder[8,9]. The identification of causal rare variants in familial PD has contributed enormously to our current understanding of the disease, ultimately yielding drug targets, biomarkers and key insights into disease mechanisms[1,10–12]. Currently, seven genes have been classified as definitely associated with familial PD[2,13]. The discovery of these familial PD genes has been achieved through various family-based study designs, including linkage analysis, homozygosity mapping and segregation filtering[1,10,12]. However, in many cases, conventional family-based study designs are insufficient to identify causal variants due to a combination of genetic heterogeneity across families, reduced penetrance and limited sample size within families. Rare variant association testing methods, such as gene burden analysis, provide an alternative strategy for discovering rare genetic risk factors[14]. Instead of leveraging familial structure, these methods leverage case–control differences in cumulative rare variant frequencies. This approach has recently been used to study rare genetic variation in idiopathic PD[15], but thus far, targeted analyses of familial PD cases have not been performed. In previous work, we demonstrated substantial power gains for disease gene discovery in amyotrophic lateral sclerosis by restricting rare variant association testing to familial cases and controls[16–18]. The rationale of selecting familial cases is to increase sensitivity by enriching

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BMC Neurology

CASE REPORT

Open Access

Duropathy as a rare motor neuron disease mimic: from bibrachial amyotrophy to infratentorial superficial siderosis

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Abstract

Background Bibrachial amyotrophy associated with an extradural CSF collection and infratentorial superficial siderosis (SS) are rare conditions that may occasionally mimic ALS. Both disorders are assumed to be due to dural tears.

Case presentation A 53-year-old man presented with a 7-year history of slowly progressive asymmetric bibrachial amyotrophy. Initially, a diagnosis of atypical motor neuron disease (MND) was made. At re-evaluation 11 years later, upper limb wasting and weakness had further progressed and were accompanied by sensorineural hearing loss. MRI of the brain and spine demonstrated extensive supra- and infratentorial SS (including the surface of the whole spinal cord) as well as a ventral longitudinal intraspinal fluid collection (VLISFC) extending along almost the entire thoracic spine. Osteodegenerative changes were observed at C5-C7 level, with osteophytes protruding posteriorly. The bony spurs at C6-C7 level were hypothesized to have lesioned the dura, causing a CSF leak and thus a VLISFC. Review of the MRI acquired at first evaluation showed that the VLISFC was already present at that time (actually beginning at C7 level), whereas the SS was not. 19 years after the onset of upper limb weakness, the patient additionally developed parkinsonism. Response to levodopa, brain scintigraphy with ^{123}I-ioflupane and brain MRI with nigrosome 1 evaluation were consistent with idiopathic Parkinson's disease (PD). On the latest follow-up 21 years after symptom onset, the VLISFC was unchanged, as were upper arm weakness and wasting.

Conclusions Based on the long-term follow-up, we could establish that, while the evidence of the VLISFC was concomitant with the clinical presentation of upper limb amyotrophy and weakness, the radiological signs of SS appeared later. This suggests that SS was not per se the cause of the ALS-like clinical picture, but rather a long-term sequela of a dural leak. The latter was instead the causative lesion, giving rise to a VLISFC which compressed the cervical motor roots. Dural tears can actually cause several symptoms, and further studies are needed to elucidate the pathophysiological correlates of "duropathies". Finally, as iron metabolism has been implicated in PD, the co-occurrence of PD with SS deserves further investigation.

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Author contributions
The study was conceived by FV. VI performed a literature search and wrote the manuscript. FV critically reviewed the pathophisiological hypotheses presented in the discussion. FV, CC, AS, GC and AE provided the neuroradiological images. CC and AE discussed the diagnostic hypotheses from the neuroradiological point of view. EC and EC contributed neurochemical data. FV, VS and NT critically reviewed the manuscript. All authors approved the final version of the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of IRCCS Istituto Auxologico Italiano (code 2021_05_18_04). The patient whose case is described in this manuscript provided written informed consent for participation.

Consent for publication
The patient whose case is described in this manuscript provided written informed consent for publication.

Competing interests
The authors declare no competing interests.

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References

1. Fearnley JM, Stevens JM, Rudge P. Superficial siderosis of the central nervous system. Brain. 1995;118(Pt 4):1051–66.
2. Kharytaniuk N, Cowley P, Sayal P, et al. Classical infratentorial superficial siderosis of the central nervous system: pathophysiology, clinical features and management. Pract Neurol; 2022.
3. Hamill RC. Report of a case of melanosis of the brain, cord and meninges. J Nerv Ment Dis 1908; 35(9).
4. Castro-Gomez S, Binder J, Schievelkamp AH, Heneka MT. CNS superficial siderosis mimicking a motor neuron disease. Brain Sci 2022;12(11).
5. Deguchi K, Honjo N, Takata T, Touge T, Masaki T. Flail arm syndrome mimic caused by hemosiderin deposition in the anterior horn. Acta Neurol Belg. 2020;120(6):1487–9.
6. Driver-Dunckley ED, Hoxworth JM, Patel NP, Bosch EP, Goodman BP. Superficial siderosis mimicking amyotrophic lateral sclerosis. J Clin Neuromuscul Dis. 2010;11(3):137–44.
7. Kumar N, Fogelson JL, Morris JM, Pichelmann MA. Superficial siderosis should be included in the differential diagnosis of motor neuron disease. Neurologist. 2012;18(3):139–45.
8. Payer M, Sottas C, Bonvin C. Superficial siderosis of the central nervous system: secondary progression despite successful surgical treatment, mimicking amyotrophic lateral sclerosis. Case report and review. Acta Neurochir (Wien). 2010;152(8):1411–6.
9. Turner B, Wills AJ. Superficial siderosis associated with anterior horn cell dysfunction. J Neurol Neurosurg Psychiatry. 2002;72(2):274–5.
10. Kumar N, Cohen-Gadol AA, Wright RA, Miller GM, Piepgras DG, Ahlskog JE. Superficial Siderosis Neurol. 2006;66(8):1144–52.
11. Takai K, Taniguchi M. Superficial siderosis of the central nervous system associated with ventral dural defects: bleeding from the epidural venous plexus. J Neurol. 2021;268(4):1491–4.
12. Kumar N. Superficial siderosis: a clinical review. Ann Neurol. 2021;89(6):1068–79.
13. Wilson D, Chatterjee F, Farmer SF, et al. Infratentorial superficial siderosis: classification, diagnostic criteria, and rational investigation pathway. Ann Neurol. 2017;81(3):333–43.
14. Kumar N. Beyond superficial siderosis: introducing duropathies. Neurology. 2012;78(24):1992–9.
15. Schievink WI, Maya MM, Jean-Pierre S, Nuno M, Prasad RS, Moser FG. A classification system of spontaneous spinal CSF leaks. Neurology. 2016;87(7):673–9.
16. Dobrocky T, Nicholson P, Hani L, et al. Spontaneous intracranial hypotension: searching for the CSF leak. Lancet Neurol. 2022;21(4):369–80.
17. Schievink WI, Maya MM. Spinal meningeal diverticula, spontaneous intracranial hypotension, and superficial siderosis. Neurology. 2017;88(9):916–7.
18. Schievink WI, Maya M, Moser F, Nuno M. Long-term risks of persistent ventral spinal CSF leaks in SIH: superficial siderosis and Bibrachial Amyotrophy. Neurology. 2021;97(19):e1964–70.
19. Brus-Ramer M, Dillon WP. Idiopathic thoracic spinal cord herniation: retrospective analysis supporting a mechanism of diskogenic dural injury and subsequent tamponade. AJNR Am J Neuroradiol. 2012;33(1):52–6.
20. Petzold A, Worthington V, Appleby I, Kerr ME, Kitchen N, Smith M. Cerebrospinal fluid ferritin level, a sensitive diagnostic test in late-presenting subarachnoid hemorrhage. J Stroke Cerebrovasc Dis. 2011;20(6):489–93.
21. Mihaylova T, Biondo A, Zak I, Lewis RA. Anterior horn cell loss from subdural hygroma: a consequence of spontaneous spinal fluid leak. J Neurol Sci. 2011;305(1–2):156–9.
22. Quattrocchi S, Bonan L, Cirillo L et al. Bibrachial amyotrophy as a rare manifestation of intraspinal fluid collection: a case report and systematic review. Neurol Sci 2023.
23. Deluca GC, Boes CJ, Krueger BR, Mokri B, Kumar N. Ventral intraspinal fluid-filled collection secondary to CSF leak presenting as bibrachial amyotrophy. Neurology. 2011;76(16):1439–40.
24. Iwase R, Kanouchi T, Yoshii T, et al. Diverging pathophysiology in superficial siderosis with proximal upper limb amyotrophy. J Neurol Sci. 2022;436:120248.
25. Morishima R, Takai K, Ando T, Nakata Y, Shimizu T, Taniguchi M. Brachial multisegmental amyotrophy caused by cervical anterior horn cell disorder associated with a spinal CSF leak: a report of five cases. J Neurol. 2019;266(11):2679–84.
26. Takahashi Y, Kodaira M, Yamada M, et al. Anterior horn damage in brachial multisegmental amyotrophy with superficial siderosis and dural tear: an autopsy case report. BMC Neurol. 2023;23(1):129.
27. Preethish-Kumar V, Vengalil S, Tiwari S, et al. Ventral longitudinal intraspinal fluid collection: rare presentation as brachial amyotrophy and intracranial hypotension. J Spinal Cord Med. 2019;42(1):45–50.
28. Hirayama K. Juvenile muscular atrophy of distal upper extremity (Hirayama disease). Intern Med. 2000;39(4):283–90.
29. Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: a systematic review and meta-analysis. Mov Disord. 2014;29(13):1583–90.
30. Rosenthal P. [Siderosis o the marginal zones of the central nervous system]. Dtsch Z Nervenheilkd. 1958;178(4):431–72.
31. Mochizuki H, Choong CJ, Baba K. Parkinson's disease and iron. J Neural Transm (Vienna). 2020;127(2):181–7.
32. Rieder CR, dos Santos Souza MP, de Freitas RM, Fricke D. Superficial siderosis of the central nervous system associated with parkinsonism. Parkinsonism Relat Disord. 2004;10(7):443–5.

Publisher's Note

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RESEARCH ARTICLE

Optineurin in patients with Amyotrophic Lateral Sclerosis associated to atypical Parkinsonism in Tunisian population

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is a heterogeneous disorder and the phenotypic variability goes far beyond the used clinical stratification parameter. Evidence has emerged that ALS may coexist with distinct neurodegenerative diseases in single cases. We aim to study the clinical features of two familial cases of ALS carriers of two distinct variants harbored in the Optineurin (*OPTN*) gene. We included definite familial ALS followed up in the Department of Neurology of Razi University Hospital, Tunisia, and selected according to Byrne criteria. Preliminary screening for the four main ALS genes (*SOD1*, *C9ORF72*, *TARDBP*, *FUS*) was conducted. Given the negative results, we proceeded to NGS target-re-sequencing with a custom panel including genes associated with ALS-FTD, Alzheimer's, and Parkinson's diseases. Both families are carriers of two different *OPTN* variants and they present very different ALS clinical features. The first family comprises two siblings diagnosed with ALS and Corticobasal syndrome (ALS-CBS) at an early age of onset and carriers of *OPTN* p.E135X in the homozygous state. The proband for the second family was diagnosed with ALS at an early age of onset presenting as progressive muscular atrophy with rapid progression. Genetic analysis revealed the presence of the homozygous variant p.R520H.Our findings highlight the peculiarity of genetic Tunisian drift. Indeed, genes with a recessive mode of inheritance may explain part of ALS diversity in clinical features. Therefore, the screening of the *OPTN* gene is highly recommended among inbreeding populations such as the Tunisian one.

Keywords: *Amyotrophic Lateral Sclerosis, optineurin, atypical Parkinsonism*

Introduction

Amyotrophic lateral sclerosis (ALS) is a multi-systemic neurodegenerative disease characterized by a progressive degeneration of motor neurons in both brain and spinal cord (1). The constantly evolving effort in understanding ALS nature and etiology has gradually led to the present vision of this fatal disease as a multifactorial one, including genetic and environmental risk factors and affecting several cell pathways (2).

The functional convergence of all these diverse entities in determining ALS clinical features (age of onset, progression, survival, etc.) is still poorly understood (3). However, the common pathological hallmark of ALS is the presence of ubiquitinated and phosphorylated TDP-43 protein that aggregates into soluble inclusions in affected brain tissues (4).

Recent evidences highlight the oligogenic and polygenic basis of ALS that might explain its clinical features diversity (5,6). Along with these reports, several causative ALS genes emerged (7) which were associated, with the exception of *SOD1* and *FUS*, with the presence of TDP-43-positive neuronal cytoplasmic inclusions, pointing to the idea that these single genes could be an upstream cause for TDP-43 pathology in ALS (8).

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Association of Amyotrophic Lateral Sclerosis and Dopa-responsive dystonia in a Tunisian patient

ARTICLE INFO

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ABSTRACT

Dopa-responsive dystonia (DRD) is an autosomal dominant disease with parkinsonian and dystonic symptoms caused by *GCH1* gene pathogenic variants affecting dopamine synthesis. The present case report is the first to link DRD with childhood-onset with ALS, suggesting that complex inheritance patterns in the North African population may contribute to multiple disorders.

Dopa-responsive dystonia (DRD) is an autosomal dominant disease involving both nigrostriatal dopaminergic and non-dopaminergic pathways. It is caused by pathogenic variants in the Guanosine Triphosphate Cyclohydrolase-1 gene (*GCH1*) and more than 200-pathogenic variants have been reported. However,DRD is a disease with genetic complexity as the penetrance of *GCH1*,involved in dopamine synthesis, was estimated approximately to be only 30 %.Moreover,*GCH1*-dificiency exhibits a highly heterogeneous expressivity leading to varied phenotypic presentations with the increasing risk of Parkinson disease(PD) occurrence. DRD is characterized by marked diurnal fluctuations and a strong response to small doses of levodopa. However,the atypical presentation—where childhood dystonia evolves into Parkinsonism with age—suggests that DRD patients may develop additional neurodegenerative diseases at different stages of life [1].

Amyotrophic Lateral Sclerosis (ALS) is a complex and clinically heterogeneous disease characterized by progressive degeneration of upper and lower motor neurons. Intriguingly,ALS,dementia,and Parkinsonism can occur together in the same patient, likely due to overlapping misfolded proteins. This association is especially predictable in highly inbred populations, such as Tunisians.

In the present report,we explore the clinical and genetic characteristics of a familial case of DRD with childhood-onset,who developed also ALS in her forties and died after 7 years of ALS diagnosis(47 years-old).

We describe a Tunisian family where the index case(III-9),from an inbred family, developed DRD phenotype and had a daughter diagnosed with DRD,too (Fig. 1-A). The case is a female who complained from difficulties in walking and a "bizarre" gait a few minutes after waking-up since the age of 3 years-old. The initial neurological examination, at age of 10 years-old,showed only lower limb dystonia while walking. However,no dystonia or abnormal movements were observed at rest. Brain MRI,Copper test,and routine laboratory analysis were normal.

She was treated with 75-mg/day of levodopa and responded perfectly, showing total improvement. Sanger sequencing of the *GCH1* gene (Fig. 1-B) revealed a heterozygous p. G203R pathogenic variants, leading to a diagnosis of DRD.

At 44 years-old,she visited our center with a 6-month history of cramping movements in her trunk, arms, and legs. Her symptoms also included generalized fasciculations, worsening difficulty with walking, and slowed movements. Neurological examination showed signs of upper and lower motor neuron impairment, including brisk reflexes, bilateral Hoffman's signs, atrophy of the intrinsic hand muscles and mild tongue atrophy,and fasciculations in the forearms.

Electromyography revealed diffuse motor neuropathy with bulbar region involvement, while Motor Evoked Potentials from transcranial magnetic stimulation showed upper motor neuron involvement. Thus,a definitive diagnosis of ALS was made.

We assessed the patient's cognitive features using the Arabic Edinburgh Cognitive and Behavioral ALS Screen (ECAS-AR), resulting in a total score of 76, slightly above the cut-off of 75. The ECAS-AR revealed impaired performance on ALS-specific tests (49/100), while the MMSE and FAB scores were normal.

Given the typical DRD features and a definite ALS diagnosis, we first screened the four main ALS-associated genes (*SOD1*,*FUS,TARDBP,* and *C9orf72*) and no pathogenic variants were found. Consequently,we conducted an extensive analysis using a custom NGS panel covering 48-genes linked to ALS-FTD spectrum, dementia, and PD. A variant of unknown significance (VUS) was identified in Parkinson disease protein 7 gene(*DJ1/PARK7* p. T110A),and another in Valosine Containing Protein gene *VCP*,p.I27V was found (Fig. 1-C).

Additionally, the daughter was diagnosed with DRD at the age of 4 years-old and the genetic testing of *GCH1* revealed the presence of the same heterozygous p. G203R pathogenic variants present in the mother. The daughter, who is 19 years-old, has shown no signs of ALS or PD during her follow-up thus far.

This is the first report of childhood-onset DRD associated with ALS. We describe a case from an inbred Tunisian family where the index patient developed definite ALS, and her daughter exhibited dystonia due to the same *GCH1* gene variant. The association of DRD and ALS may be explained by pathological mechanisms, as tyrosine deficiency is known to cause DRD. Elevated nitrotyrosine levels have been linked to both sporadic and familial ALS (sALS, and fALS), with increased free nitrotyrosine found in the spinal cords of transgenic ALS mice [2]. Furthermore, *GCH1* has been shown to disrupt tyrosine homeostasis and activate innate immune mechanisms in the brain [3]. This suggests that the *GCH1* variant may impair tyrosine functions, indicating a shared pathway that could contribute to the childhood-onset association of ALS

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genes for improved genotype-phenotype correlation.

CRediT authorship contribution statement

Imen Kacem: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Ikram Sghaier:** Writing – original draft, Methodology, Formal analysis, Data curation. **Hanene Ben Rhouma:** Writing – review & editing, Data curation. **Antonia Ratti:** Writing – review & editing, Formal analysis. **Nicola Ticozzi:** Writing – review & editing, Formal analysis. **Vincenzo Silani:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Neziha Gouider-Khouja:** Writing – review & editing, Data curation. **Riadh Gouider:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Conceptualization.

Ethics approval

The subject conformed to the principles outlined in the Declaration of Helsinki and the study have been performed with permission of the Razi hospital ethic committee. The patient was informed about the purposes of the study and gave her written consent to participate.

Data sharing

None.

Funding statement

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Declaration of competing interest

Prof. Gouider Riadh is the associate editor of European Journal of Neurology and the Neurological Sciences Journal. He has been serving as constant reviewer in Neurophysiology and Neurological Disorders Journal since 2013 and receives and has received research support from the Tunisian Ministry of Higher Education and scientific research/ and the Tunisian Ministry of Health. Prof Silani Vincenzo is in the Editorial Board of Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, European Neurology, American Journal of Neurodegenerative Diseases, Frontiers in Neurology, and Exploration of Neuroprotective Therapy. He received compensation for consulting services and/or speaking activities from AveXis, Cytokinetics, Italfarmaco, Novartis paharma AG, and Zambon Biotech SA. For the rest of co-authors that they have no known competing financial interests or personal relationships that could have appeared to influence the present work.

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References

[1] H. Ichinose, T. Suzuki, H. Inagaki, T. Ohye, T. Nagatsu, Molecular genetics of dopa-responsive dystonia, Biol. Chem. 380 (1999), https://doi.org/10.1515/BC.1999.175.

[2] H. Peluffo, J.J. Shacka, K. Ricart, C.G. Bisig, L. Martìnez-Palma, O. Pritsch, A. Kamaid, J.P. Eiserich, J.P. Crow, L. Barbeito, A.G. Estèvez, Induction of motor neuron apoptosis by free 3-nitro-L-tyrosine, J. Neurochem. 89 (2004) 602–612, https://doi.org/10.1046/j.1471-4159.2004.02363.x.

[3] H. Larbalestier, M. Keatinge, L. Watson, E. White, S. Gowda, W. Wei, K. Koler, S. A. Semenova, A.M. Elkin, N. Rimmer, S.T. Sweeney, J. Mazzolini, D. Sieger, W. Hide, J. McDearmid, P. Panula, R.B. MacDonald, O. Bandmann, GCH1 deficiency activates brain innate immune response and impairs tyrosine hydroxylase homeostasis, J. Neurosci. 42 (2022) 702–716, https://doi.org/10.1523/JNEUROSCI.0653-21.2021.

[4] P.L. McGeer, J.C. Steele, The ALS/PDC syndrome of Guam: potential biomarkers for an enigmatic disorder, Prog Neurobiol 95 (2011) 663–669, https://doi.org/10.1016/j.pneurobio.2011.04.001.

[5] G. Annesi, G. Savettieri, P. Pugliese, M. D'Amelio, P. Tarantino, P. Ragonese, V. La Bella, T. Piccoli, D. Civitelli, F. Annesi, B. Fierro, F. Piccoli, G. Arabia, M. Caracciolo, I.C. Cirò Candiano, A. Quattrone, DJ-1 mutations and parkinsonism-dementia-amyotrophic lateral sclerosis complex, Ann. Neurol. 58 (2005) 803–807, https://doi.org/10.1002/ana.20666.

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BRIEF REPORT

De novo *FRMD5* Missense Variants in Patients with Childhood-Onset Ataxia, Prominent Nystagmus, and Seizures

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ABSTRACT: Background: *FRMD5* variants were recently identified in patients with developmental delay, ataxia, and eye movement abnormalities.
Objectives: We describe 2 patients presenting with childhood-onset ataxia, nystagmus, and seizures carrying pathogenic de novo *FRMD5* variants. Weighted gene co-expression network analysis (WGCNA) was performed to gain insights into the function of *FRMD5* in the brain.
Methods: Trio-based whole-exome sequencing was performed in both patients, and CoExp web tool was used to conduct WGCNA.
Results: Both patients presented with developmental delay, childhood-onset ataxia, nystagmus, and seizures. Previously unreported findings were diffuse choreoathetosis and dystonia of the hands (patient 1) and areas of abnormal magnetic resonance imaging signal in the white matter (patient 2). WGCNA showed that *FRMD5* belongs to gene networks involved in neurodevelopment and oligodendrocyte function.
Conclusions: We expanded the phenotype of *FRMD5*-related disease and shed light on its role in brain function and development. We recommend including *FRMD5* in the genetic workup of childhood-onset ataxia and nystagmus. © 2024 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: ataxia; *FRMD5*; nystagmus; genetics; weighted gene co-expression network analysis

FERM domain-containing proteins (FDCP) are a group of proteins that play a crucial role in anchoring the cytoskeleton to the plasma membrane, thereby regulating cell motility and interaction with the extracellular environment.[1]

FRMD5 is an FDCP found in the adherens junctions, which interacts with the cell adhesion molecules p120-catenin and E-cadherin. Functional studies suggest its involvement in tumor progression and tissue invasion.[2]

Until recently, no human diseases were associated with variants in the *FRMD5* gene. However, Lu and colleagues identified rare de novo variants in *FRMD5* in 8 patients characterized by developmental delay, ataxia, nystagmus, and opsoclonus.[3]

In this study, we present the first independent confirmation of the causative role of de novo *FRMD5* variants in childhood-onset ataxia, nystagmus, intellectual disability, and seizures. Furthermore, we observed previously unreported clinical features, including diffuse choreoathetosis and dystonic posturing of hands (patient 1) and hyperintense magnetic resonance imaging (MRI) white matter (WM) lesions (patient 2).

Weighted gene co-expression network analysis (WGCNA) indicates that *FRMD5* may play a role in regulating oligodendrocyte function, myelination, and neurodevelopment across multiple brain regions.

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RESEARCH ARTICLE OPEN ACCESS

Association of Vascular Risk Factors and Cerebrovascular Pathology With Alzheimer Disease Pathologic Changes in Individuals Without Dementia

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Abstract

Background and Objectives

Vascular risk factors (VRFs) and cerebral small vessel disease (cSVD) are common in patients with Alzheimer disease (AD). It remains unclear whether this coexistence reflects shared risk factors or a mechanistic relationship and whether vascular and amyloid pathologies have independent or synergistic influence on subsequent AD pathophysiology in preclinical stages. We investigated links between VRFs, cSVD, and amyloid levels ($A\beta_{1\text{-}42}$) and their combined effect on downstream AD biomarkers, that is, CSF hyperphosphorylated tau ($P\text{-}tau_{181}$), atrophy, and cognition.

Methods

This retrospective study included nondemented participants (Clinical Dementia Rating < 1) from the European Prevention of Alzheimer's Dementia (EPAD) cohort and assessed VRFs with the Framingham risk score (FRS) and cSVD features on MRI using visual scales and white matter hyperintensity volumes. After preliminary linear analysis, we used structural equation modeling (SEM) to create a "cSVD severity" latent variable and assess the direct and indirect effects of FRS and cSVD severity on $A\beta_{1\text{-}42}$, $P\text{-}tau_{181}$, gray matter volume (baseline and longitudinal), and cognitive performance (baseline and longitudinal).

Results

A total cohort of 1,592 participants were evaluated (mean age = 65.5 ± 7.4 years; 56.16% F). We observed positive associations between FRS and all cSVD features (all $p < 0.05$) and a negative association between FRS and $A\beta_{1\text{-}42}$ ($\beta = -0.04 \pm 0.01$). All cSVD features were negatively associated with CSF $A\beta_{1\text{-}42}$ (all $p < 0.05$). Using SEM, the cSVD severity fully mediated the association between FRS and CSF $A\beta_{1\text{-}42}$ (indirect effect: $\beta = -0.03 \pm 0.01$), also when omitting vascular amyloid-related markers. We observed a significant indirect effect of cSVD severity on $P\text{-}tau_{181}$ (indirect effect: $\beta = 0.12 \pm 0.03$), baseline and longitudinal gray matter volume (indirect effect: $\beta = -0.10 \pm 0.03$; $\beta = -0.12 \pm 0.05$), and baseline cognitive performance (indirect effect: $\beta = -0.16 \pm 0.03$) through CSF $A\beta_{1\text{-}42}$.

Discussion

In a large nondemented population, our findings suggest that cSVD is a mediator of the relationship between VRFs and CSF $A\beta_{1\text{-}42}$ and affects downstream neurodegeneration and cognitive impairment. We provide evidence of VRFs indirectly affecting the pathogenesis of AD, highlighting the importance of considering cSVD burden in memory clinics for AD risk evaluation and as an early window for intervention. These results stress the role of VRFs and cerebrovascular pathology as key biomarkers for accurate design of anti-amyloid clinical trials and offer new perspectives for patient stratification.

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underlying etiologies[43]; future studies could use tau PET to better disentangle the effect of vascular factors on such pathologic mechanisms. It is important to note that the absence of neuropathologic data limited the assessment of independent contributions of CAA and arteriolosclerosis markers. However, in our sensitivity analysis, we showed that excluding lobar CMBs and PVS-CS, considered as CAA neuroradiologic indices in the most recent criteria,[27] did not change the results of our main analysis. This suggests that arteriolosclerosis-related neuroradiologic abnormalities have a driving role in the observed associations, independently of CAA.

Taken together, our results highlight the important role of cSVD in early amyloid deposition and related events, including tau pathology and atrophy. Furthermore, the data suggest a route whereby VRFs can link through cSVD to promote the amyloid pathologic cascade of events in susceptible individuals. Overall, these findings suggest that VRFs and cSVD represent integral components of the early stages of the biological cascade that leads to neurodegeneration in AD and stress the importance of monitoring and controlling VRFs, not ignoring brain cSVD features, and accelerating the testing of agents that could improve the vascular dysfunction in cSVD as a way to help prevent development of AD.

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ORIGINAL ARTICLE

Association of *APOE* genotype and cerebrospinal fluid Aβ and tau biomarkers with cognitive and motor phenotype in amyotrophic lateral sclerosis

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Abstract

Objective: Little is known about amyotrophic lateral sclerosis (ALS)-nonspecific cognitive deficits – most notably memory disturbance – and their biological underpinnings. We investigated the associations of the Alzheimer's disease (AD) genetic risk factor *APOE* and cerebrospinal fluid (CSF) biomarkers Aβ and tau proteins with cognitive and motor phenotype in ALS.

Methods: *APOE* haplotype was determined in 281 ALS patients; for 105 of these, CSF levels of Aβ42, Aβ40, total tau (T-tau), and phosphorylated tau (P-tau181) were quantified by chemiluminescence enzyme immunoassay (CLEIA). The Edinburgh Cognitive and Behavioural ALS Screen (ECAS) was employed to evaluate the neuropsychological phenotype.

Results: *APOE*-E4 allele was associated with worse ECAS memory score (median, 14.0 in carriers vs. 16.0 in non-carriers) and lower CSF Aβ42 (−0.8 vs. 0.1, log-transformed values) and Aβ42/40 ratio (−0.1 vs. 0.3). Some 37.1% of ALS patients showed low Aβ42 levels,

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RESEARCH ARTICLE

Mutations in the tail and rod domains of the neurofilament heavy-chain gene increase the risk of ALS

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Abstract

Objective: Neurofilament heavy-chain gene (*NEFH*) variants are associated with multiple neurodegenerative diseases, however, their relationship with ALS has not been robustly explored. Still, *NEFH* is commonly included in genetic screening panels worldwide. We therefore aimed to determine if *NEFH* variants modify ALS risk. **Methods**: Genetic data of 11,130 people with ALS and 7,416

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Disease disclosure in the workplace in people living with rheumatic diseases: an exploratory study

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SUMMARY

Objective. Rheumatic musculoskeletal diseases (RMDs) are the causes of frequent absence from work and loss of productivity. As (in)visible diseases, it is up to the individuals to decide if disclosing their diagnosis, with important repercussions also within the workplace. Still little is known about disease disclosure in the workplace (DD-W) in patients with RMDs. This study aimed to investigate socio-demographic, clinical, and psychological predictors of DD-W among working patients with RMDs.

Methods. A cross-sectional Italian national study captured DD-W in people with RMDs. An online survey was developed using *ad-hoc* questions and scientific questionnaires to explore demographics and work-related, clinical, and psychological factors. Stepwise logistic regressions were run to identify significant predictors of DD-W.

Results. A total of 250 working rheumatic patients completed the survey; 81.2% of the participants enacted DD-W. DD-W behaviors were predicted by perceived visibility of the RMD (p=0.008), work type (p=0.022), general DD behaviors (p<0.001), and perceived family support (p=0.023). Among RMD patients, psoriatic arthritis participants had higher probabilities of DD-W (p=0.02), whereas lower probabilities were detected in fibromyalgia patients (p=0.003). Lower disease duration corresponded in the sample to higher probabilities of DD-W (p=0.036).

Conclusions. The majority of RMD patients in this study enacted DD-W. DD-W was associated with medical, occupational, and psychological factors, supporting the multidimensionality of the process. Further research on the subject might help foster better DD-W decision-making processes for RMD patients while promoting intervention strategies in education, policy, and culture.

***Key words**: Rheumatic disease, invisible disability, chronic disease, disease disclosure, health disclosure.*

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INTRODUCTION

Rheumatic musculoskeletal diseases (RMDs) are a diverse group of medical conditions (over 200) affecting people of any age and causing significant morbidity, comorbidity, and mortality (1, 2). RMDs affect joints, muscles, bones, and inner organs, are characterized by pain and inflammation, and are associated with functional impairments leading to disability in severe cases (2). RMDs are among the most frequent causes of absence from work and loss of work productivity, workability, and work participation in the working population (3-5). Moreover, some RMDs are commonly associated with fatigue (6), anxiety, and depression symptoms (7-9), which further worsen the impact of such chronic conditions on the quality of life and workability

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The Single-Matrix Digit Cancellation Test, a Screener for Selective Attention Deficits: Standardization in an Italian Population Sample and Clinical Usability in Acute Stroke Patients

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Keywords
Selective attention · Stroke · Digit cancellation test · Cognitive screening · Neuropsychology

Abstract
Introduction: This study aimed at validating and providing Italian norms for the Single-Matrix Digit Cancellation Test (SMDCT), a cancellation task to screen for selective attention deficits, as well as providing clinical usability evidence for it in acute stroke patients. ***Methods:*** The SMDCT stimulus is a specular, 4-quadrant, horizontally oriented matrix, across which target distribution is homogeneous. Both accuracy (-A) and time (-T) outcomes were computed. *N* = 263 healthy participants (HPs) and *N* = 76 acute stroke patients were recruited. *N* = 108 HPs also underwent the Mini-Mental State Examination, Frontal Assessment Battery (FAB), and Trail-Making Test (TMT), while patients were further assessed by the Mental Performance in Acute Stroke (MEPS). Regression-based norms were derived (equivalent scores). Construct and factorial validity, as well as case-control discrimination, were tested. ***Results:*** The matrix was underpinned by a two-component structure reflecting left and right hits. The SMDCT-T and -A were associated with TMT and FAB scores, respectively. Education predicted the SMDCT-A/-T, whereas age predicted the SMDCT-T only. In patients, the SMDCT converged with the MEPS, also accurately discriminating them from HPs. An index of right-left difference differentiated right- from left-damaged patients. ***Conclusions:*** The SMDCT is a valid and normed screener for selective attention deficits, encompassing measures of both accuracy and time, whose adoption is encouraged in acute stroke patients. Relatedly, the horizontal disposition of its matrix does allow for the qualitative report of either leftward of rightward biases due to underlying visual or attentional-representational deficits in this population.

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Hypothalamic involvement in multiple system atrophy: A structural MRI study

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ARTICLE INFO

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ABSTRACT

Objective: To investigate hypothalamic atrophy and its clinical correlates in multiple system atrophy (MSA) in-vivo.
Background: MSA is characterized by autonomic dysfunction and parkinsonian/cerebellar manifestations. The hypothalamus regulates autonomic and homeostatic functions and is also involved in memory and learning processes.
Methods: 11 MSA, 18 Parkinson's Disease (PD) and 18 Healthy Controls (HC) were included in this study. A validated and automated hypothalamic segmentation tool was applied to 3D-T1-weighted images acquired on a 3T MRI scanner. MSA hypothalamic volumes were compared to those of PD and HC. Furthermore, the association between hypothalamic volumes and scores of autonomic, depressive, sleep and cognitive manifestations were investigated.
Results: Posterior hypothalamus volume was reduced in MSA compared to controls ($t = 2.105$, $p = 0.041$) and PD ($t = 2.055$, $p = 0.046$). Total hypothalamus showed a trend towards a reduction in MSA vs controls ($t = 1.676$, $p = 0.101$). Reduced posterior hypothalamus volume correlated with worse MoCA scores in the parkinsonian (MSA + PD) group and in each group separately, but not with autonomic, sleep, or depression scores.
Conclusions: In-vivo structural hypothalamic involvement may be present in MSA. Reduced posterior hypothalamus volume, which includes the mammillary bodies and lateral hypothalamus, is associated with worse cognitive functioning. Larger studies on hypothalamic involvement in MSA and its clinical correlates are needed.

1. Introduction

Autonomic dysfunction is characteristic of multiple system atrophy (MSA), along with parkinsonism and/or cerebellar ataxia [1]. In MSA, autonomic dysfunction is mainly due to the neurodegenerative process affecting the central autonomic network [2]. The hypothalamus is a hub of this network and regulates many homeostatic functions, such as blood pressure, neuroendocrine regulation, and the sleep/wake cycle [3,4]. More recently, a role in memory and learning has also been shown [5–8]. In MSA, the hypothalamus displays characteristic glial and neuronal alpha-synuclein inclusion pathology along with neuronal loss [9–11].

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BRIEF REPORT

Analysis of normal *C9orf72* repeat length as possible disease modifier in amyotrophic lateral sclerosis

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Abstract

The *C9orf72* hexanucleotide repeat (HR) expansion is the main genetic cause of amyotrophic lateral sclerosis (ALS), with expansion size from 30 to >4000 units. Normal *C9orf72* HR length is polymorphic (2–23 repeats) with alleles >8 units showing a low frequency in the general population. This study aimed to investigate if the normal *C9orf72* HR length influences *C9orf72* gene expression and acts as disease modifier in ALS patients negative for *C9orf72* mutation (ALS-C9Neg). We found that the distribution of HR alleles was similar in 325 ALS-C9Neg and 303 healthy controls. Gene expression analysis in blood revealed a significant increase of total *C9orf72* and V3 mRNA levels in ALS-C9Neg carrying two long alleles (L/L; ≥8 units) compared to patients homozygous for the 2-unit short allele (S/S). However, HR allele genotypes (L/L, S/L, S/S) correlated with no clinical parameters. Our data suggest that normal *C9orf72* HR length does not act as disease modifier in ALS-C9Neg despite increasing gene expression.

Keywords: *amyotrophic lateral sclerosis, C9orf72, gene expression, disease modifier*

Introduction

The intronic hexanucleotide repeat (HR) expansion of the *C9orf72* gene is the main genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (1). Conventionally, the *C9orf72* pathogenic threshold is >30 repeats, although mutated patients may carry alleles up to 4000 HRs (2). The *C9orf72*-associated pathomechanisms are the haploinsufficiency of *C9orf72* protein, due to reduced transcription of the main V2 isoform from exon 1b downstream of HR expansion, and the accumulation of repeat-containing RNAs transcribed from the upstream exon 1a and of dipeptide repeat proteins through repeat-associated non-AUG translation of V1/V3 transcripts (1).

Normal *C9orf72* HR alleles are polymorphic (2–23 units) with a trimodal distribution (2, 5, 8 units) (3). Alleles >8 repeats have a low frequency both in ALS cases and healthy controls (CTR), and they have been already investigated as risk factors in different neurodegenerative diseases (4). A previous functional assay showed that 9-, 17- and 24-unit alleles reduced luciferase gene transcription in a length-dependent manner compared to the 2-unit allele (5), but whether normal HR length also influences *C9orf72* gene expression in ALS patients' biosamples remains uninvestigated. Indeed, the purpose of this study was to assess whether normal HR length may influence *C9orf72* gene expression acting as disease modifier in ALS patients without *C9orf72* mutation (ALS-C9Neg).

Materials and methods

Our study included 325 ALS-C9Neg diagnosed according to the revised El Escorial criteria, and

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CASE REPORT Open Access

Further insights into anti-IgLON5 disease: a case with complex clinical presentation

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Abstract

Background Anti-IgLON5 disease is an autoimmune encephalitis overlapping with neurodegenerative disorders due to pathological accumulation of hyperphosphorylated tau. It is characterized by several clinical manifestations determined by involvement of different brain areas, and mild response to first-line immunotherapies. We report a case of anti-IgLON5 disease with a multifaceted semiology and an unusually good response to glucocorticoid monotherapy.

Case presentation A 68-year-old man with type 2 diabetes was evaluated for an 8-month history of progressive gait disorder causing frequent falls. He also suffered from obstructive sleep apneas and complained of dysphonia, dysarthria, occasional dysphagia, urinary incontinence, and upper limb action tremor. Neurological examination demonstrated bilateral eyelid ptosis, limitation of ocular horizontal smooth pursuit movements, slow horizontal saccades, and lack of inhibition of the vestibulo-ocular reflex during rapid horizontal head torsions. The patient also displayed involuntary, slow, rhythmic movements of the left periorbital and perioral muscles, spreading to the ipsilateral hemipalate and hemitongue, along with bilateral negative upper limb myoclonus. There were proximal muscle wasting in the upper limbs, proximal weakness of the four limbs, and diffuse fasciculations. Ataxia of stance and gait and of the four limbs was noted. MRI of the brain and spine was unremarkable; nerve conduction studies revealed a chronic, predominantly demyelinating, sensory-motor polyneuropathy, probably due to diabetes. Routine CSF examination was unrevealing and serum GFAP level was 89.6 pg/mL; however, the autoimmunity tests revealed a high-titer positivity for anti-IgLON5 autoantibodies in both CSF and serum, leading to the diagnosis of anti-IgLON5 disease. Symptoms improved significantly after intravenous methylprednisolone.

Conclusions Hemifacial and hemiorolingual myorhythmia along with peculiar oculomotor abnormalities characterizes the multifaceted clinical picture of our case. The complex semiology of our patient may reflect multifocal targeting of the autoimmune process or sequential spreading of tau inclusions in different brain areas. Our patient's optimal response to glucocorticoid monotherapy could be underpinned by a slightly different phenotype in which autoimmunity plays a greater pathogenic role than tauopathy, with a lower burden of tau deposition. In

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References

1. Graus F, Titulaer MJ, Balu R, Benseler S, Bien CG, Cellucci T, et al. A clinical approach to diagnosis of autoimmune encephalitis. Lancet Neurol. 2016;15:391–404.
2. Flanagan EP, Geschwind MD, Lopez-Chiriboga AS, Blackburn KM, Turaga S, Binks S, et al. Autoimmune encephalitis misdiagnosis in adults. JAMA Neurol. 2023;80:30–9.
3. Dalmau J, Graus F. Antibody-mediated encephalitis. N Engl J Med. 2018;378:840–51.
4. Madetko N, Marzec W, Kowalska A, Przewodowska D, Alster P, Koziorowski D. Anti-IgLON5 disease - the current state of knowledge and further perspectives. Front Immunol. 2022:13:852215.
5. Honorat JA, Komorowski L, Josephs KA, Fechner K, St Louis EK, Hinson SR, et al. IgLON5 antibody: Neurological accompaniments and outcomes in 20 patients. Neurol Neuroimmunol Neuroinflamm. 2017;4:e385.
6. Gaig C, Graus F, Compta Y, Högl B, Bataller L, Brüggemann N, et al. Clinical manifestations of the anti-IgLON5 disease. Neurology. 2017;88:1736–43.
7. Baizabal-Carvallo JF, Cardoso F, Jankovic J. Myorhythmia: phenomenology, etiology, and treatment. Mov Disord. 2015;30:171–9.
8. Sabater L, Gaig C, Gelpi E, Bataller L, Lewerenz J, Torres-Vega E, et al. A novel non-rapid-eye movement and rapid-eye-movement parasomnia with sleep breathing disorder associated with antibodies to IgLON5: a case series, characterisation of the antigen, and post-mortem study. Lancet Neurol. 2014;13:575–86.
9. El-Abassi R, Soliman MY, Williams F, England JD. Whipple's disease. J Neurol Sci. 2017;377:197–206.
10. Mecklenburg J, Moos V, Moter A, Siebert E, Nave AH, Schneider T, et al. The spectrum of central nervous system involvement in Whipple's disease. Eur J Neurol. 2023;30:3417–29.
11. Grüter T, Möllers FE, Tietz A, Dargvainiene J, Melzer N, Heidbreder A, et al. Clinical, serological and genetic predictors of response to immunotherapy in anti-IgLON5 disease. Brain. 2023;146:600–11.
12. Werner J, Jelcic I, Schwarz EI, Probst-Müller E, Nilsson J, Schwizer B, et al. Anti-IgLON5 disease: a new bulbar-onset motor neuron mimic syndrome. Neurol Neuroimmunol Neuroinflamm. 2021;8:e962.
13. Gelpi E, Höftberger R, Graus F, Ling H, Holton JL, Dawson T, et al. Neuropathological criteria of anti-IgLON5-related tauopathy. Acta Neuropathol. 2016;132:531–43.
14. Berger-Sieczkowski E, Endmayr V, Haider C, Ricken G, Jauk P, Macher S, et al. Analysis of inflammatory markers and tau deposits in an autopsy series of nine patients with anti-IgLON5 disease. Acta Neuropathol. 2023;146:631–45.
15. Chung DC, Roemer S, Petrucelli L, Dickson DW. Cellular and pathological heterogeneity of primary tauopathies. Mol Neurodegener. 2021;16:57.
16. Ryding M, Gamre M, Nissen MS, Nilsson AC, Okarmus J, Poulsen AAE, et al. Neurodegeneration induced by anti-IgLON5 antibodies studied in induced pluripotent stem cell-derived human neurons. Cells. 2021;10:837.
17. Erro ME, Sabater L, Martínez L, Herrera M, Ostolaza A, García de Gurtubay I, et al. Anti-IGLON5 disease: a new case without neuropathologic evidence of brainstem tauopathy. Neurol Neuroimmunol Neuroinflamm. 2019;7:e651.
18. Lee SY, Shoji H, Shimozawa A, Aoyagi H, Sato Y, Tsumagari K, et al. Phenotypic insights into anti-IgLON5 disease in IgLON5-deficient mice. Neurol Neuroimmunol Neuroinflamm. 2024;11:e200234.
19. Sabater L, Planagumà J, Dalmau J, Graus F. Cellular investigations with human antibodies associated with the anti-IgLON5 syndrome. J Neuroinflammation. 2016;13:226.
20. Nissen MS, Blaabjerg M. Anti-IgLON5 disease: a case with 11-year clinical course and review of the literature. Front Neurol. 2019;10:1056.

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ORIGINAL COMMUNICATION

Prevalence and motor-functional correlates of frontotemporal-*spectrum* disorders in a large cohort of non-demented ALS patients

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Abstract

Background This study aimed at (1) delivering generalizable estimates of the prevalence of frontotemporal-*spectrum* disorders (FTSDs) in non-demented ALS patients and (2) exploring their motor-functional correlates.

Methods $N = 808$ ALS patients without FTD were assessed for motor-functional outcomes—*i.e.*, disease duration, severity (ALSFRS-R), progression rate (ΔFS), and stage (King's and Milano–Torino—MiToS—systems)—cognition—via the cognitive section of the Edinburgh Cognitive and Behavioural ALS Screen (ECAS)—and behaviour—via the ECAS-Carer Interview. Neuropsychological phenotypes were retrieved via Strong's revised *criteria*—*i.e.*, ALS cognitively and behaviourally normal (ALScbn) or cognitively and/or behaviourally impaired (ALSci/bi/cbi).

Results Defective ECAS-Total performances were detected in ~ 29% of patients, with the ECAS-Executive being failed by the highest number of patients (~ 30%), followed by the ECAS-Language, -Fluency, and -Memory (~ 15–17%) and -Visuospatial (~ %8). Apathy was the most frequent behavioural change (~ 28%), followed by loss of sympathy/empathy (~ 13%); remaining symptoms were reported in < 4% of patients. The distribution of Strong's classifications was as follows: ALScbn: 46.7%; ALSci/bi/cbi: 22.9%/20.0%/10.4%. Multinomial regressions on Strong's classifications revealed that lower ALSFRS-R scores were associated with a higher probability of ALSbi and ALScbi classifications ($p \leq .008$). Higher King's and MiToS stages were associated with a higher probability of ALSbi classification ($p \leq .031$).

Conclusions FTSDs affect ~ 50% of non-demented ALS patients, with cognitive deficits being as frequent as behavioural changes. A higher degree of motor-functional involvement is associated with worse behavioural outcomes—with this link being weaker for cognitive deficits.

Keywords Amyotrophic lateral sclerosis · Frontotemporal degeneration · Neuropsychology · Epidemiology

Background

It is thoroughly acknowledged that a non-negligible proportion of non-demented amyotrophic lateral sclerosis (ALS) patients may present with frontotemporal-*spectrum* disorders (FTSDs) [1, 2].

However, currently available prevalence estimates of cognitive (*i.e.*, ~ 35–50%) [3–5] and behavioural (*i.e.*, ~ 25–40%) involvement [6, 7] in this population are moderately heterogeneous—possibly as a result of (1) the paucity of large-scale studies and (2) the unsystematic employment of gold-standard, ALS-specific measures

Barbara Poletti, Edoardo Nicolò Aiello, Monica Consonni, and Barbara Iazzolino share the first authorship.

Vincenzo Silani, Giuseppe Lauria, Adriano Chiò and Nicola Ticozzi share the last authorship.

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Deep brain stimulation; verbal fluency; Parkinson's disease; total electrical energy delivered; surgery

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Can total electrical energy (TEED) after subthalamic DBS alter verbal fluency in Parkinson's disease patients? A preliminary evidence

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Abstract

Objective. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) improves motor outcomes in Parkinson's disease (PD) but may have adverse long-term effects on specific cognitive domains. The aim of this study was to investigate the association between total electrical energy (TEED) delivered by DBS and postoperative changes in verbal fluency.

Methods. Seventeen PD patients undergoing bilateral STN-DBS were assessed with the Alternate Verbal Fluency Battery (AVFB), which includes phonemic (PVF), semantic (SVF), and alternate verbal fluency (AVF) tests, before surgery (T0) and after 6 (T1) and 12 months (T2). Bilateral TEED and average TEEDM were recorded at T1 and T2. For each AVFB measurement, changes from T0 to T1 (Δ-01) and from T0 to T2 (Δ-02) were calculated.

Results. At T1, PVF (p = 0.007) and SVF scores (p = 0.003) decreased significantly. TEED measures at T1 and T2 were unrelated to Δ-01 and Δ-02 scores, respectively. However, an inverse, marginally significant association was detected between the TEEDM and Δ-01 scores for the AVF (p = 0.041, against an $\alpha_{adjusted}$ = 0.025).

Conclusions. In conclusion, the present reports provide preliminary evidence that TEED may not be responsible or only slightly responsible for the decline in VF performance after STN-DBS in PD.

Introduction

While deep brain stimulation (DBS) of the subthalamic nucleus (STN) does ameliorate motor outcomes in Parkinson's disease (PD), such a surgical treatment might entail detrimental, long-term effects on specific cognitive domains – *i.e.* language, memory, and executive functioning.[1]

Data on the neuropsychological outcome of STN-DBS usually do not show global cognitive deterioration,[2] with the exception of a specific sample composed of elderly patients, who are more at risk of decompensation,[3,4] and patients who suffered from preoperative cognitive difficulties.[5] Therefore, comparisons between preoperative and postoperative (3 to 12 months after surgery) assessments have shown less consistent effects on global cognition and other cognitive tasks, while postoperative declines in verbal fluency[6–8] have been reported.

To the aim of detecting post-DBS cognitive decline, verbal fluency (VF) tests have been thoroughly shown to be suitable.[9] However, little is known about the underpinnings of VF changes after STN-DBS, with both disease- and treatment-related causes having been postulated.[10]

Among treatment-related factors possibly accounting for post-STN-DBS changes in VF, the total electrical energy delivered (TEED)[11] to the STN has been to this day largely neglected. Indeed, to the best of the authors' knowledge, only one study[12] has recently approached this matter: here, an association between increased TEED to the left STN and a decrease in semantic

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Original Article

NEK1 haploinsufficiency worsens DNA damage, but not defective ciliogenesis, in *C9ORF72* patient-derived iPSC-motoneurons

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Abstract

The hexanucleotide G_4C_2 repeat expansion (HRE) in *C9ORF72* gene is the major cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), leading to both loss- and gain-of-function pathomechanisms. The wide clinical heterogeneity among *C9ORF72* patients suggests potential modifying genetic and epigenetic factors. Notably, *C9ORF72* HRE often co-occurs with other rare variants in ALS/FTD-associated genes, such as *NEK1*, which encodes for a kinase involved in multiple cell pathways, including DNA damage response and ciliogenesis. In this study, we generated induced pluripotent stem cells (iPSCs) and differentiated motoneurons (iPSC-MNs) from an ALS patient carrying both *C9ORF72* HRE and a *NEK1* loss-of-function mutation to investigate the biological effect of *NEK1* haploinsufficiency on *C9ORF72* pathology in a condition of oligogenicity. Double mutant *C9ORF72/NEK1* cells showed increased pathological *C9ORF72* RNA foci in iPSCs and higher DNA damage levels in iPSC-MNs compared to single mutant *C9ORF72* cells, but no effect on DNA damage response. When we analysed the primary cilium, we observed a defective ciliogenesis in *C9ORF72* iPSC-MNs which was not worsened by *NEK1* haploinsufficiency in the double mutant iPSC-MNs. Altogether, our study shows that *NEK1* haploinsufficiency influences differently DNA damage and cilia length, potentially acting as a modifier at biological level in an *in vitro* ALS patient-derived disease model of *C9ORF72* pathology.

Keywords: ALS; *C9ORF72*; *NEK1*; iPSC-motoneurons; primary cilium

Introduction

The hexanucleotide G_4C_2 repeat expansion (HRE) in the first intron of *C9ORF72* gene represents the major genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) (OMIM_#105550) [1, 2]. *C9ORF72* HRE-associated pathomechanisms comprise both loss- and gain-of-function conditions, the latter characterized by the formation of toxic HRE-containing RNA foci and the translation of dipeptide-repeat proteins (DPRs) which altogether lead to alterations of several cellular pathways [3]. The wide clinical heterogeneity observed among *C9ORF72* patients, encompassing disease manifestation (ALS/FTD), age of onset, progression rate and clinical symptoms, suggests the potential influence of additional genetic and epigenetic modifying factors. Among the genetic modifiers, the oligogenicity condition in *C9ORF72* carriers has already been described, although its correlation with specific clinical features still remains uncertain [4–6]. Of note, *C9ORF72* HRE is frequently found in combination with rare variants in other ALS and/or FTD-associated genes, including *TARDBP*, *SOD1*, *FUS* and other minor genes, such as *NEK1* [7, 8]. *NEK1* heterozygous loss-of-function (LOF) variants and the missense p.Arg261His variant have been identified in about 3% of both familial and sporadic ALS cases [9, 10]. *NEK1* encodes for a serine/threonine tyrosine kinase involved in the maintenance of genomic stability and DNA damage response (DDR) [11], cell cycle regulation [12, 13], mitochondrial activity [14] and ciliogenesis [15, 16], although the biological effects of ALS-related *NEK1* mutations on these pathways remain largely unexplored.

Different studies have already used induced-pluripotent stem cells (iPSCs) and differentiated motoneurons (iPSC-MNs) to investigate the effect of *C9ORF72* HRE [17] and *NEK1* LOF mutations on DNA damage and DDR [18] and on nucleo-cytoplasmic transport [19]. However, the combined effect of distinct ALS gene variants has been poorly investigated functionally so far and patient-derived iPSC-MNs represent a suitable disease model for this purpose.

Results

Generation and characterization of iPSCs from the double mutant *C9ORF72/NEK1* ALS patient

By a genetic screening of an Italian ALS cohort (*unpublished data*) we identified a patient carrying *C9ORF72* HRE and a

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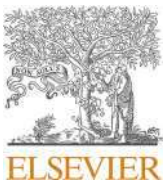

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Continuity of treatment in ALS: Benefits and challenges of maintaining riluzole over the course of the disease

ARTICLE INFO

Keywords
ALS
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Formulation
Riluzole
Film
Orodispersible

1. Introduction

The regular and consistent administration of medication – taken accurately and as prescribed by the treating physician – is a cornerstone of chronic disease management [1–3]. Multiple analyses have demonstrated that non-adherence to medication is associated with suboptimal health outcomes [1–3]. However, the reasons contributing to non-adherence are complex and varied [2,3]. Furthermore, adherence – the extent to which a patient's behaviour aligns with their physician's prescribing instructions [1,3] – is not the only factor when considering continuity of treatment. A chronic disease that remains undetected or mis-diagnosed, for example, cannot be treated until the condition is successfully identified [4]. And in some settings – particularly progressive and neurodegenerative illnesses – maintaining treatment continuity can be difficult despite a willingness to adhere due to the practical challenges presented by the patient's symptoms, functional decline and requirement for external assistance [3,5–8].

Every chronic disease presents its own unique challenges with regard to maintaining treatment continuity, but amyotrophic lateral sclerosis (ALS) possesses several attributes that make avoiding treatment interruption a particularly difficult prospect.

2. Background

ALS is a relentlessly progressive neurodegenerative disease for which there is currently no cure. Median survival is approximately 3 years following symptom onset, and death is mostly attributed to respiratory muscle failure [9].

Despite extensive research, pharmacological treatment options are limited. At present, riluzole remains the only licensed therapy for ALS in the EU [9,10]. The American FDA approved intravenously-administered edaravone in 2017 (and an oral formulation in 2022) and a sodium phenylbutyrate/taurursodiol combination in 2022 [11] – but riluzole remains the gold-standard of available treatment options, as evidenced by the fact that in clinical trials, new agents tend to be compared with riluzole or used as an add-on treatment [12,13]. Recent high-profile study failures investigating more recently-developed ALS therapies have further highlighted the importance of riluzole.

The pivotal clinical trials that resulted in riluzole's approval demonstrated significant, albeit modest improvements in median survival of 2–3 months, translating to a 9% increase in survival probability for one year [9,14,15]. However, subsequent real-world data analyses have suggested a more substantial benefit of between 6 and 19 months [15,16]. Such a discrepancy may be due to the fact that participants in the pivotal trials tended to have longstanding, possibly treatment-resistant disease [16]. In the fixed-dose and dose-ranging trials, the mean time from diagnosis to treatment was 2.2 and 1.8 years, respectively [16]. Given median survival is only 3 years [9] and patients are now more typically prescribed riluzole approximately 9–14 months after symptom onset [16] any survival benefit demonstrated from these trials is likely an underestimation.

The specific stage of disease at which riluzole provides the most benefit has also been the subject of further investigation and debate. It was once believed that riluzole had little effect in the latter stages of ALS – indeed, guidelines published by the European Federation of Neurological Societies (EFNS) over a decade ago reinforced this notion [17].

It is certainly apparent that the treatment effect of riluzole is more pronounced when given in the earlier stages of disease – most notably in bulbar-onset patients [18,19]. And its modest but significant effect in slowing the decline of bulbar and limb function (as evaluated via modified Norris scales) suggests a tangible functional advantage of earlier treatment [14].

However, other studies have demonstrated that the benefit of riluzole occurs predominantly in the *advanced* stages, prolonging survival in

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analysis of data from a dose-ranging study, Lancet Neurol. 17 (2018) 416–422, https://doi.org/10.1016/S1474-4422(18)30054-1.
[21] A.D. de Jongh, R.P.A. van Eijk, L.H. van den Berg, Evidence for a multimodal effect of riluzole in patients with ALS? J. Neurol. Neurosurg. Psychiatry 90 (10) (2019) 1183–1184, https://doi.org/10.1136/jnnp-2018-320211.
[22] K. Kibum, S. Kim, M. Katana, et al., Riluzole is Associated With Reduced Risk of Heart Failure, medRxiv 12.28.23300552, 2023, https://doi.org/10.1101/2023.12.28.23300552.
[23] A.A. Taylor, C. Fournier, M. Polak, et al., Predicting disease progression in amyotrophic lateral sclerosis, Ann. Clin. Transl. Neurol. 3 (11) (2016) 866–875, https://doi.org/10.1002/acn3.348.
[24] S. Jahandideh, A.A. Taylor, D. Beaulieu, et al., Longitudinal modeling to predict vital capacity in amyotrophic lateral sclerosis, Amyotroph. Lateral Scler. Frontotemporal. Degener. 19 (3–4) (2017) 294–302, https://doi.org/10.1080/21678421.2017.1418003.
[25] R.G. Huber, S. Pandey, D. Chhangani, et al., Identification of potential pathways and biomarkers linked to progression in ALS, Ann. Clin. Transl. Neurol. 10 (2) (2022) 150–165, https://doi.org/10.1002/acn3.51697.
[26] H. Seibold, A. Zeileis, T. Hothorn, Model-based recursive partitioning for subgroup analyses, Int. J. Biostat. 12 (1) (2016) 45–63, https://doi.org/10.1515/ijb-2015-0032.
[27] H. Seibold, A. Zeileis, T. Hothorn, Individual treatment effect prediction for amyotrophic lateral sclerosis patients, Stat. Methods Med. Res. 27 (10) (2018) 3104–3125, https://doi.org/10.1177/0962280217693034.
[28] S. Giometto, M. Finocchietti, O. Paoletti, CAESAR study group, et al., Adherence to riluzole therapy in patients with amyotrophic lateral sclerosis in three Italian regions -the CAESAR study, Pharmacoepidemiol. Drug Saf. 33 (1) (2024) e5736, https://doi.org/10.1002/pds.5736.
[29] A. Geronimo, R.M. Albertson, J. Noto, Z. Simmons, Ten years of riluzole use in a tertiary ALS clinic, Muscle Nerve 65 (6) (2022) 659–666, https://doi.org/10.1002/mus.27541.
[30] P. Van Damme, A. Al-Chalabi, P.M. Andersen, et al., European academy of neurology (EAN) guideline on the management of amyotrophic lateral sclerosis in collaboration with European reference network for neuromuscular diseases (ERN EURO-NMD), Eur. J. Neurol. 12 (2024) e16264, https://doi.org/10.1111/ene.16264.
[31] H. Mitsumoto, E.J. Kasarskis, Z. Simmons, Hastening the diagnosis of amyotrophic lateral sclerosis, Neurology 99 (2022) 60–68, https://doi.org/10.1212/WNL.0000000000200799.
[32] J. Caga, S. Hsieh, P. Lillo, et al., The impact of cognitive and behavioral symptoms on ALS patients and their caregivers, Front. Neurol. 10 (2019) 192, https://doi.org/10.3389/fneur.2019.00192.
[33] G. Colombo, R. Artico, D. Barbareschi, Riluzole Oral suspension for the treatment of amyotrophic lateral sclerosis: texture and compatibility with food thickeners evaluation, J 3 (2020) 275–288, https://doi.org/10.3390/j3030021.
[34] E. Onesti, I. Schettino, M.C. Gori, et al., Dysphagia in amyotrophic lateral sclerosis: impact on patient behavior, diet adaptation, and riluzole management, Front. Neurol. 8 (2017) 94, https://doi.org/10.3389/fneur.2017.00094.
[35] A. Casiraghi, A. Gentile, I. Marjanovic, A. Chiò, Crushing riluzole tablets: evaluation of loss of powder and active principle in a home-simulation experiment, Amyotroph. Lateral Scler. Frontotemporal. Degener. 24 (2023) 634–640, https://doi.org/10.1080/21678421.2023.2245860.
[36] A.M. Dyer, A. Smith, Riluzole 5 mg/mL oral suspension: for optimized drug delivery in amyotrophic lateral sclerosis, Drug Des. Dev. Ther. 11 (2017) 59–64, https://doi.org/10.2147/DDDT.S123776.
[37] B.R. Brooks, P. Bettica, S. Cazzaniga, Riluzole oral suspension: bioavailability following percutaneous gastrostomy tube-modeled administration versus direct oral administration, Clin. Ther. 41 (2019) 2490–2499, https://doi.org/10.1016/j.clinthera.2019.09.016.
[38] Teglutik Summary of Product Characteristics, 2024.
[39] Emylif Summary of Product Characteristics, 2024.
[40] A.F.D. Di Stefano, M.M. Radicioni, A. Segantin, et al., Randomised, 2-sequence, 4-period replicate cross-over bioequivalence study of a new riluzole orodispersible film vs. a reference tablet in healthy volunteers, J. Bioeq. Stud. 8 (1) (2022) 101. Available at: https://www.annexpublishers.co/full-text/JBS/8101/Randomised-2-Sequence-4-Period-Replicate.php.
[41] J. Wymer, S. Apple, A. Harrison, B.A. Hill, Pharmacokinetics, bioavailability, and swallowing safety with riluzole oral film, Clin. Pharmacol. Drug Dev. 12 (2023) 57–64, https://doi.org/10.1002/cpdd.1168.

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GUIDELINES

European Academy of Neurology (EAN) guideline on the management of amyotrophic lateral sclerosis in collaboration with European Reference Network for Neuromuscular Diseases (ERN EURO-NMD)

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Funding information
ERN Euro-NMD; European Academy of Neurology; ALS Liga Belgium; EUpALS; ENCALS

Abstract

Background: This update of the guideline on the management of amyotrophic lateral sclerosis (ALS) was commissioned by the European Academy of Neurology (EAN) and prepared in collaboration with the European Reference Network for Neuromuscular Diseases (ERN EURO-NMD) and the support of the European Network for the Cure ALS (ENCALS) and the European Organization for Professionals and Patients with ALS (EUpALS).

Methods: Grading of Recommendations Assessment, Development, and Evaluation (GRADE) methodology was used to assess the effectiveness of interventions for ALS. Two systematic reviewers from Cochrane Response supported the guideline panel. The working group identified a total of 26 research questions, performed systematic reviews, assessed the quality of the available evidence, and made specific recommendations. Expert consensus statements were provided where insufficient evidence was available.

Results: A guideline mapping effort revealed only one other ALS guideline that used GRADE methodology (a National Institute for Health and Care Excellence [NICE] guideline). The available evidence was scarce for many research questions. Of the 26 research questions evaluated, the NICE recommendations could be adapted for 8 questions. Other recommendations required updates of existing systematic reviews or de novo reviews. Recommendations were made on currently available disease-modifying treatments, multidisciplinary care, nutritional and respiratory support, communication aids, psychological support, treatments for common ALS symptoms (e.g., muscle cramps, spasticity, pseudobulbar affect, thick mucus, sialorrhea, pain), and end-of-life management.

For Affiliation refer page on 12

Resuscitation orders, and Lasting Power of Attorney when interventions such as gastrostomy and NIV are planned. [++]

- Provide additional support as the end of life approaches, for example, additional psychological, social, or nursing care to enable informal carers and family to reduce their carer responsibilities and spend time with the person with ALS. [++]
- Towards the end of life, ensure there is prompt access to the following, if not already provided:
  - A method of communication that meets the person's needs, such as an AAC system
  - Specialist palliative care
  - Equipment, if needed, such as syringe drivers, suction machines, riser–recliner chair, hospital bed, commode, and hoist
  - Anticipatory medicines, including opioids and benzodiazepines to treat breathlessness, anxiety and antimuscarinic medicines to treat problematic saliva and respiratory secretions. [++]
- Offer bereavement support to family members and/or carers (as appropriate). [++]
- Take into account the spiritual support needs of the patient and their family and/or carers (as appropriate). [new recommendation added by EAN]. [++]

Remarks:

Discuss Advance Decisions to Refuse Treatment, Do Not Attempt Resuscitation orders, and Lasting Power of Attorney according to the local law. [added by EAN].

Discuss euthanasia and assisted suicide in countries where it is legal. [added by EAN].

CONCLUSIONS

This guideline on the management of ALS is an update of the EFNS guideline published in 2012. It contains updated recommendations on the management of ALS and includes recommendations on new emerging therapies for ALS. The landscape of ALS therapies is rapidly changing and further updates will be prepared when new evidence becomes available.

AUTHOR CONTRIBUTIONS

Philip Van Damme: Conceptualization; funding acquisition; writing – original draft; investigation; supervision; validation; data curation; project administration. **Ammar Al-Chalabi:** Conceptualization; writing – review and editing; validation; investigation. **Peter M. Andersen:** Conceptualization; validation; writing – review and editing; investigation. **Adriano Chiò:** Conceptualization; validation; writing – review and editing; investigation. **Philippe Couratier:** Conceptualization; validation; writing – review and editing; investigation. **Mamede De Carvalho:** Conceptualization; investigation; validation; writing – review and editing. **Orla Hardiman:** Conceptualization; investigation; validation; writing – review and editing. **Magdalena Kuzma-Kozakiewicz:** Conceptualization; investigation; validation; writing – review and editing. **Albert Ludolph:** Conceptualization; investigation; validation; writing – review and editing. **Christopher J. McDermott:** Conceptualization; investigation; validation; writing – review and editing. **Jesus S. Mora:** Conceptualization; investigation; validation; writing – review and editing. **Susanne Petri:** Conceptualization; investigation; validation; writing – review and editing. **Katrin Probyn:** Writing – original draft; methodology; validation; software; formal analysis; data curation; conceptualization; investigation; project administration; visualization. **Evy Reviers:** Validation; writing – review and editing; funding acquisition. **François Salachas:** Conceptualization; investigation; validation; writing – review and editing. **Vincenzo Silani:** Conceptualization; investigation; validation; writing – review and editing. **Ole-Bjorn Tysnes:** Conceptualization; investigation; validation; writing – review and editing. **Leonard H. van den Berg:** Conceptualization; investigation; validation; writing – review and editing; funding acquisition. **Gemma Villanueva:** Conceptualization; investigation; writing – original draft; methodology; validation; visualization; software; formal analysis; data curation; supervision; project administration. **Markus Weber:** Conceptualization; investigation; validation; writing – review and editing.

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ORIGINAL ARTICLE

Cerebrospinal fluid and blood neurofilament light chain levels in amyotrophic lateral sclerosis and frontotemporal degeneration: A meta-analysis

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Abstract

Background and purpose: Neurofilament light chain (NFL) has been shown to be increased in amyotrophic lateral sclerosis (ALS) and, to a lesser extent, in frontotemporal dementia (FTD). A meta-analysis of NFL in ALS and FTD was performed.

Methods: Available studies comparing cerebrospinal fluid and blood NFL levels in ALS versus neurologically healthy controls (NHCs), other neurological diseases (ONDs) and ALS mimics, as well as in FTD and related entities (behavioural variant of FTD and frontotemporal lobar degeneration syndromes) versus NHCs, ONDs and other dementias were evaluated.

Results: In ALS, both cerebrospinal fluid and blood levels of NFL were higher compared to other categories. In FTD, behavioural variant of FTD and frontotemporal lobar degeneration syndromes, NFL levels were consistently higher compared to NHCs; however, several comparisons with ONDs and other dementias did not demonstrate significant differences.

Discussion: Amyotrophic lateral sclerosis is characterized by higher NFL levels compared to most other conditions. In contrast, NFL is not as good at discriminating FTD from other dementias.

KEYWORDS
amyotrophic lateral sclerosis (ALS), biomarkers, cerebrospinal fluid, frontotemporal dementia (FTD), neurofilament light chain (NFL)

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper motor neurons of the motor cortex and lower motor neurons of the brainstem and spinal cord. It causes progressive paralysis of voluntary muscles leading to death after a median time of 3 years from symptom onset. Approximately 10% of cases are familial, caused by genetic mutations in one of >30 genes, usually with autosomal dominant inheritance. The diagnosis is mainly clinical and is supported by electromyography [1].

Frontotemporal dementia (FTD) is the third most common form of degenerative dementia after Alzheimer's disease (AD) and dementia with Lewy bodies. It has a strong genetic component, with up to one-third of cases having a positive family history, most commonly

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