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"CENTRO DINO FERRARI"
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NEUROMUSCOLARI E NEURODEGENERATIVE

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" CENTRO DINO FERRARI"

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Ataluren in patients with nonsense mutation Duchenne muscular dystrophy (ACT DMD): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial

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Summary

Background Duchenne muscular dystrophy (DMD) is a severe, progressive, and rare neuromuscular, X-linked recessive disease. Dystrophin deficiency is the underlying cause of disease; therefore, mutation-specific therapies aimed at restoring dystrophin protein production are being explored. We aimed to assess the efficacy and safety of ataluren in ambulatory boys with nonsense mutation DMD.

Methods We did this multicentre, randomised, double-blind, placebo-controlled, phase 3 trial at 54 sites in 18 countries located in North America, Europe, the Asia-Pacific region, and Latin America. Boys aged 7–16 years with nonsense mutation DMD and a baseline 6-minute walk distance (6MWD) of 150 m or more and 80% or less of the predicted normal value for age and height were randomly assigned (1:1), via permuted block randomisation (block size of four) using an interactive voice-response or web-response system, to receive ataluren orally three times daily (40 mg/kg per day) or matching placebo. Randomisation was stratified by age (<9 years *vs* ≥9 years), duration of previous corticosteroid use (6 months to <12 months *vs* ≥12 months), and baseline 6MWD (<350 m *vs* ≥350 m). Patients, parents and caregivers, investigational site personnel, PTC Therapeutics employees, and all other study personnel were masked to group allocation until after database lock. The primary endpoint was change in 6MWD from baseline to week 48. We additionally did a prespecified subgroup analysis of the primary endpoint, based on baseline 6MWD, which is reflective of anticipated rates of disease progression over 1 year. The primary analysis was by intention to treat. This study is registered with ClinicalTrials.gov, number NCT01826487.

Findings Between March 26, 2013, and Aug 26, 2014, we randomly assigned 230 patients to receive ataluren (n=115) or placebo (n=115); 228 patients comprised the intention-to-treat population. The least-squares mean change in 6MWD from baseline to week 48 was –47·7 m (SE 9·3) for ataluren-treated patients and –60·7 m (9·3) for placebo-treated patients (difference 13·0 m [SE 10·4], 95% CI –7·4 to 33·4; p=0·213). The least-squares mean change for ataluren versus placebo in the prespecified subgroups was –7·7 m (SE 24·1, 95% CI –54·9 to 39·5; p=0·749) in the group with a 6MWD of less than 300 m, 42·9 m (15·9, 11·8–74·0; p=0·007) in the group with a 6MWD of 300 m or more to less than 400 m, and –9·5 m (17·2, –43·2 to 24·2; p=0·580) in the group with a 6MWD of 400 m or more. Ataluren was generally well tolerated and most treatment-emergent adverse events were mild to moderate in severity. Eight (3%) patients (n=4 per group) reported serious adverse events; all except one event in the placebo group (abnormal hepatic function deemed possibly related to treatment) were deemed unrelated to treatment.

Interpretation Change in 6MWD did not differ significantly between patients in the ataluren group and those in the placebo group, neither in the intention-to-treat population nor in the prespecified subgroups with a baseline 6MWD of less than 300 m or 400 m or more. However, we recorded a significant effect of ataluren in the prespecified subgroup of patients with a baseline 6MWD of 300 m or more to less than 400 m. Baseline 6MWD values within this range were associated with a more predictable rate of decline over 1 year; this finding has implications for the design of future DMD trials with the 6-minute walk test as the endpoint.

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Introduction

Duchenne muscular dystrophy (DMD) is a severe, progressive, and rare neuromuscular, X-linked recessive disease.[1] Corticosteroids and better coordinated care have improved outcomes in patients with DMD in the past few decades,[2,3] but these approaches do not specifically target dystrophin deficiency—the underlying cause of disease.[4] Mutation-specific therapies aimed at restoring dystrophin protein production are therefore being explored. Ataluren promotes readthrough of a nonsense mutation to produce full-length functional dystrophin protein.[4–7] About 10–15% of patients with

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Declaration of interests

CMM has acted as a consultant on clinical trials of Duchenne muscular dystrophy (DMD) for BioMarin, Catabasis, Eli Lilly, Italfarmaco, Mitobridge, Pfizer, PTC Therapeutics, Santhera Pharmaceuticals, and Sarepta Therapeutics, and has received research support for clinical trials from BioMarin, Eli Lilly, PTC Therapeutics, and Sarepta Therapeutics. CC has collaborated on clinical trials with Acceleron, Biogen, BioMarin, Eli Lilly, Ionis Pharmaceuticals, Pfizer, and PTC Therapeutics. RSF has acted as a consultant for AveXis, Biogen, BioMarin, Catabasis, Eli Lilly, Ionis Pharmaceuticals, Mitobridge, Novartis, PTC Therapeutics, Roche, Sarepta Therapeutics, and Summit Therapeutics, and has received research support for clinical trials from Bristol-Myers Squibb, Cytokinetics, PTC Therapeutics, ReveraGen BioPharma, Sarepta Therapeutics, Santhera Pharmaceuticals, and Summit Therapeutics. KMF has acted as a consultant for Audentes Therapeutics, Italfarmaco, Marathon Pharmaceuticals, PTC Therapeutics, Santhera Pharmaceuticals, Sarepta Therapeutics, Tivorsan; has served as a site investigator for Abeona Therapeutics, Akashi Therapeutics, BioMarin, and PTC Therapeutics; and receives research support unrelated to this work from the National Institutes of Health (National Institute of Arthritis and Musculoskeletal and Skin Diseases, and National Institute of Neurological Disorders and Stroke) and CureDuchenne. NG is a site principal investigator for the PTC Therapeutics extension study of ataluren in DMD and has acted as a consultant and/or advisory board member for BioMarin, Biogen, Bristol-Myers Squibb, Eli Lilly, Italfarmaco, PTC Therapeutics, Roche, and Summit Therapeutics. PH has acted as a consultant for Marathon Pharmaceuticals, PTC Therapeutics, and Sarepta Therapeutics. AK has received speaker fees from PTC Therapeutics. JK has acted as a consultant for AveXis, Biogen, Ionis Pharmaceuticals, PTC Therapeutics, and Roche, and has received research support for taking part in clinical research from Biogen, BioMarin, GlaxoSmithKline, Ionis Pharmaceuticals, Novartis, PTC Therapeutics, Roche, Santhera Pharmaceuticals, and Trophos. FM has received consulting fees from Akashi Therapeutics, Biogen, BioMarin, Catabasis, Italfarmaco, Pfizer, PTC Therapeutics, Roche, Sarepta Therapeutics, and Tivorsan, and is supported by the National Institute of Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust, and University College London. ANO has received speaker and consulting fees from PTC Therapeutics. US is a site principal investigator for the PTC Therapeutics extension study of ataluren in DMD and for the GlaxoSmithKline–Prosensa studies on exon skipping, and has acted as an advisory board member for PTC Therapeutics. TS has received speaking and expert consultancy fees from Biogen, BioMarin, and PTC Therapeutics. PBS has received speaking fees from Catalyst Pharmaceuticals, Grifols, and PTC Therapeutics; has acted as an ad-hoc consultant for Genentech and Ultragenyx; has acted as an advisory board member for AveXis, BioBlast, Biogen, BioMarin, Catabasis, Cytokinetics, Marathon Pharmaceuticals, and Novartis; and has received research support from Biogen, Catabasis Pharmaceuticals, Ionis Pharmaceuticals, Marathon Pharmaceuticals, Novartis, PTC Therapeutics, and Ultragenyx. HLS has acted as a consultant for PTC Therapeutics. MT has received lecture fees from PTC Therapeutics and has acted as a consultant on DMD clinical trials for PTC Therapeutics and BioMarin, and an advisory board member for AveXis. JJV has received consulting fees from BioMarin, Genzyme, Pfizer, and PTC Therapeutics. TV has acted as an advisory board member for Prosensa-BioMarin and Tarix Orphan, and has acted as a consultant for BioMarin, Debiopharm, FibroGen, Laboratoires Servier, Santhera Pharmaceuticals, and Sarepta Therapeutics. BW has acted as an advisory board member for BioMarin, Gilead Sciences, and Sarepta Therapeutics, and has received research support from, Akashi, Eli Lilly, Pfizer, Prosensa-BioMarin, PTC Therapeutics, and Sarepta Therapeutics. EM has acted as an advisory board member for AveXis, Biogen,

Safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal muscular atrophy: a randomised, double-blind, placebo-controlled phase 2 trial

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Summary

Background Spinal muscular atrophy (SMA) is a progressive motor neuron disease causing loss of motor function and reduced life expectancy, for which limited treatment is available. We investigated the safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 SMA.

Methods This randomised, double-blind, placebo-controlled, phase 2 study was done in 22 neuromuscular care centres in Belgium, France, Germany, Italy, Netherlands, Poland, and the UK. Safety and efficacy of olesoxime were assessed in patients aged 3–25 years with genetically confirmed type 2 or non-ambulatory type 3 SMA. A centralised, computerised randomisation process allocated patients (2:1 with stratification by SMA type and centre) to receive olesoxime (10 mg/kg per day) in an oral liquid suspension or placebo for 24 months. Patients, investigators assessing outcomes, and sponsor study personnel were masked to treatment assignment. The primary outcome measure was change from baseline compared with 24 months between the two treatment groups in functional domains 1 and 2 of the Motor Function Measure (MFM D1+D2) assessed in the full analysis population. A shorter, 20-item version of the MFM, which was specifically adapted for young children, was used to assess patients younger than 6 years. Safety was assessed in the intention-to-treat population. The trial is registered with ClinicalTrials.gov, number NCT01302600.

Findings The trial was done between Nov 18, 2010, and Oct 9, 2013. Of 198 patients screened, 165 were randomly assigned to olesoxime (n=108) or placebo (n=57). Five patients in the olesoxime group were not included in the primary outcome analysis because of an absence of post-baseline assessments. The change from baseline to month 24 on the primary outcome measure was 0·18 for olesoxime and –1·82 for placebo (treatment difference 2·00 points, 96% CI –0·25 to 4·25, p=0·0676). Olesoxime seemed to be safe and generally well tolerated, with an adverse event profile similar to placebo. The most frequent adverse events in the olesoxime group were pyrexia (n=34), cough (n=32), nasopharyngitis (n=25), and vomiting (n=25). There were two patient deaths (one in each group), but these were not deemed to be related to the study treatment.

Interpretation Olesoxime was safe at the doses studied, for the duration of the trial. Although the primary endpoint was not met, secondary endpoints and sensitivity analyses suggest that olesoxime might maintain motor function in patients with type 2 or type 3 SMA over a period of 24 months. Based on these results, olesoxime might provide meaningful clinical benefits for patients with SMA and, given its mode of action, might be used in combination with other drugs targeting other mechanisms of disease, although additional evidence is needed.

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Introduction

Spinal muscular atrophy (SMA) is a rare and severely debilitating neuromuscular disease that manifests predominantly in infancy and childhood.[1,2] In type 2 and type 3 SMA, the deterioration of motor function results in substantial disability and in patients and a high burden for their caregivers.[3] SMA is caused by loss-of-function mutations in the Survival of Motor Neuron 1 (*SMN1*) gene. The absence of the *SMN1* gene results in insufficient levels of SMN protein in cells, which particularly affect motor neurons and neuromuscular junctions, leading to muscle weakness, hypotonia, and atrophy.[1,4]

Although reduced SMN protein levels impair many fundamental neuronal processes and are the triggering event in all SMA types, the downstream pathological consequences of atrophy and denervation are also related to mitochondrial dysfunction, which also affects other cell types.[5–8] Given their role on energy production, mitochondria are vital for cells with a high energy demand, including motor neurons and muscle fibres that are central to the pathophysiology of SMA.[5,7,9,10]

Current therapies in clinical development have aimed to increase SMN production systemically, either by replacing SMN1 (eg, gene therapy with AVXS-101) or by SMN2 splicing modulators (eg, RO6885247, RO7034067, and LMI070). Nusinersen, a SMN2 splicing modulator for intrathecal administration, has been approved by the US Food and Drug Administration for treatment of SMA.[11]

Biallelic *C1QBP* Mutations Cause Severe Neonatal-, Childhood-, or Later-Onset Cardiomyopathy Associated with Combined Respiratory-Chain Deficiencies

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Complement component 1 Q subcomponent-binding protein (C1QBP; also known as p32) is a multi-compartmental protein whose precise function remains unknown. It is an evolutionary conserved multifunctional protein localized primarily in the mitochondrial matrix and has roles in inflammation and infection processes, mitochondrial ribosome biogenesis, and regulation of apoptosis and nuclear transcription. It has an N-terminal mitochondrial targeting peptide that is proteolytically processed after import into the mitochondrial matrix, where it forms a homotrimeric complex organized in a doughnut-shaped structure. Although C1QBP has been reported to exert pleiotropic effects on many cellular processes, we report here four individuals from unrelated families where biallelic mutations in *C1QBP* cause a defect in mitochondrial energy metabolism. Infants presented with cardiomyopathy accompanied by multisystemic involvement (liver, kidney, and brain), and children and adults presented with myopathy and progressive external ophthalmoplegia. Multiple mitochondrial respiratory-chain defects, associated with the accumulation of multiple deletions of mitochondrial DNA in the later-onset myopathic cases, were identified in all affected individuals. Steady-state C1QBP levels were decreased in all individuals' samples, leading to combined respiratory-chain enzyme deficiency of complexes I, III, and IV. *C1qbp*$^{-/-}$ mouse embryonic fibroblasts (MEFs) resembled the human disease phenotype by showing multiple defects in oxidative phosphorylation (OXPHOS). Complementation with wild-type, but not mutagenized, *C1qbp* restored OXPHOS protein levels and mitochondrial enzyme activities in *C1qbp*$^{-/-}$ MEFs. C1QBP deficiency represents an important mitochondrial disorder associated with a clinical spectrum ranging from infantile lactic acidosis to childhood (cardio)myopathy and late-onset progressive external ophthalmoplegia.

Introduction

Mitochondrial disorders are an extremely heterogeneous group of inborn errors of metabolism and encompass a wide range of clinical presentations, such that approximately 300 disease-associated genes have been identified to date.[1,2] Mitochondrial dysfunction mainly affects organs with high energy requirements, such as the brain, central nervous system, muscle, and heart. The broad clinical and genetic presentation of mitochondrial disorders makes the molecular diagnosis challenging. Mutations can directly affect oxidative phosphorylation (OXPHOS) subunits or indirectly impair OXPHOS activity by disturbing mitochondrial homeostasis. Next-generation sequencing techniques (gene panels and exome and genome sequencing) are proving to be an appropriate tool for the diagnosis of this broad clinical group. However, any diagnostic approach continues to rely upon deep

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S2) to childhood myopathy, PEO, and later peripheral neuropathy (in proband S3) to adult-onset myopathy with PEO (in proband S4). All individuals presented with major cardiac symptoms, which resulted in early death in the neonatal form and were rather stable in individuals with later presentation. A very recently established cardiomyocyte-specific deletion of *C1qbp* resulted in contractile dysfunction, cardiac dilatation, and cardiac fibrosis and thereby confirmed an important function of C1QBP in the heart.[38] Decreased COXI and COXIII expression confirmed the mitochondrial dysfunction that resulted in cardiomyopathy at the age of 2 months and a median lifespan of approximately 14 months.[38]

In addition, the two individuals with a late disease onset presented with PEO and variable mtDNA deletions. The clinical manifestation of disorders with deletions in the mitochondrial genome is heterogeneous but often includes PEO.[2] In the group of disorders with multiple mtDNA deletions, cardiomyopathy is a rare symptom. It is rarely reported in individuals with variants in *POLG* (MIM: 258450)[39–41] and *TWNK* (MIM: 609286)[42] and has been reported in just a single individual with pathogenic variants in *MGME1* (MIM: 615084).[43] It is more commonly associated with autosomal-recessive deficiency of *SLC25A4* (cardiomyopathy types of the disease [MIM: 617184 and 615418]), another mtDNA maintenance gene, although variable mtDNA deletions are usually associated with dominant pathogenic variants in this gene.

Numerous functions in various cellular organelles have been reported for C1QBP.[16] The clinical manifestation of our cohort of probands with C1QBP deficiency was mainly attributed to defects in mitochondrial energy metabolism. No signs of immunologic dysfunction could be associated with the complement system.

Given our observations, the main functions of C1QBP reside within the mitochondrial compartment. However, the exact mechanism leading to a reduction of OXPHOS enzymes remains unclear, especially in the neonatal form.

In summary, we present four individuals with *C1QBP* mutations characterized by combined respiratory-chain deficiency and increased lactate. Disease onset was variable, including intrauterine onset, oligohydramnios, and neonatal cardiomyopathy leading to early death. Later onset in childhood or adulthood was associated with exercise intolerance, PEO, and multiple mtDNA deletions. Cardiomyopathy was found in all forms, which is a relatively unusual presentation in individuals with multiple mtDNA deletions. Peripheral neuropathy seems to be an issue; however, the central nervous system seems to be spared.

Supplemental Data

Supplemental Data include one figure and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2017.08.015.

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Web Resources

ExAC Browser, http://exac.broadinstitute.org/
GeneReviews, DiMauro, S., and Hirano, M. (1993). Mitochondrial DNA Deletion Syndromes, https://www.ncbi.nlm.nih.gov/books/NBK1203/
MutationTaster, http://www.mutationtaster.org
OMIM, https://www.omim.org
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/bgi.shtml
PROVEAN, http://provean.jcvi.org/genome_submit_2.php?species=human
RCSB Protein Data Bank, https://www.rcsb.org/pdb/home/home.do
UCSC Genome Browser, https://genome.ucsc.edu/
UnitProt, http://www.uniprot.org/

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REVIEW Open Access

Congenital myopathies: clinical phenotypes and new diagnostic tools

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Abstract

Congenital myopathies are a group of genetic muscle disorders characterized clinically by hypotonia and weakness, usually from birth, and a static or slowly progressive clinical course. Historically, congenital myopathies have been classified on the basis of major morphological features seen on muscle biopsy. However, different genes have now been identified as associated with the various phenotypic and histological expressions of these disorders, and in recent years, because of their unexpectedly wide genetic and clinical heterogeneity, next-generation sequencing has increasingly been used for their diagnosis. We reviewed clinical and genetic forms of congenital myopathy and defined possible strategies to improve cost-effectiveness in histological and imaging diagnosis.

Keywords: Congenital myopathy, Next generation sequencing, Muscle MRI, Muscle biopsy

Background

The term congenital myopathy refers to a group of clinically, genetically and histologically heterogeneous diseases that mainly affect muscle tissue. The presence of particular histopathological alterations on muscle biopsy distinguishes these conditions from other neuromuscular disorders. Congenital myopathy is caused by genetically determined defects in structural proteins of muscle and classified on the basis of muscle biopsy findings [1]. The onset generally occurs in the neonatal period. Although the precise epidemiology of congenital myopathy is not known, it has an estimated incidence of around 1:25,000, and has been reported to account for 14% of all cases of neonatal hypotonia [2]. Although the classification of congenital myopathy is under constant review as more genes are identified and associated with its various phenotypic and histological expressions, for the moment it continues to be based mainly on the features seen on muscle biopsy. Accordingly, congenital myopathy can be divided into the following five forms:

1. nemaline myopathy (subtypes: rod, core-rod, cap and zebra body myopathy);
2. core myopathy (subtypes: central core and multiminicore myopathy);
3. centronuclear myopathy (subtypes: myotubular myopathy and autosomal centronuclear myopathy);
4. congenital fiber-type disproportion myopathy;
5. myosin storage myopathy

This paper describes the different congenital myopathy disease types, focusing, in particular, on their diagnosis through muscle biopsy, their muscle MRI features, and the use of genetic testing based on cutting-edge gene analysis technologies (next-generation sequencing, NGS). Although adult-onset sporadic nemaline myopathy, spheroid body myopathy, sarcotubular myopathy and reducing body myopathy were all initially regarded as forms of congenital myopathy, they were recently excluded from the official classification [1] on the basis of expert opinion, which deemed that they may be more appropriately grouped with other neuromuscular disorders. For example, spheroid body myopathy caused by mutations in *TRIM32* and sarcotubular myopathy caused by mutations in *MYOT* may more correctly be grouped with the limb-girdle muscular dystrophies.

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Ethics approval and consent to participate
This study was approved by the ethics committee of IRCCS Stella Maris, Pisa, Italy and other participating institutions.

Consent for publication
All the procedures complied with the Helsinki Declaration of 1975. DNA, morphological, MRI and clinical studies were performed with parental written informed consent.

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

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Development of Therapeutics for C9ORF72 ALS/FTD-Related Disorders

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Abstract The identification of the hexanucleotide repeat expansion (HRE) GGGGCC (G4C2) in the non-coding region of the *C9ORF72* gene as the most frequent genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) has opened the path for advances in the knowledge and treatment of these disorders, which remain incurable. Recent evidence suggests that HRE RNA can cause gain-of-function neurotoxicity, but haploinsufficiency has also been hypothesized. In this review, we describe the recent developments in therapeutic targeting of the pathological expansion of *C9ORF72* for ALS, FTD, and other neurodegenerative disorders. Three approaches are prominent: (1) an antisense oligonucleotides/RNA interference strategy; (2) using small compounds to counteract the toxic effects directly exerted by RNA derived from the repeat transcription (foci), by the translation of dipeptide repeat proteins (DPRs) from the repeated sequence, or by the sequestration of RNA-binding proteins from the *C9ORF72* expansion; and (3) gene therapy, not only for silencing the toxic RNA/protein, but also for rescuing haploinsufficiency caused by the reduced transcription of the *C9ORF72* coding sequence or by the diminished availability of RNA-binding proteins that are sequestered by RNA foci. Finally, with the perspective of clinical therapy, we discuss the most promising progress that has been achieved to date in the field.

Keywords Hexanucleotide repeat expansion · Haploinsufficiency · Antisense oligonucleotides

Introduction

Amyotrophic lateral sclerosis (ALS) is an incurable and invariably fatal neurodegenerative condition characterized by the progressive loss of both upper and lower motor neurons in the cortex, brainstem, and spinal cord; it clinically results in progressive paralysis and death within 3–5 years of its onset, often due to respiratory failure [1]. No effective therapy is available for this disease. The only drug approved by the FDA and EMA is riluzole, which extends the median lifespan by only 3 months [1]. Thus, the discovery of clinically effective therapies is urgently needed. The vast majority of cases are sporadic (sALS) of unknown origin, while 5–10 % are familial (fALS), often with autosomal dominant inheritance [2]. The disease pathogenesis is multilayered, given that several pathways have been identified as key elements both in the onset and in the progression of the disease. Although several elements have been investigated as possible targets for treatment advancement, the lack of a clear understanding of the causes of ALS, particularly in cases of sALS, has hampered the search for a cure [1]. However, the genetic forms of ALS can offer a solid basis for research since, at least in these cases, the etiopathogenic primum movens is known. The first causative genetic mutations were described in Cu–Zn superoxide dismutase 1 (SOD1) gene in 1993 [3], and due to several genome sequencing projects, many of the genes responsible for ALS (>30 genes so far) have been described [4]. The identification of a hexanucleotide repeat expansion (HRE)

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Therapeutic Strategies Under Development Targeting Inflammatory Mechanisms in Amyotrophic Lateral Sclerosis

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Abstract Amyotrophic lateral sclerosis (ALS) is a neurological disease characterized by the progressive loss of cortical, bulbar, and spinal motor neurons (MNs). The cardinal manifestation of ALS is a progressive paralysis which leads to death within a time span of 3 to 5 years after disease onset. Despite similar final output of neuronal death, the underlying pathogenic causes are various and no common cause of neuronal damage has been identified to date. Inflammation-mediated neuronal injury is increasingly recognized as a major factor that promotes disease progression and amplifies the MN death-inducing processes. The neuroimmune activation is not only a physiological reaction to cell-autonomous death but is an active component of nonautonomous cell death. Such injury-perpetuating phenomenon is now proved to be a common mechanism in many human disorders characterized by progressive neurodegeneration. Therefore, it represents an interesting therapeutic target. To date, no single cell population has been proved to play a major role. The existing evidence points to a complex cross talk between resident immune cells and nonresident cells, like monocytes and T lymphocytes, and to a dysregulation in cytokine profile and in phenotype commitment. After a summary of the most important mechanisms involved in the inflammatory reaction in ALS, this review will focus on novel therapeutic tools that rely on tackling inflammation to improve motor function and survival. Herein, completed, ongoing, or planned clinical trials, which aim to modify the rapidly fatal course of this disease, are discussed. Anti-inflammatory compounds that are currently undergoing preclinical study and novel suitable molecular targets are also mentioned.

Keywords ALS · Inflammation · Microglia · Astrocytes · Anti-inflammatory drugs · ALS progression · Neurodegeneration · Motor neurons

Abbreviations

ALS	Amyotrophic lateral sclerosis
A-SMase	Acid sphingomyelinase
ABC	ATP-binding cassette
ALSFRS-R	ALS function rating scale revised
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP1	Activator protein 1
APP	Amyloid precursor protein
Arg1	Arginase 1
ATP	Adenosine triphosphate
AUC	Area under curve
BDNF	Brain-derived neurotrophic factor
KIT	Receptor tyrosine-kinase
C(max)	Maximum serum concentration
C/EBP	CCAAT-enhancer-binding protein
C9ORF72	Chromosome 9 open reading frame 72
CAFS	Combined assessment of function and survival
CB2	Cannabinoid receptor 2
CCAAT	Cytidine-cytidine-adenosine-adenosine-thymidine
CD	Cluster of differentiation
Chi3l3	Chitinase-3-like-3
CNS	Central nervous system
COX	Cyclooxygenase
CRP	C-reactive protein

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MicroRNA-Directed Neuronal Reprogramming as a Therapeutic Strategy for Neurological Diseases

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Abstract The loss of neurons due to injury and disease results in a wide spectrum of highly disabling neurological and neurodegenerative conditions, given the apparent limited capacity of endogenous repair of the adult central nervous system (CNS). Therefore, it is important to develop technologies that can promote de novo neural stem cell and neuron generation. Current insights in CNS development and cellular reprogramming have provided the knowledge to finely modulate lineage-restricted transcription factors and microRNAs (miRNA) to elicit correct neurogenesis. Here, we discuss the current knowledge on the direct reprogramming of somatic non-neuronal cells into neural stem cells or subtype specific neurons in vitro and in vivo focusing on miRNA driven reprogramming. miRNA can allow rapid and efficient direct phenotype conversion by modulating gene networks active during development, which promote global shifts in the epigenetic landscape pivoting cell fate decisions. Furthermore, we critically present state-of-the-art and recent advances on miRNA therapeutics that can be applied to the diseased CNS. Together, the advances in our understanding of miRNA role in CNS development and disease, recent progress in miRNA-based therapeutic strategies, and innovative drug delivery methods create novel perspectives for meaningful therapies for neurodegenerative disorders.

Keywords Neuronal repair · Therapeutics · microRNA · Reprogramming · Neural stem cells · Neurons

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Background

The loss of neuronal cell populations is a key feature that underlies different neurological and neurodegenerative diseases, which severely affect the life of many patients [1, 2]. The vast majority of these conditions still lack effective therapies. Since the disability is due to the critical loss of neurons, a rational approach aims to therapeutically induce neurogenesis compensating for the amount of dead cells [3–5]. The adult CNS is apparently incapable of major repair capacity given its inability to effectively replace neuronal circuitries and damaged tissues. The reasons of this defect are largely undetermined and it occurs despite the presence in the CNS of specific areas in which are located progenitor cells, which hold a certain degree of regenerative ability [6, 7]. However, the demonstration of self-renewing stem/progenitor cell populations in the adult CNS has raised the hypothesis to artificially manipulate their potential for an effective endogenous CNS regeneration after injuries [3–5].

On the other hand, the regenerative efficacy of transplanted neuronal stem and progenitor cells has been increasingly analyzed, also in clinical trials, but this approach is still in its infancy and likely requires invasive cell administration to the CNS [8]. As alternative, in vivo direct reprogramming of somatic CNS cells into neural stem cells (NSCs) or directly into specific neuronal subtype has been suggested as a possible approach for tissue repairing, overcoming the limits related to invasive cell transplantation. Many of the experimental efforts focus on converting glial cells into stem cell, progenitor or fully differentiated neurons. Glial cells are the most abundant cells in the adult brain and thus could represent a suitable target [1, 9].

In 2006, Takahashi and Yamanaka modified the paradigm of immutable terminal cell lineage commitment, demonstrating the capacity of a combination of defined factors central for

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many existing serotypes. They are able to enter and integrate in the nucleus of nondividing cells and they do not elicit a significant immunological response [60].

Taking this into consideration, gene transfer will become permanent and the potential side effect of miRNA overexpression has to be carefully considered. A possible strategy to reduce the risk could comprehend the selection of miRNAs that are already highly expressed and proven to be well tolerated in normal tissues.

Conclusion and Future Perspectives

miRNA can regulate the expression of a wide range of target genes by multiple mechanisms well beyond RNAi alone, both by directly interacting with the gene promoter and by epigenetic action through the modification of the DNA methylation [61].

The understanding of miRNA role in neurogenesis and reprogramming is rapidly evolving with the potential to significantly modify in the near future the methodologies of direct cell somatic conversion in vitro and in vivo. At the present, the most frequently employed miRNAs are that with a clear role in neurogenesis, like miRNA-124 and miRNA-9, but novel combination can be explored and applied at different time points of cell fate conversion.

miRNA-based strategies allow a rapid and efficient cell reprogramming due to miRNA broad impact on the cell gene expression pattern. miRNAs can limit the need of transcription factors, and thanks to the ongoing technological advances, this may lead to the realization of proficient non-viral, non-integrating direct reprogramming strategies in vitro and in vivo for therapeutic purpose. However, given the complexity of the reprogramming process and the potential broad effect of miRNAs, a complete knowledge of miRNA mechanisms and effects is needed for their effective and safe application in the clinical setting.

In conclusion, miRNA-mediated reprogramming may represent a promising tool to generate novel neuronal cells for the development of therapeutics for neurological diseases.

AAVs, adeno-associated viruses; ABM, Ascl1, Brn2, and Myt1L; CNS, central nervous system; iMSNs, induced medium spiny neurons; iNs, induced neurons; iPSCs, induced pluripotent stem cells; miRNA, microRNA; ncRNAs, noncoding RNAs; NSCs, neural stem cells; RISC, RNA-induced silencing complex; RNAi, RNA interference

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Author Contributions IF and SC conceived the idea, revised all the literature, and contributed to all parts. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interests The authors declare that they have no conflict of interest.

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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Neuromuscular excitability changes produced by sustained voluntary contraction and response to mexiletine in myotonia congenita

Modifications d'excitabilité neuromusculaire produites par une contraction volontaire prolongée et réponse à la mexilétine dans la myotonie congénitale

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KEYWORDS
Chloride conductance;
Myotonia;
Natural activity;
Sodium channel;
Weakness

Summary
Objective. — To investigate the cause of transient weakness in myotonia congenita (MC) and the mechanism of action of mexiletine in reducing weakness.
Methods. — The changes in neuromuscular excitability produced by 1 min of maximal voluntary contractions (MVC) were measured on the amplitude of compound muscle action potentials (CMAP) in two patients with either recessive or dominant MC, compared to control values obtained in 20 healthy subjects. Measurements were performed again in MC patients after mexiletine therapy.

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miRNA in spinal muscular atrophy pathogenesis and therapy

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Abstract

Spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease characterized by the selective death of lower motor neurons in the brain stem and spinal cord. SMA is caused by mutations in the survival motor neuron 1 gene (*SMN1*), leading to the reduced expression of the full-length SMN protein. microRNAs (miRNAs) are small RNAs that regulate post-transcriptional gene expression. Recent findings have suggested an important role for miRNAs in the pathogenesis of motor neuron diseases, including SMA. Motor neuron-specific miRNA dysregulation in SMA might be implicated in their selective vulnerability. In this study, we discuss recent findings regarding the consequences of SMN defects on miRNAs and their target mRNAs in motor neurons. Taken together, these data suggest that cell-specific changes in miRNAs are not only involved in the SMA motor neuron phenotype but can also be used as biomarkers and therapeutic targets.

Keywords: spinal muscular atrophy • microRNA • biomarkers

Introduction

Spinal muscular atrophy (SMA) is a severe neurodegenerative disease with autosomal recessive transmission [1, 2]. SMA represents the first genetic cause identified for infant mortality with an incidence of approximately one in 11,000 live births [3]. The progressive degeneration of lower motor neurons located in the brain stem and spinal cord leads to muscular weakness and, at later stages, to complete paralysis [1, 2].

SMA is determined by mutations (predominantly homozygous deletions) in the survival motor neuron 1 gene (*SMN1,* MIM#600354) which encodes the full-length form of the SMN protein [4]. The *SMN1* paralogous gene, *SMN2*, predominantly encodes a truncated and unstable isoform through alternative splicing of exon 7. Only 10% of the transcript encodes a full-length protein that can partially balance the *SMN1* absence. The number of copies of *SMN2* in the patient's

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THE ITALIAN LIMB GIRDLE MUSCULAR DYSTROPHY REGISTRY: RELATIVE FREQUENCY, CLINICAL FEATURES, AND DIFFERENTIAL DIAGNOSIS

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ABSTRACT: *Introduction:* Limb girdle muscular dystrophies (LGMDs) are characterized by high molecular heterogeneity, clinical overlap, and a paucity of specific biomarkers. Their molecular definition is fundamental for prognostic and therapeutic purposes. *Methods:* We created an Italian LGMD registry that included 370 molecularly defined patients. We reviewed detailed retrospective and prospective data and compared each LGMD subtype for differential diagnosis purposes. *Results:* LGMD types 2A and 2B are the most frequent forms in Italy. The ages at disease onset, clinical progression, and cardiac and respiratory involvement can vary greatly between each LGMD subtype. In a set of extensively studied patients, targeted next-generation sequencing (NGS) identified mutations in 36.5% of cases. *Conclusion*: Detailed clinical characterization combined with muscle tissue analysis is fundamental to guide differential diagnosis and to address molecular tests. NGS is useful for diagnosing forms without specific biomarkers, although, at least in our study cohort, several LGMD disease mechanisms remain to be identified.

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Additional supporting information may be found in the online version of this article.

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; CK, creatine kinase; ECG, electrocardiogram; EF, ejection fraction; EMG, electromyography; FVC, forced vital capacity; FEV_1, forced expiratory volume; IHC, immunohistochemistry; LGMD, limb girdle muscular dystrophy; MFM, motor function measure; MRC, Medical Research Council; 6MWT, 6-minute walking test; NGS, next-generation sequencing; PCR, polymerase chain reaction; RBBB, right bundle branch blocks; SF, shortening fraction; TIGEM, Telethon Institute of Genetic and Medicine; WES, whole exome sequencing; WB, Western blot

Key words: differential diagnosis; genotype–phenotype correlations; limb girdle muscular dystrophy; natural history; next-generation sequencing

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Limb girdle muscular dystrophies (LGMDs) are heterogeneous genetic disorders characterized by progressive muscle impairment, predominantly involving proximal and limb girdle muscles with onset after independent ambulation is achieved, and by histological signs of degeneration and regeneration in muscles.[1,2] The clinical spectrum of LGMDs can vary from more severe infantile forms with early loss of independent ambulation to milder adult-onset and slowly progressive weakness. Cardiac and respiratory involvement can be variably present, and cognitive impairment is usually absent. The estimated incidence of LGMDs in northern England is 2.27 per 100,000,[3] but updated epidemiological data in Italy are lacking.

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Mutations in *TMEM230* are rare in autosomal dominant Parkinson's disease

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A recent linkage analysis in a large autosomal dominant Parkinson's disease (ADPD) kindred identified a new locus for ADPD on chr.20p13. A missense Arg141Leu mutation within the *TMEM230* gene has been suggested as causative of the disease [1]. Additional variants in the same gene have been found in two young onset US PD cases and seven Chinese PD patients, of which five were homozygous for the new identified mutation.

Here we reported the results of a sequencing analysis of the *TMEM230* gene in 86 Italian familial PD Caucasian patients compatible with an AD inheritance (two or more PD cases in at least two consecutive generations) collected between 2012 and 2016. Other patients with PD but without a history of AD inheritance were not included in this study. Demographic data of the patients are reported in Supplementary Table 1. All patients were collected at our Hospital, IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, and the clinical diagnosis of PD was based on the presence of at least two of the following signs: bradykinesia, resting tremor and rigidity, along with a positive response to levodopa treatment and absence of other causes of parkinsonism. All subjects were screened for SNCA, GBA, and common LRRK2 mutations, but we did not remove those carrying pathogenic variants from the analysis. The ethics committee of IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico approved the study and all patients provided written informed consent. To detect mutations, we performed a polymerase chain reaction of all exons and intron-exon boundaries of the *TMEM230* gene (isoforms NM_001009923.1 and NM_014145) using primers reported in Supplementary Table 2. Sanger sequencing of all exons was performed. The identified variants were annotated according to the longer cDNA sequence deposited in Genbank (accession number NM_001009923.1) and their frequencies were checked in dbSNP.

No pathogenic variants were identified. Two intronic (c.174+5G > C, c.412-44G > A) and four exonic known polymorphisms were detected (p.Met64Thr, p.Pro102Pro, p.Lys103Lys, p.Ala110Ala) (Supplementary Table 3).

These results suggest that *TMEM230* mutations are not a frequent cause of PD with AD inheritance in the Italian population. In the original cloning paper a single mutation was found to be present in several PD patients of Chinese ancestry, some in homozygous state, suggesting a high frequency of this variant in familial cases from that specific population. Further genetic analyses in other populations are warranted to detect other possible population-specific *TMEM230* mutations. Furthermore, the additional variants reported in US patients lacking a clear co-segregation need to be confirmed in other studies. In this regard, additional screening in familial cases would be important in order to assess the co-segregation of *TMEM230* variants with PD.

Disclosure statement

The authors declare no competing financial or personal interests that can influence the presented work. All authors have approved the final article.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.parkreldis.2017.03.007.

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Genetic correlation between amyotrophic lateral sclerosis and schizophrenia

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We have previously shown higher-than-expected rates of schizophrenia in relatives of patients with amyotrophic lateral sclerosis (ALS), suggesting an aetiological relationship between the diseases. Here, we investigate the genetic relationship between ALS and schizophrenia using genome-wide association study data from over 100,000 unique individuals. Using linkage disequilibrium score regression, we estimate the genetic correlation between ALS and schizophrenia to be 14.3% (7.05–21.6; $P=1\times10^{-4}$) with schizophrenia polygenic risk scores explaining up to 0.12% of the variance in ALS ($P=8.4\times10^{-7}$). A modest increase in comorbidity of ALS and schizophrenia is expected given these findings (odds ratio 1.08–1.26) but this would require very large studies to observe epidemiologically. We identify five potential novel ALS-associated loci using conditional false discovery rate analysis. It is likely that shared neurobiological mechanisms between these two disorders will engender novel hypotheses in future preclinical and clinical studies.

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ORIGINAL COMMUNICATION

Revisiting mitochondrial ocular myopathies: a study from the Italian Network

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Abstract Ocular myopathy, typically manifesting as progressive external ophthalmoplegia (PEO), is among the most common mitochondrial phenotypes. The purpose of this study is to better define the clinical phenotypes associated with ocular myopathy. This is a retrospective study on a large cohort from the database of the "Nation-wide Italian Collaborative Network of Mitochondrial Diseases". We distinguished patients with ocular myopathy as part of a multisystem mitochondrial encephalomyopathy (PEO-encephalomyopathy), and then PEO with isolated ocular myopathy from PEO-plus when PEO was associated with additional features of multisystemic involvement. Ocular myopathy was the most common feature in our cohort of mitochondrial patients. Among the 722 patients with a definite genetic diagnosis, ocular myopathy was observed in 399 subjects (55.3%) and was positively associated with mtDNA single deletions and *POLG* mutations. Ocular myopathy as manifestation of a multisystem mitochondrial encephalomyopathy (PEO-encephalomyopathy, $n = 131$) was linked to the m.3243A>G mutation, whereas the other "PEO" patients ($n = 268$) were associated with mtDNA single deletion and *Twinkle* mutations. Increased lactate was associated with central neurological involvement. We then defined, among the PEO group, as "pure PEO" the patients with isolated ocular myopathy and "PEO-plus" those with ocular myopathy and other features of neuromuscular and multisystem involvement, excluding central nervous system. The male proportion was significantly lower in pure PEO than PEO-plus. This study reinforces

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MicroRNA Metabolism and Dysregulation in Amyotrophic Lateral Sclerosis

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Abstract MicroRNAs (miRNAs) are a subset of endogenous, small, non-coding RNA molecules involved in the post-transcriptional regulation of eukaryotic gene expression. Dysregulation in miRNA-related pathways in the central nervous system (CNS) is associated with severe neuronal injury and cell death, which can lead to the development of neurodegenerative disorders, such as amyotrophic lateral sclerosis (ALS). ALS is a fatal adult onset disease characterized by the selective loss of upper and lower motor neurons. While the pathogenesis of ALS is still largely unknown, familial ALS forms linked to TAR DNA-binding protein 43 (*TDP-43*) and fused in sarcoma (*FUS*) gene mutations, as well as sporadic forms, display changes in several steps of RNA metabolism, including miRNA processing. Here, we review the current knowledge about miRNA metabolism and biological functions and their crucial role in ALS pathogenesis with an in-depth analysis on different pathways. A more precise understanding of miRNA involvement in ALS could be useful not only to elucidate their role in the disease etiopathogenesis but also to investigate their potential as disease biomarkers and novel therapeutic targets.

Keywords Amyotrophic lateral sclerosis · ALS · microRNA · miRNA · Central nervous system · CNS

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Introduction

Amyotrophic lateral sclerosis (ALS) represents one of the most common late-onset neurodegenerative disorders [1]. The neuropathological features are characterized by the progressive loss of somatic motor neurons in the spinal cord, which innervate all voluntary muscles in the body. This process clinically results in the progressive paralysis of the muscular functions. In addition, bulbar symptoms, such as dysphagia and dysarthria, related to the degeneration of lower brain stem motor neurons may arise during the disease course. Death usually occurs within a few years from onset due to respiratory failure [1, 2]. To date, the only approved compound for ALS treatment is riluzole that can only modestly increase survival by a few months [1].

ALS classified as sporadic (sALS) represents the majority of the diagnoses while familial ALS (fALS) accounts for only 10% of the cases [3, 4]. However, 10% of initially diagnosed sALS subjects display gene mutations [5]. The most common ALS-causative genes include chromosome 9 open reading frame 72 (*C9orf72*), Cu^{2+}/Zn^{2+} superoxide dismutase (*SOD1*), TAR DNA-binding protein 43 (*TARDBP*), and fused in sarcoma/translocated in liposarcoma (*FUS/TLS*) [4, 6, 7] (see Table 1 for the whole list). Interestingly, many ALS-linked genes, particularly *TARDBP* and *FUS*, are involved in RNA metabolism, including microRNA (miRNA) processing [44, 45].

MiRNAs are tissue-specific, small non-coding RNAs that are expressed in different viruses, animals, and plants [46–50]. They are widespread and highly conserved molecules representing approximately 1–2% of non-protein-coding genes [46, 47]. In particular, they are involved in the inhibition and degradation of messenger RNAs (mRNAs) thwarting their expression by pairing with them [46, 49]. Because of their involvement in the development, function, and survival of different types of mature neurons in organisms [51],

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Effects of short-to-long term enzyme replacement therapy (ERT) on skeletal muscle tissue in late onset Pompe disease (LOPD)

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Effects of short-to-long term enzyme replacement therapy (ERT) on skeletal muscle tissue in late onset Pompe disease (LOPD)

Aims: Pompe disease is an autosomal recessive lysosomal storage disorder resulting from deficiency of acid α-glucosidase (GAA) enzyme. Histopathological hallmarks in skeletal muscle tissue are fibre vacuolization and autophagy. Since 2006, enzyme replacement therapy (ERT) is the only approved treatment with human recombinant GAA alglucosidase alfa. We designed a study to examine ERT-related skeletal muscle changes in 18 modestly to moderately affected late onset Pompe disease (LOPD) patients along with the relationship between morphological/biochemical changes and clinical outcomes. Treatment duration was short-to-long term. **Methods**: We examined muscle biopsies from 18 LOPD patients at both histopathological and biochemical level. All patients underwent two muscle biopsies, before and after ERT administration respectively. The study is partially retrospective because the first biopsies were taken before the study was designed, whereas the second biopsy was always performed after at least 6 months of ERT administration. **Results**: After ERT, 15 out of 18 patients showed improved 6-min walking test (6MWT; $P = 0.0007$) and most of them achieved respiratory stabilization. Pretreatment muscle biopsies disclosed marked histopathological variability, ranging

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Correction added on 11 September 2017, after first online publication: The author Simona Saredi has been correctly updated on this version.

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Genome-wide RNA-seq of iPSC-derived motor neurons indicates selective cytoskeletal perturbation in Brown–Vialetto disease that is partially rescued by riboflavin

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Riboflavin is essential in numerous cellular oxidation/reduction reactions but is not synthesized by mammalian cells. Riboflavin absorption occurs through the human riboflavin transporters RFVT1 and RFVT3 in the intestine and RFVT2 in the brain. Mutations in these genes are causative for the Brown–Vialetto–Van Laere (BVVL), childhood-onset syndrome characterized by a variety of cranial nerve palsies as well as by spinal cord motor neuron (MN) degeneration. Why mutations in RFVTs result in a neural cell–selective disorder is unclear. As a novel tool to gain insights into the pathomechanisms underlying the disease, we generated MNs from induced pluripotent stem cells (iPSCs) derived from BVVL patients as an *in vitro* disease model. BVVL-MNs explained a reduction in axon elongation, partially improved by riboflavin supplementation. RNA sequencing profiles and protein studies of the cytoskeletal structures showed a perturbation in the neurofilament composition in BVVL-MNs. Furthermore, exploring the autophagy–lysosome pathway, we observed a reduced autophagic/mitophagic flux in patient MNs. These features represent emerging pathogenetic mechanisms in BVVL-associated neurodegeneration, partially rescued by riboflavin supplementation. Our data showed that this therapeutic strategy could have some limits in rescuing all of the disease features, suggesting the need to develop complementary novel therapeutic strategies.

Riboflavin (7,8-dimethyl-10-ribityl-isoalloxazine; vitamin B2) is a water-soluble group B vitamin and the precursor of the coenzymes flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)[1]. They are essential cofactors in different metabolic processes, including carbohydrate, amino acid, and lipid metabolism and the electron transport chain[2]. Riboflavin cannot be readily synthesized in mammalian cells, so it must be absorbed from the diet via riboflavin transporters (RFVTs)[3–5]. RFVT1 and RFVT3 are predominantly expressed in the intestine and RFVT2 in the brain. The absence of riboflavin in the diet causes a range of developmental and growth disorders[6]. Furthermore, riboflavin supplementation is useful in the treatment of inborn errors of metabolism, such as mild multiple acyl-CoA dehydrogenation defect (MADD) and some mitochondrial diseases[7,8].

In 2010, it was established that autosomal recessive mutations in the riboflavin transporter genes *SLC52A2* (coding for *RFT3*, *RFVT2*) and *SLC52A3* (alias *C20orf54*, coding for *RFT2*, *RFVT3*) are responsible for the neurodegenerative disorder previously identified as Brown–Vialetto–Van Laere (BVVL) or Fazio Londe (FL) syndrome[9–14]. *RFVT1* does not seem to be associated with a human disease (or as an alternative lethal at embryonic stage), as no patients with this deficiency have been described.

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Anti-sulfatide reactivity in patients with celiac disease

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ORIGINAL ARTICLE

Anti-sulfatide reactivity in patients with celiac disease

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ABSTRACT

Objective: To explore a possible significance of the presence of anti-ganglioside and anti-sulfatide antibodies in sera of adult patients with celiac disease (CD) in different clinical scenario.

Methods: We selected 22 adult patients with newly diagnosed CD and 20 age–sex matched non-CD controls. Patients' serum was tested – before and after at least 6 months on a gluten-free diet (GFD) – for anti-GM1, GM2, GM3, GD1a, GD1b, GD3, GT1a, GT1b, GQ1b and sulfatide IgM, IgG and IgA auto-antibodies, by means of a dot blot technique and enzyme-linked immunosorbent assay (ELISA).

Results: We found the presence of auto-antibodies in untreated patients. In particular, anti-sulfatide IgG antibodies were present in 8 (36%) patients independently of the presence of neurological symptoms. Anti-sulfatide IgA antibodies were present in 3 (19%) patients. During GFD, anti-sulfatide IgG disappeared in all the patients, whereas IgA were observed in 2 patients. Anti-sulfatide, anti-GM1 and anti-GM2 IgM antibodies were also observed in 2 patients on a GFD. All the other auto-antibodies were absent and no demographic or clinical parameters were associated. Non-CD controls did not present any auto-antibody.

Conclusions: We found anti-sulfatide IgG antibodies in CD patients on a gluten-containing diet. Anti-sulfatide IgA antibodies persisted during GFD together with the occurrence of other IgM auto-antibodies. These data suggest a possible link between gluten and IgG auto-antibodies.

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Auto-antibodies; celiac disease; anti-sulfatide reactivity; neurological disorders; gluten-free diet

Introduction

Celiac disease (CD) is a common autoimmune enteropathy triggered by the ingestion of gluten proteins in genetically susceptible individuals carrying the HLA type II DQ2 and/or DQ8 haplotypes. Although the small bowel is the main targeted organ and localization of the inflammatory damage, CD is considered a systemic disorder as different tissues and organs, including the central and peripheral nervous systems, are involved. Approximately, 10% of CD patients show neurological symptoms,[1] in particular cerebellar ataxia, peripheral neuropathy (PN) and epilepsy.[2–4]

The pathogenesis of the neurological complications in CD is still unclear; vitamin deficiency could be considered in case of malabsorption and extensive small-bowel atrophy. However, this mechanism does not justify all the cases, especially those with normal levels of vitamins and without a clear malabsorptive syndrome. It could be supposed that systemic inflammation and the consequent increase in cytokines and auto-antibodies levels would lead to an immune reaction against nervous system components. Moreover, the response of neurological symptoms to a gluten-free diet (GFD) remains controversial. From this point of view, if neurological symptoms are independent of GFD, the presence of a genetic background facilitating the onset of multiple autoimmune diseases (including neurological autoimmune disorders) with CD may represent a co-factor.[5]

The presence of anti-ganglioside antibodies in the serum of CD patients may explain neurological symptoms in CD, as recently supposed by different researchers; in fact, IgG antibodies against gangliosides have been found in the sera of adult CD patients with PN.[6]

Gangliosides are glycosphingolipids (GM1, GM2, GM3, GD1a, GD1b, GD3, GT1a, GT1b, GQ1b) which contain sialic acid and are present in large amounts in the nervous system, especially in myelin. They can be antigenic targets in a variety of autoimmune neuropathies. The identification of anti-ganglioside antibodies in large cohorts of patients with a wide range of acute and chronic peripheral neuropathies and their association with particular clinical phenotypes have been reported.[7] The possible involvement of anti-sulfatide antibodies in CD remains unknown. Sulfatide is a common glycolipid in the peripheral nerve myelin and dorsal root ganglia [8] and may be targeted by IgM auto-antibodies associated with the onset of sensory axonal neuropathy or predominantly demyelinating sensorimotor neuropathy.[9]

Others authors confirmed the presence of anti-ganglioside IgG antibodies in CD patients but did not find any correlation

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Linezolid-induced lactic acidosis: the thin line between bacterial and mitochondrial ribosomes

Alessandro Santini , Dario Ronchi, Manuela Garbellini, Daniela Piga & Alessandro Protti

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Linezolid-induced lactic acidosis: the thin line between bacterial and mitochondrial ribosomes

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Globus pallidus internus deep brain stimulation in PINK-1 related Parkinson's disease: A case report

Deep brain stimulation (DBS) is the surgical procedure of choice in patients with advanced Parkinson's disease. The best target for stimulation, either the subthalamic nucleus (STN) or the globus pallidus internus (GPi), is still a matter of debate. The largest clinical trial comparing the two surgical targets showed no difference in motor outcomes as measured by UPDRS-III. The authors suggested that in choosing between STN and GPi clinicians should take into account a constellation of motor and non-motor features, as well as technical considerations such as the center's surgical experience with each target [1]. Nonetheless, based on more recent evidences, GPi stimulation is generally reserved for patients with cognitive decline, psychiatric features and severe axial involvement [2].

Genetic forms of Parkinson's disease, which are reportedly over-represented in DBS case series, respond to STN-DBS and GPi-DBS with comparable results to those found with idiopathic Parkinson's disease [3–5]. However, most of the studied populations had mutations in leucine-rich repeat kinase 2 (LRRK-2), glucocerebrosidase (GBA) and parkin. Homozygous PTEN-induced putative kinase 1 (PINK-1) mutations are the second most frequent cause of early-onset Parkinson's disease, responsible of 1–8% of sporadic early-onset Parkinson's disease [6]. Due to its rare occurrence, few reports exist concerning PINK-1 patients who underwent DBS, all of which refer to STN-DBS [3,4].

In this report, we describe the case of a 49-year-old Filipino woman whose symptoms started at age 30 with progressive impairment of gait that developed into shuffling due to painful proximal lower limb dystonia. She further developed bradykinesia, more prominent in her lower limbs, but no tremor. Symptoms markedly improved with levodopa and dopamine agonists. The diagnosis of Parkinson's disease was made and genetic analysis was positive for a homozygous L347P mutation of PINK-1 gene. Motor fluctuations began at age 42 followed by freezing of gait and invalidating dyskinesias, despite several trials with a combination of levodopa, dopamine agonists, iMAOs, COMT-inhibitors and amantadine. Interestingly, the patient did not demonstrate non-motor features of Parkinson's disease (hyposmia, postural hypotension, constipation, depression, or anxiety) and never developed cognitive decline, impulse control disorders, or dopa dysregulation syndrome.

After a thorough neuropsychological evaluation and a positive levodopa loading test, the patient was considered for DBS treatment. GPi was chosen as the target based on the prominent dystonia and the severe, disabling dyskinesias. The patient's MDS-UPDRS III score prior to the procedure was 44/132 when off medication and 33/132 when on medication. Her MDS-UPDRS IV score was 16/24. The levodopa equivalent daily dose (LEDD) before surgery was 1029 mg.

GPi targeting was performed with a 3 T MRI followed by a stereotactic CT scan. Surgery was performed under local anaesthesia with intraoperative electrophysiological target localization. The DBS leads (Vercise™ Lead Tungsten Stylet DB-2201-30DC, Boston Scientific, Valencia, California, USA) were placed in both GPis without intraoperative complications. Three days after electrode placement, an implantable pulse generator (Vercise™ DB-1110-C, Boston Scientific, Valencia, California, USA) was placed subcutaneously in the subclavian region under general anaesthesia and without complications. After lead implantation and before DBS activation, dyskinesias were markedly reduced, and amantadine was subsequently stopped, reducing LEDD at discharge to 779 mg.

The DBS was activated four weeks later with double monopolar stimulation parameters set at a total amplitude of 3 mA, a frequency of 130 Hz and a pulse width of 60 μs bilaterally. Neurological examination showed an improvement of gait due to significant reduction of dystonia in the lower limbs, rare turn hesitation, reduced dyskinesias and improvement of bradykinesia. The patient's MDS-UPDRS III score was 32/132 in the on medication/on stimulation state and her MDS-UPDRS IV score was 0/24. At two months after DBS activation, the patient required an adjustment of the stimulation parameters with an incremental increase in amplitude in both targets to 5 mA.

To the best of our knowledge, this report describes the first case of a PINK-1 patient treated successfully with GPi-DBS. The patient's most debilitating symptoms were severe lower limb dystonia that caused marked impairment of gait and dyskinesias. These symptoms are the main reason why we chose to target the GPi. Our choice proved successful as the patient experienced marked symptom relief without fluctuations and her painful dystonia almost completely resolved, improving her quality of life greatly. In conclusion, we suggest consideration of GPi targeting in patients with prevalent dystonic parkinsonism, such as those with autosomal recessive early-onset Parkinson's disease, where dystonia is frequently one of the most disabling motor symptoms.

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CASE REPORT
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Progressive Encephalomyelitis with Rigidity and Myoclonus Associated With Anti-GlyR Antibodies and Hodgkin's Lymphoma: A Case Report

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Introduction: A 60-year-old man presented with a 6-month history of low back pain and progressive rigidity of the trunk and lower limbs, followed by pruritus, dysphonia, hyperhydrosis, and urinary retention. Brain and spinal imaging were normal. EMG showed involuntary motor unit hyperactivity. Onconeural, antiglutamic acid decarboxylase (anti-GAD), voltage-gated potassium channel, and dipeptidyl peptidase-like protein 6 (DPPX) autoantibodies were negative. CSF was negative. Symptoms were partially responsive to baclofen, gabapentin, and clonazepam, but he eventually developed severe dysphagia. Antiglycine receptor (anti-GlyR) antibodies turned out positive on both serum and CSF. A plasmapheresis cycle was completed with good clinical response. A PET scan highlighted an isolated metabolically active axillary lymphnode that turned out to be a classic type Hodgkin lymphoma (HL), in the absence of bone marrow infiltration nor B symptoms. Polychemotherapy with ABVD protocol was completed with good clinical response and at 1-year follow-up the neurological examination is normal.

Background: Progressive encephalomyelitis with rigidity and myoclonus (PERM) is a rare and severe neurological syndrome characterized by muscular rigidity and spasms as well as brain stem and autonomic dysfunction. It can be associated with anti-GAD, GlyR, and DPPX antibodies. All of these autoantibodies may be variably associated with malignant tumors and their response to immunotherapy, as well as to tumor removal, is not easily predictable.

Conclusion: Progressive encephalomyelitis with rigidity and myoclonus has already been described in association with HL, but this is the first case report of a HL manifesting as anti-GlyR antibodies related PERM. Our report highlights the importance of malignancy screening in autoimmune syndromes of suspected paraneoplastic origin.

Keywords: progressive encephalomyelitis with rigidity and myoclonus, glycine receptor antibodies, paraneoplastic syndromes, stiff person syndrome, Hodgkin's lymphoma

SCIENTIFIC REPORTS

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The *GBAP1* pseudogene acts as a ceRNA for the glucocerebrosidase gene *GBA* by sponging miR-22-3p

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Mutations in the *GBA* gene, encoding lysosomal glucocerebrosidase, represent the major predisposing factor for Parkinson's disease (PD), and modulation of the glucocerebrosidase activity is an emerging PD therapy. However, little is known about mechanisms regulating *GBA* expression. We explored the existence of a regulatory network involving *GBA*, its expressed pseudogene *GBAP1*, and microRNAs. The high level of sequence identity between *GBA* and *GBAP1* makes the pseudogene a promising competing-endogenous RNA (ceRNA), functioning as a microRNA sponge. After selecting microRNAs potentially targeting both transcripts, we demonstrated that miR-22-3p binds to and down-regulates *GBA* and *GBAP1*, and decreases their endogenous mRNA levels up to 70%. Moreover, over-expression of *GBAP1* 3′-untranslated region was able to sequester miR-22-3p, thus increasing *GBA* mRNA and glucocerebrosidase levels. The characterization of *GBAP1* splicing identified multiple out-of-frame isoforms down-regulated by the nonsense-mediated mRNA decay, suggesting that *GBAP1* levels and, accordingly, its ceRNA effect, are significantly modulated by this degradation process. Using skin-derived induced pluripotent stem cells of PD patients with *GBA* mutations and controls, we observed a significant *GBA* up-regulation during dopaminergic differentiation, paralleled by down-regulation of miR-22-3p. Our results describe the first microRNA controlling *GBA* and suggest that the *GBAP1* non-coding RNA functions as a *GBA* ceRNA.

The glucocerebrosidase gene (*GBA*) encodes for the enzyme glucocerebrosidase (GCase), which catalyzes the hydrolysis of the membrane glucosylceramide (GlcCer) to ceramide and glucose. GCase is mainly a lysosomal enzyme and only partly associated with the outer surface of the cell membrane[1]. GCase deficiency leads to the accumulation of the substrate, responsible for the multi-organ clinical manifestations of Gaucher's disease (MIM #606463)[2], one of the most common lysosomal storage disorders[3]. While biallelic mutations in *GBA* are responsible for Gaucher's disease, heterozygous *GBA* variants have been repeatedly associated with susceptibility to Parkinson's Disease (PD)[4,5]. Importantly, Gaucher's and PDs have been connected due to the clinical observation of parkinsonism and Lewy Bodies (LB) pathology in a fraction of patients with Gaucher's disease[6]. Compared with the general population, patients with the milder form of Gaucher's disease (type 1) have a 20-fold increased lifetime risk of developing parkinsonism[7], whereas the odds ratio for any *GBA* mutation in PD patients compared to controls was greater than 5 in a multi-center analysis including more than 5000 cases and 4000 controls[8]. Several studies confirmed that *GBA* mutations, in particular the two most common ones (p.N370S and p.L444P), are more frequent in PD patients than in healthy controls, demonstrating that genetic lesions in this gene are a

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Chapter 1
Leucine-Rich Repeat Kinase (*LRRK2*) Genetics and Parkinson's Disease

Edoardo Monfrini and Alessio Di Fonzo

Abstract The discovery of *LRRK2* mutations as a cause of Parkinson's disease (PD), including the sporadic late-onset form, established the decisive role of genetics in the field of PD research. Among *LRRK2* mutations, the G2019S, mostly lying in a haplotype originating from a common Middle Eastern ancestor, has been identified in different populations worldwide. The G2385R and R1628P variants represent validated risk factors for PD in Asian populations. Here, we describe in detail the origin, the present worldwide epidemiology, and the penetrance of *LRRK2* mutations. Furthermore, this chapter aims to characterize other definitely/probably pathogenic mutations and risk variants of *LRRK2*. Finally, we provide some general guidelines for a *LRRK2* genetic testing and counseling. In summary, *LRRK2* discovery revolutionized the understanding of PD etiology and laid the foundation for a promising future of genetics in PD research.

Keywords Leucine-rich repeat kinase 2 • *LRRK2* • Dardarin • Parkinson's disease • PARK8 • Parkinson's disease genetics • Familial Parkinson's disease • *LRRK2* mutations

Until the discovery of leucine-rich repeat kinase 2 (*LRRK2*) mutations as a genetic cause of Parkinson's disease (PD), the hereditary influences on PD were limited to observation of rare autosomal dominant familial cases harboring highly penetrant *SNCA* (alpha-synuclein) mutations and juvenile or young onset autosomal recessive forms carrying *PRKN*, *PINK1*, and *DJ-1* mutations. This scenario was more suggestive of a minor role played by genetic factors in PD, especially considering the common sporadic late-onset form. The innovative finding of *LRRK2* low penetrant mutations in common forms of PD revolutionized this outdated view.

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A de novo *C19orf12* heterozygous mutation in a patient with MPAN

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Heterozygous mutation
De novo mutation

Mitochondrial Membrane Protein-Associated Neurodegeneration (MPAN or NBIA4) is caused by biallelic mutations in the *C19orf12* gene. MPAN accounts for the largest proportion of NBIA after PKAN (*PANK2* mutations) and PLAN (*PLA2G6* mutations). It is characterized by juvenile-onset spastic paraparesis, levodopa-unresponsive parkinsonism, dystonia and neuropsychiatric symptoms. Additional features are optic atrophy, dysphagia, dysarthria, and motor axonal neuropathy. Brain MRI displays T2-weighted symmetrical hypointensities in globus pallidus (GP) and substantia nigra (SN) [1]. Missense, frameshift and nonsense mutations were found in exons 2 and 3 of *C19orf12* (NM_001031726 transcript). One splice-site mutation (c.194-2A>G) was identified in intron 2 (Fig. 1a). Specific variants were found to be frequent in MPAN cases of selected populations (c.204_214del - p.G69Rfs*10 in Eastern Europeans and p.T11M in Turkish) [2,3]. Here we report a case of NBIA with a novel *C19orf12* mutation with molecular evidence of de novo occurrence.

The relevant ethical authorities approved the study and written informed consent was obtained from all involved subjects. The proband is the only child of non-consanguineous Italian parents. Familial history was negative for neurological disorders (Fig. 1b). She was born at term by spontaneous delivery after an uneventful pregnancy. Motor development was at first normal, while a delay in language development was reported at the age of 3. Height-weight growth has been always at the lower normal limits (3–10 centiles). At the age of 5 she developed a progressive imbalanced gait associated with lower limbs rigidity and later onset of right hand dystonia forced her to use the left hand to write. She developed mild hirsutism at 9 years (pubis and limbs) and precocious puberty. Ocular involvement was present and included low vision, hypermetropia and astigmatism. She attends high school with a support teacher for learning disability (IQ 74). She came to the attention of our outpatient clinic at the age of 16 years. The neurological examination showed moderate dysarthria, cervical dystonia, dysdiadochokinesia, mild intentional and postural upper limbs tremor, lower limbs spastic hypertonia associated with movement-exacerbated dystonic postures of feet, patellar hyperreflexia and bilateral Babinski sign. Typical radiological findings of SN and GP hypointensity in SWI, T2* and T2-weighted MRI with a T2-hyperintense medial medullary lamina (MML) were present, with the additional finding of subthalamic nucleus involvement. No cortical or cerebellar atrophy was evident (Fig. 1d). Electroencephalogram and electroretinogram were normal, while visual evoked potential displayed an increased latency of P100 wave bilaterally with reduced amplitude and EMG showed diffuse axonal motor neuropathy. Fundus examination revealed optic atrophy.

Genetic analysis revealed a novel heterozygous *C19orf12* c.265_266delAT (NM_001031726) - p.M89Gfs*12 (NP_001026896) mutation in the proband, but not in her parents (Fig. 1b–c). False paternity was excluded using eight polymorphic short tandem repeats (STR) on chromosome X. This suggests the p.M89Gfs*12 being a de novo mutation. Sanger sequencing of all exons and intron-exons boundaries of *PANK2*, *PLA2G6*, *C19orf12*, *FA2H*, *WDR45*, *COASY*, *CP*, and *FTL*, and full-length amplification followed by sequencing of *PANK2* and *WDR45* transcript did not detect other pathogenic mutations or rearrangements.

The amount of *C19orf12* transcripts was measured by quantitative reverse-transcription PCR in lymphocytes of patient, parents and controls. This assay failed to detect a reduced mRNA quantity in the proband cDNA, suggesting the absence of degradation through nonsense-mRNA decay. Sequence analysis of *C19orf12* transcripts revealed the presence of the heterozygous c.265_266delAT mutation in the proband and ruled-out splicing aberrations on both mutated and non-mutated alleles.

In order to explore a possible mosaicism, the mutation was studied in DNA from several tissues (hair, saliva and urine). The presence of the p.M89Gfs*12 in all tissues suggests a germ-line mosaicism in a parent.

MPAN is considered an autosomal recessive disorder; however, five monoallelic *C19orf12* mutations have been already described [1,3,4]. A second occult mutation was suspected to be present in these cases. Deletion screening and promoter regions sequencing in some of these cases did not identified a second mutation [1,3,4]. Interestingly, all these mutations localize on exon 3 (G69R, p.Q86*, p.A94Cfs*8, p.A120Gfs*32 and p.L99fs*102), including deleterious frameshift/stop mutations on the *C19orf12* C-terminal domain, as in the case described in this study. Moreover, in a family with a heterozygous p.A120Gfs*32 mutation family history suggested a possible autosomal-dominant inheritance; indeed, the father of the patient, who died at age 47 following a long course of progressive dementia with parkinsonism, showed a typical MPAN neuropathology at postmortem examination [3].

Several lines of evidence support the pathogenic role of the identified variant reported here: the association with clinical-radiological features of MPAN, the de novo occurrence of the variant, the lack of additional mutations or aberrant splice sites on *C19orf12* transcripts and the absence of genetic mutations in

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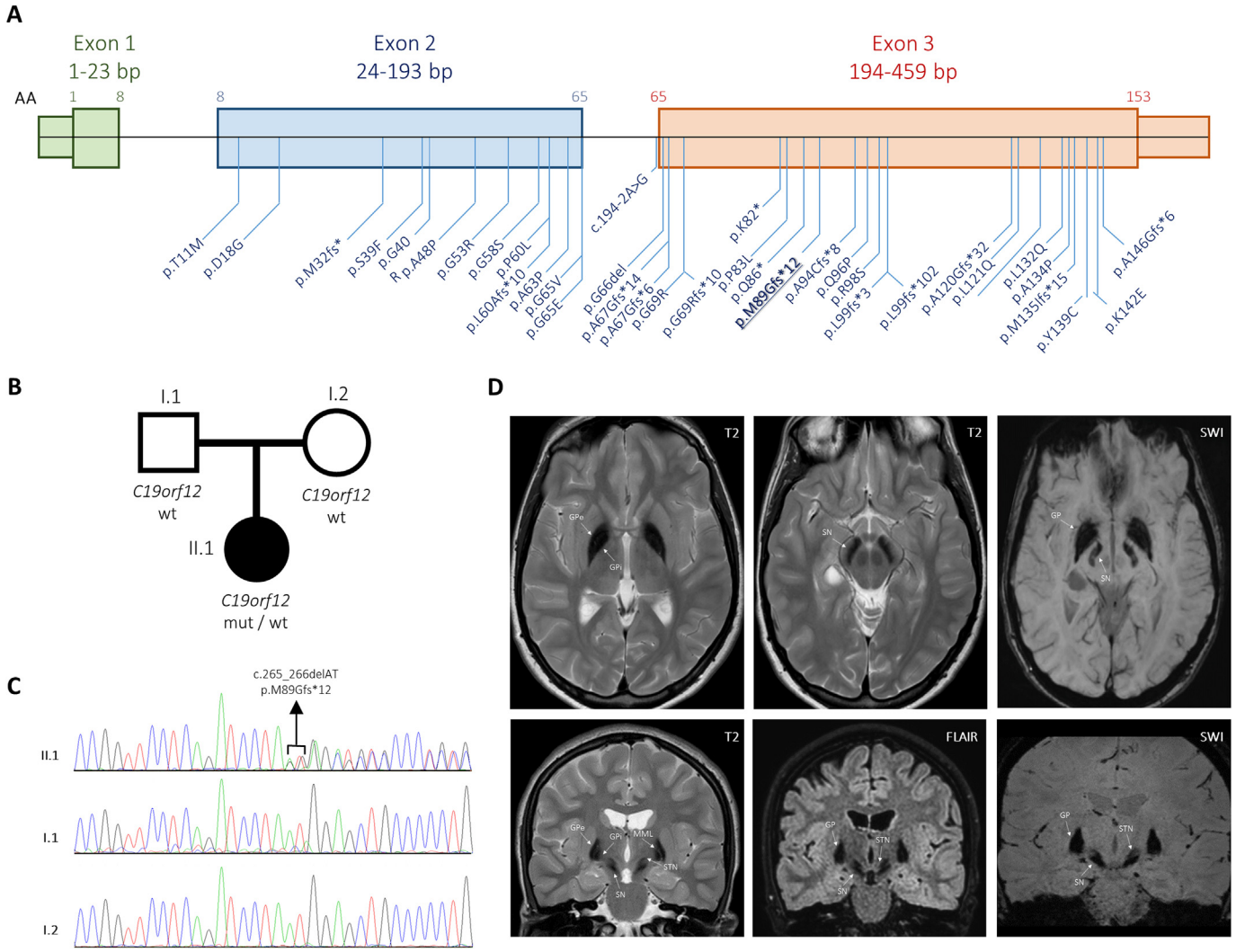

Fig. 1. A) Representation of the *C19orf12* gene mutations reported in MPAN phenotypes so far. B) Pedigree of the family under study. Black symbol denotes affected individual. C) Electropherograms of the mutation c.265_266delAT of *C19orf12* gene in the proband (II.1) and wild type sequence in her parents (I.1 and I.2). D) Patient Brain MRI (T2, FLAIR and SWI sequence), performed at the age of 16, showing hypointensity of substantia nigra, globus pallidus and interestingly, subthalamic nucleus. (Abbreviations: wt = Wild type; mut = Mutated; GP = Globus Pallidus; GPe = External Globus Pallidus; GPi = Internal Globus Pallidus; SN = Substantia Nigra; STN = Subthalamic nucleus; MML = Medial Medullary Lamina).

other NBIA genes. The lack of a second mutation may indicate a dominant negative effect of this variant. Alternatively, the combination of this p.M89Gfs*12 with mutations in other still unknown causative genes may be hypothesized. In this view, studies on additional MPAN cases with monoallelic mutations are required in order to explore their role in NBIA.

Financial disclosure

The authors have no conflicts of interests to declare.

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Ethics

The relevant ethical authorities approved the study and writ informed consent was obtained from all involved subjects.

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Short communication

X-linked Parkinsonism with Intellectual Disability caused by novel mutations and somatic mosaicism in *RAB39B* gene

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ABSTRACT

Background: RAB39B pathogenic variants cause X-linked Parkinsonism associated with Intellectual Disability, known as Waisman syndrome, a very rare disorder that has been mainly identified through exome sequencing in large Parkinson's disease cohorts.
In this study we searched for pathogenic variants in *RAB39B* in two Italian families affected by X-linked early-onset Parkinsonism and Intellectual Disability.
Methods: Three patients received neurological evaluation and underwent *RAB39B* sequencing.
Results: Two novel *RAB39B* frameshift variants were found to result in the absence of RAB39B protein (family 1: c.137dupT; family 2: c.371delA). Patients showed unilateral rest tremor and bradykinesia; one of them also displayed an early-onset postural tremor. Paramagnetic substance deposition in the substantia nigra, globus pallidi, red nucleus, putamen and pulvinar was assessed by brain imaging. Two patients also showed moderate calcification of globus pallidi.
Conclusion: In this study we highlight the evidence that X-linked early-onset Parkinsonism associated with Intellectual Disability occurs as a pattern of clinical and neuroimaging features attributable to *RAB39B* pathogenic variants.

1. Introduction

RAB39B pathogenic variants were first identified in patients affected by X-linked Intellectual Disability (XLID) in comorbidity with autism spectrum disorder, macrocephaly and epilepsy [1,2]. Moreover, additional evidence showed a causative role of *RAB39B* in the pathogenesis of XLID and early-onset Parkinsonism, also referred to as Waisman syndrome (WSMN, OMIM 311510) [3–7].

RAB39B is a member of the RAB (an acronym of *ras* genes from *rat brain*) GTPases belonging to the RAS oncogene superfamily. It is responsible for the control of intracellular vesicular trafficking in neuronal cells [8]. To date, the role of *RAB39B* has been defined in the pathogenesis of Intellectual Disability (ID) [9] but how the absence of this protein could impact on Parkinsonism still remains unknown.

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ORIGINAL ARTICLE

The Italian dementia with Lewy bodies study group (DLB-SINdem): toward a standardization of clinical procedures and multicenter cohort studies design

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Abstract Dementia with Lewy bodies (DLB) causes elevated outlays for the National Health Systems due to high institutionalization rate and patients' reduced quality of life and high mortality. Furthermore, DLB is often misdiagnosed as Alzheimer's disease. These data motivate harmonized multicenter longitudinal cohort studies to improve clinical management and therapy monitoring. The Italian DLB study group of the Italian Neurological Society for dementia (SINdem) developed and emailed a semi-structured questionnaire to 572 national dementia centers (from primary to tertiary) to prepare an Italian large longitudinal cohort. The questionnaire surveyed: (1) prevalence and incidence of DLB; (2) clinical assessment; (3) relevance and availability of diagnostic tools; (4) pharmacological management of cognitive, motor, and behavioural disturbances; (5) causes of hospitalization, with specific focus on delirium and its treatment. Overall, 135 centers (23.6 %) contributed to the survey. Overall, 5624 patients with DLB are currently followed by the 135 centers in a year (2042 of them are new patients). The percentage of DLB patients was lower (27 ± 8 %) than that of Alzheimer's disease and frontotemporal dementia (56 ± 27 %) patients. The majority of the centers (91 %) considered the clinical and neuropsychological assessments as the most relevant

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MULTIPLE SCLEROSIS JOURNAL MSJ

Original Research Paper

CSF β-amyloid as a putative biomarker of disease progression in multiple sclerosis

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Abstract

Background: Neurodegeneration plays a major role in determining disability in multiple sclerosis (MS) patients. Hence, there is increasing need to identify reliable biomarkers, which could serve as prognostic measure of disease progression.

Objectives: To assess whether cerebrospinal fluid (CSF) tau and β-amyloid (Aβ) levels were altered in newly diagnosed MS patients and correlated with disability. Moreover, we investigated whether these CSF biomarkers associate with macroscopic brain tissue damage measures.

Methods: CSF Aβ and tau levels were determined by enzyme-linked immunosorbent assay in CSF samples from 48 newly diagnosed MS patients, followed-up clinically for 3 years by recording their Expanded Disability Status Scale score at 6-month intervals, and 45 controls. All patients underwent magnetic resonance imaging at baseline and at the end of follow-up to quantify their lesion load (LL).

Results: CSF Aβ levels were significantly reduced in patients compared to controls ($p < 0.001$). Lower CSF Aβ levels at baseline were a disability predictor at 3-year follow-up ($p = 0.009$). CSF tau levels correlated with T2- and T1-LL ($p < 0.001$).

Conclusion: CSF Aβ reduction is a promising biomarker of neurodegeneration and may predict patients' clinical outcome. Therefore, CSF Aβ should be considered as a potential biomarker of prognostic value.

Keywords: Multiple sclerosis, neurodegeneration, biomarker, beta-amyloid, tau

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Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS), characterized by demyelination, axonal degeneration/loss, and gliosis, whose underlying pathophysiology still remains largely unclear. Although MS is traditionally regarded as a white matter (WM) demyelinating disease, there is now substantial evidence indicating axonal and neuronal loss as critically involved in MS pathophysiology since early clinical stages.[1] Additionally, these neurodegenerative aspects play a major role in determining accumulation of permanent physical and cognitive disabilities.[2] The mechanisms underlying axonal damage in MS still need to be fully clarified, and, currently, there are no reliable prognostic biomarkers of disease progression. However, imaging surrogates, such as brain atrophy on magnetic resonance imaging (MRI) and retinal nerve fiber layer (RNFL) thinning on optical coherence tomography (OCT), can be used for prognostic purposes. A multicenter study including more than 800 MS patients and using OCT showed that RNFL thinning under a specific threshold is associated with a significantly increased risk of clinical worsening over 5 years.[3]

MRI is an invaluable tool for the diagnostic work-up of MS patients. By simply collecting conventional magnetic resonance (MR) images, clinicians may apply current criteria for an early diagnosis of MS and monitor disease activity over time. In contrast, no strong correlation has been found between conventional MRI measures, such as T2- and T1-lesion loads (LL), and

SCIENTIFIC REPORTS

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Extracellular proteasome-osteopontin circuit regulates cell migration with implications in multiple sclerosis

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Osteopontin is a pleiotropic cytokine that is involved in several diseases including multiple sclerosis. Secreted osteopontin is cleaved by few known proteases, modulating its pro-inflammatory activities. Here we show by *in vitro* experiments that secreted osteopontin can be processed by extracellular proteasomes, thereby producing fragments with novel chemotactic activity. Furthermore, osteopontin reduces the release of proteasomes in the extracellular space. The latter phenomenon seems to occur *in vivo* in multiple sclerosis, where it reflects the remission/relapse alternation. The extracellular proteasome-mediated inflammatory pathway may represent a general mechanism to control inflammation in inflammatory diseases.

Osteopontin (OPN), a component of bone matrix and a soluble pleiotropic cytokine, plays a pivotal role in several diseases such as tumors, myocardial and kidney dysfunctions, and autoimmune diseases. OPN has a particular relevance in multiple sclerosis (MS), a disease in which the autoimmune response targets the myelin sheaths of the central nervous system (CNS)[1]. Indeed, in MS secreted OPN stimulates the expression of Th1 and Th17 cytokines, inhibits apoptosis of autoreactive T cells, and regulates leukocyte adhesion, migration, and trafficking into the CNS by binding to CD44 and various integrins[2]. Increased concentrations of OPN occur in peripheral

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BRAIN
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Cognitive reserve and *TMEM106B* genotype modulate brain damage in presymptomatic frontotemporal dementia: a GENFI study

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Frontotemporal dementia is a heterogeneous neurodegenerative disorder with around a third of cases having autosomal dominant inheritance. There is wide variability in phenotype even within affected families, raising questions about the determinants of the progression of disease and age at onset. It has been recently demonstrated that cognitive reserve, as measured by years of formal schooling, can counteract the ongoing pathological process. The *TMEM106B* genotype has also been found to be a modifier of the age at disease onset in frontotemporal dementia patients with TDP-43 pathology. This study therefore aimed to elucidate the modulating effect of environment (i.e. cognitive reserve as measured by educational attainment) and genetic background (i.e. *TMEM106B* polymorphism, rs1990622 T/C) on grey matter volume in a large cohort of presymptomatic subjects bearing frontotemporal dementia-related pathogenic mutations. Two hundred and thirty-one participants from the GENFI study were included: 108 presymptomatic *MAPT*, *GRN*, and *C9orf72* mutation carriers and 123 non-carriers. For each subject, cortical and subcortical grey matter volumes were generated using a parcellation of the volumetric T_1-weighted magnetic resonance imaging brain scan. *TMEM106B* genotyping was carried out, and years of education recorded. First, we obtained a composite measure of grey matter volume by graph-Laplacian principal component analysis, and then fitted a linear mixed-effect interaction model, considering the role of (i) genetic status; (ii) educational attainment; and (iii) *TMEM106B* genotype on grey matter volume. The presence of a mutation was associated with a lower grey matter volume ($P = 0.002$), even in presymptomatic subjects. Education directly affected grey matter volume in all the samples ($P = 0.02$) with lower education attainment being associated with lower volumes. *TMEM106B* genotype did not influence grey matter volume directly on its own but in mutation carriers it modulated the slope of the correlation between education and grey matter volume ($P = 0.007$). Together, these results indicate that brain atrophy in presymptomatic carriers of common frontotemporal dementia mutations is affected by both genetic and environmental factors such that *TMEM106B* enhances the benefit of cognitive reserve on brain structure. These findings should be considered in evaluating outcomes in future disease-modifying trials, and support the search for protective mechanisms in people at risk of dementia that might facilitate new therapeutic strategies.

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Keywords: frontotemporal dementia; genetics; cognitive reserve; TMEM106b; structural MRI

Abbreviations: FTD = frontotemporal dementia; GS = genetic status

Introduction

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterized by neuronal loss in the frontal and temporal lobes (Hodges *et al.*, 2004; Rohrer *et al.*, 2011; Warren *et al.*, 2013). It presents clinically with behavioural symptoms, deficits of executive functions and language impairment, and in some cases, with motor neuron disease, progressive supranuclear palsy or corticobasal syndrome (Seelaar *et al.*, 2011). Up to 40% of cases have a family history of dementia, with an autosomal dominant inheritance in around a third of patients (Stevens *et al.*, 1998). Mutations within microtubule-associated protein tau (*MAPT*) (Hutton *et al.*, 1998), granulin (*GRN*) (Baker *et al.*, 2006; Cruts *et al.*, 2006), and chromosome 9 open reading frame 72 (*C9orf72*) (DeJesus-Hernandez *et al.*, 2011; Renton *et al.*, 2011) are proven major causes of genetic FTD, accounting for 10–20% of all FTD cases. *MAPT* mutations lead to FTD with neuronal tau inclusions, while *GRN* and *C9orf72* are associated with intraneuronal TAR DNA-binding protein 43 (TDP-43) inclusions (Baborie *et al.*, 2011).

Recently, it has been demonstrated in the Genetic Frontotemporal Dementia Initiative (GENFI) study that grey matter and cognitive changes can be identified 5–10 years before the expected onset of symptoms in adults at risk of genetic FTD (Rohrer *et al.*, 2015), and even earlier for those with *C9orf72* expansions. However, there is wide variation in the age at onset within families, and possible modifiers of disease progression (including genetic and environmental factors) have yet to be investigated. Such modifiers will be important for several reasons: to properly define biomarkers that can stage presymptomatic disease and track disease progression, to correctly identify individuals most suitable for clinical trials, and to reduce heterogeneity and increase the statistical power of analyses of such trials.

Cognitive reserve and genetic factors have both been proposed as moderators of the onset of disease. Cognitive reserve is a theoretical concept proposing that certain lifetime experiences, including education, individual intelligence quotient, degree of literacy, and occupational attainment, increase the flexibility, efficiency, and capacity of brain networks, thereby allowing individuals with higher cognitive reserve to sustain greater levels of brain pathology before showing clinical impairment (for a review, see Stern, 2009). In healthy individuals, higher educational attainment (Arenaza-Urquijo *et al.*, 2013) as well as cognitive enrichment (Sun *et al.*, 2016) have been related to greater volume and greater metabolism in frontotemporal regions, thus

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Review

The Enigmatic Role of Viruses in Multiple Sclerosis: Molecular Mimicry or Disturbed Immune Surveillance?

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Multiple sclerosis (MS) is a T cell driven autoimmune disease of the central nervous system (CNS). Despite its association with Epstein-Barr Virus (EBV), how viral infections promote MS remains unclear. However, there is increasing evidence that the CNS is continuously surveyed by virus-specific T cells, which protect against reactivating neurotropic viruses. Here, we discuss how viral infections could lead to the breakdown of self-tolerance in genetically predisposed individuals, and how the reactivations of viruses in the CNS could induce the recruitment of both autoaggressive and virus-specific T cell subsets, causing relapses and progressive disability. A disturbed immune surveillance in MS would explain several experimental findings, and has important implications for prognosis and therapy.

Genetic and Environmental Factors Contribute to the Risk of MS

MS is the most common inflammatory autoimmune disorder of the CNS [1,2]. It is characterized by the destruction of the protective myelin sheath of neurons, resulting in macroscopic lesions in the brain and causing progressive disability. MS can be subdivided into relapsing–remitting (RR), primary progressive (PP) or secondary progressive (SP; i.e., the RR subtype worsening over time to SP-MS) forms. RR-MS is the dominant form at disease onset, and is characterized by acute clinical attacks followed by apparent disease stability. Symptoms can be alleviated with several therapies, but, in some patients, there is no beneficial effect and the disease may evolve to a SP form. PP-MS and SP-MS remain difficult to treat and are also mechanistically poorly understood [3].

The etiology of MS is still unknown, but both genetic and environmental factors contribute to the risk of developing MS [1,2]. The major genetic risk factor maps to the human leukocyte antigen (HLA) gene cluster, and the strongest risk is conferred by HLA-DRB1*15:01 in the class II region [4,5]. The principal function of MHC class II proteins is to present peptide ligands to $CD4^+$ lymphocytes and these T cells are consequently believed to have a key pathogenic role in MS. However, the MHC class I cluster, which regulates cytotoxic lymphocyte responses, contains polymorphic regions that are associated with protection against MS [4]. Several other gene

Trends

A huge body of evidence suggests that viral infections promote MS; however, no single causal virus has been identified. Multiple viruses could promote MS via bystander effects.

Molecular mimicry is an established pathogenic mechanism in selected autoimmune diseases. It is also well documented in MS, but its contribution to MS pathogenesis is still unclear.

Bystander activation upon viral infection could be involved in the generation of the autoreactive and potentially encephalitogenic T helper (Th)-1/17 central memory ($Th1/17_{CM}$) cells found in the circulation of patients with MS.

Autoreactive $Th1/17_{CM}$ cells could expand at the cost of antiviral $Th1_{CM}$ cells in patients with MS, in particular in those undergoing natalizumab therapy, because these cells are expected to compete for the same homeostatic niche.

Autoreactive Th1/17 cells and antiviral Th1 cells are recruited to the CSF of patients with MS following attacks, suggesting that viral reactivations in the CNS induce the recruitment of pathogenic Th1/17 cells. Autoreactive Th1/17 cells in the CNS might also induce *de novo* viral reactivations in a circuit of self-induced inflammation.

polymorphisms associated with MS are involved in immune responses, in particular in the activation and homeostasis of T cells [6], consistent with the concept that MS is a T cell-driven autoimmune disease.

The importance of the environment in determining whether a genetically susceptible individual develops MS has been underlined by studies of monozygotic twins and of genetically susceptible individuals migrating from low- to high-risk areas. The strongest environmental risk factors are Vitamin D deficiency, smoking, and viral infections [7]. Interestingly, infections with helminths have been shown to have a protective effect [7,8]. Among viral infections, EBV shows the strongest association, and it was estimated that EBV-induced infectious mononucleosis increases the risk of MS to a similar degree as the strongest genetic risk factor (HLA-DRB1*15:01) [4,9–11]. In addition to EBV, several other viruses have been implicated in MS [12], in particular neurotropic viruses, including human herpes virus-6 (HHV-6) [13], herpes zoster virus [14] and John Cunningham virus (JCV) [15], but also endogenous retroviruses [16]. Based on this evidence, a possible viral etiology of MS has been proposed [9,13,15,17] and continues to stimulate intense research in the field (see Outstanding Questions).

The risk of life-threatening JCV-induced **progressive multifocal leukoencephalopathy** (PML) in patients with MS undergoing therapy with natalizumab [18], a therapeutic antibody that binds to the α4-integrin adhesion receptor and blocks lymphocyte migration to the CNS, has highlighted the importance of antiviral immune surveillance of the CNS. Indeed, the presence of a lymphatic system in the CNS has challenged the view of the CNS being an immune-privileged site [19,20], and it is now widely accepted that the CNS is surveyed and protected by antiviral T cells [21] (Box 1).

Given this updated view of immune responses in the CNS, here we discuss different models of how viral infections could promote MS, and illustrate how a defective antiviral immune surveillance could be a driving force in its pathogenesis.

The Most Widely Studied Animal Models of MS Induce CNS Inflammation in the Absence of Viral Infections

Although the epidemiological data clearly indicate that viral infections are a critical risk factor for MS, the underlying mechanisms are poorly understood [12]. Animal models that induce **experimental autoimmune encephalomyelitis** (EAE) in the absence of viral infections by priming pathogenic $CD4^+$ T cells with myelin antigens are widely used to study neuroinflammation and MS [22]. Self-tolerance has to be broken in these models by adjuvants such as CFA, which contain killed mycobacteria, intracellular pathogens that potently activate the innate immune system. Alternative models of MS, in which demyelination is induced by neurotropic viruses, such as mouse hepatitis virus or Theiler's murine encephalomyelitis virus (TMEV), are less studied, but enable researchers to address how viral infections could promote MS [23]. TMEV induces chronic inflammation and demyelination in the brain and, importantly, both virus-specific and myelin-reactive effector T cells are generated in this MS model [23]. Thus, antiviral immune responses in the CNS can result in the breakdown of self-tolerance to myelin antigens,

Box 1. CNS Immune Privilege

The notion that the CNS is a tolerogenic, 'immune-privileged' site, where immune reactions that occur in peripheral tissues are inefficient and slow, stems from seminal studies with transplanted allogenic tissues that were not or were only slowly rejected in the brain, unless animals had been immunized previously [150]. In addition, it is well known that entry of macromolecules and immune cells into the CNS from the blood is restricted by the BBB and, until recently, the CNS was also believed to lack lymphatic drainage. However, the presence of a lymphatic system of the meninges in the brain and of occasionally reactivating neurotropic viruses suggest that the CNS is constantly surveyed by the immune system, although in a manner that limits the type of collateral tissue damage that occurs in MS.

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White matter hyperintensities are seen only in *GRN* mutation carriers in the GENFI cohort

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ABSTRACT

Genetic frontotemporal dementia is most commonly caused by mutations in the progranulin *(GRN)*, microtubule-associated protein tau (*MAPT)* and chromosome 9 open reading frame 72 *(C9orf72*) genes. Previous small studies have reported the presence of cerebral white matter hyperintensities (WMH) in genetic FTD but this has not been systematically studied across the different mutations. In this study WMH were assessed in 180 participants from the Genetic FTD Initiative (GENFI) with 3D T1- and T2-weighed magnetic resonance images: 43 symptomatic (7 *GRN*, 13 *MAPT* and 23 *C9orf72*), 61 presymptomatic mutation carriers (25 *GRN*, 8 *MAPT* and 28 *C9orf72*) and 76 mutation negative non-carrier family members. An automatic detection and quantification algorithm was developed for determining load, location and appearance of WMH. Significant differences were seen only in the symptomatic *GRN* group compared with the other groups with no differences in the *MAPT* or *C9orf72* groups: increased global load of WMH was seen, with WMH located in the frontal and occipital lobes more so than the parietal lobes, and nearer to the ventricles rather than juxtacortical. Although no differences were seen in the presymptomatic group as a whole, in the *GRN* cohort only there was an association of increased WMH volume with expected years from symptom onset. The appearance of the WMH was also different in the *GRN* group compared with the other groups, with the lesions in the *GRN* group being more

Abbreviations: PS, Presymptomatic; S, Symptomatic; FTD, Frontotemporal dementia; WMH, White matter hyperintensity; IQR, Inter Quartile Range; CI, Confidence interval; TIV, Total Intracranial volume

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RESEARCH ARTICLE

A novel network analysis approach reveals DNA damage, oxidative stress and calcium/cAMP homeostasis-associated biomarkers in frontotemporal dementia

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Abstract

Frontotemporal Dementia (FTD) is the form of neurodegenerative dementia with the highest prevalence after Alzheimer's disease, equally distributed in men and women. It includes several variants, generally characterized by behavioural instability and language impairments. Although few mendelian genes (*MAPT*, *GRN*, and *C9orf72*) have been associated to the FTD phenotype, in most cases there is only evidence of multiple risk loci with relatively small effect size. To date, there are no comprehensive studies describing FTD at molecular level, highlighting possible genetic interactions and signalling pathways at the origin FTD-associated neurodegeneration. In this study, we designed a broad FTD genetic interaction map of the Italian population, through a novel network-based approach modelled on the concepts of disease-relevance and interaction perturbation, combining Steiner tree search and Structural Equation Model (SEM) analysis. Our results show a strong connection between Calcium/cAMP metabolism, oxidative stress-induced Serine/Threonine kinases activation, and postsynaptic membrane potentiation, suggesting a possible combination of neuronal damage and loss of neuroprotection, leading to cell death. In our model, Calcium/cAMP homeostasis and energetic metabolism impairments are primary causes of loss of neuroprotection and neural cell damage, respectively. Secondly, the altered postsynaptic membrane potentiation, due to the activation of stress-induced Serine/Threonine kinases, leads to neurodegeneration. Our study investigates the molecular underpinnings of these processes, evidencing key genes and gene interactions that may account for a significant fraction of

conventions followed in Fig 2.
(TIF)

S7 Fig. Disease Ontology (DO) nervous system sub-network. Sub-network extracted mapping genes annotated with DO terms descending from *Nervous System Disease* (DOID:863) and *Disease of Mental Health* (DOID:150) roots. It shows a high density (77%) of perturbed interactions, including the FTD-network backbone. Nodes and edges are labelled according to the conventions followed in Fig 2.
(TIF)

S1 Table. Maximum Likelihood Estimates (MLE) of the SEM regression parameters. SEM parameter estimation is based on the mean difference between the group variable *C* (0 = control, 1 = case) for each node, adjusted by its parents in the extracted Steiner Tree. Standard errors are calculated by bootstrap (B = 1000 resampling). Significant estimates (p-value < 0.05) are reported in bold.
(DOCX)

S2 Table. Selected ancestral bow-free covariances ($p < 0.05$, values in bold) between pairs of "target" (outgoing degree = 0) nodes of the extracted Steiner tree. Pairs of target genes that do not share a directed path are called ancestral bow-free nodes. When these genes share significant covariances, there may be an unobserved common cause perturbing their interaction. This condition is evaluated using a latent variable (LV) model in which a LV, influenced by the group variable *C* (0 = controls, 1 = cases), is connected to the two bow-free targets. A LV is designed as a significant unknown cause acting on the targets, if the *C*->LV interaction is significant (i.e., p-value(C->LV) < 0.05, in bold), and the LV model has a good fit (i.e., p-value of the Likelihood Ratio Test (LRT) ≥ 0.05, in bold).
(DOCX)

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EVIDENCE OF CNS β-AMYLOID DEPOSITION IN NASU-HAKOLA DISEASE DUE TO THE *TREM2* Q33X MUTATION

Nasu-Hakola disease (NHD), also known as polycystic lipomembranous osteodysplasia and sclerosing leukoencephalopathy, is an autosomal recessive disorder characterized by dementia and multifocal bone cysts. NHD is associated with the loss-of-function mutation of triggering receptor expressed on myeloid cells-2 gene (*TREM2*), leading to an aberrant form of TREM2.[1]

Case report. A 39-year-old woman was referred to our department complaining of a 2-year history of progressive cognitive impairment. She experienced personality changes, social inhibition, and apathy associated with memory disturbances and disorientation in time and place. She was an only child, born to nonconsanguineous parents, and had normal early development and no remarkable previous medical conditions. No positive family history for early-onset dementia or other neurologic diseases was reported.

Neuropsychological evaluation outlined moderate cognitive impairment, involving all functions examined. She scored 26/30 on the Mini-Mental State Examination (MMSE).

MRI scans showed an important diffuse, symmetric, cortical atrophy, involving especially the parietal lobes, the dorsal cingulus, the precuneus, and the superior temporal gyrus, with a relative sparing of mesial temporal regions. White matter was severely reduced and hyperintense in T2-weighted images (figure, A).

^{18}F-fluorodeoxyglucose-PET revealed a marked reduction in cortical glucose metabolism in the temporal and parietal areas, and a slight hypometabolism in the dorsal cingulus and in the precuneus (figure, B).

CSF analysis was consistent only for low levels of β-amyloid (Aβ = 597 pg/mL). This finding was confirmed by florbetapir-amyloid-PET (tracer binding preferentially to Aβ fibrils), which showed a heavily whitened increased signal in the gray matter of the inferior frontal and occipital lobes (figure, C).

As symptoms at presentation and imaging were not typical for a specific dementing syndrome (cognitive abnormalities, early white matter pathology) and considering the early age at onset and the lack of positive family history, we hypothesized the presence of a genetic recessive cause or a de novo mutation. Therefore, we took advantage of a next-generation sequencing technique (supplemental material at Neurology.org) covering the spectrum of common and rare dementias, which revealed the homozygous Q33X mutation in *TREM2*, further confirmed by direct sequencing. The diagnosis of NHD was supported by the radiographic detection of multiple asymptomatic cystic bone lesions of hands and feet.

Both the patient's parents, aged 72 years, resulted heterozygous carriers of the same mutation. *APOE* genotype was 3/3 for both the patient and her mother, 3/4 for her father.

The parents showed no cognitive impairment (MMSE = 30/30 for both of them). Florbetapir-PET evidenced Aβ deposition in the frontal, occipital, and lateral temporal lobes and in the cingulus and in the precuneus (figure, D and E). The mother of the patient underwent X-rays, which demonstrated the absence of cystic bone lesions of hands and feet.

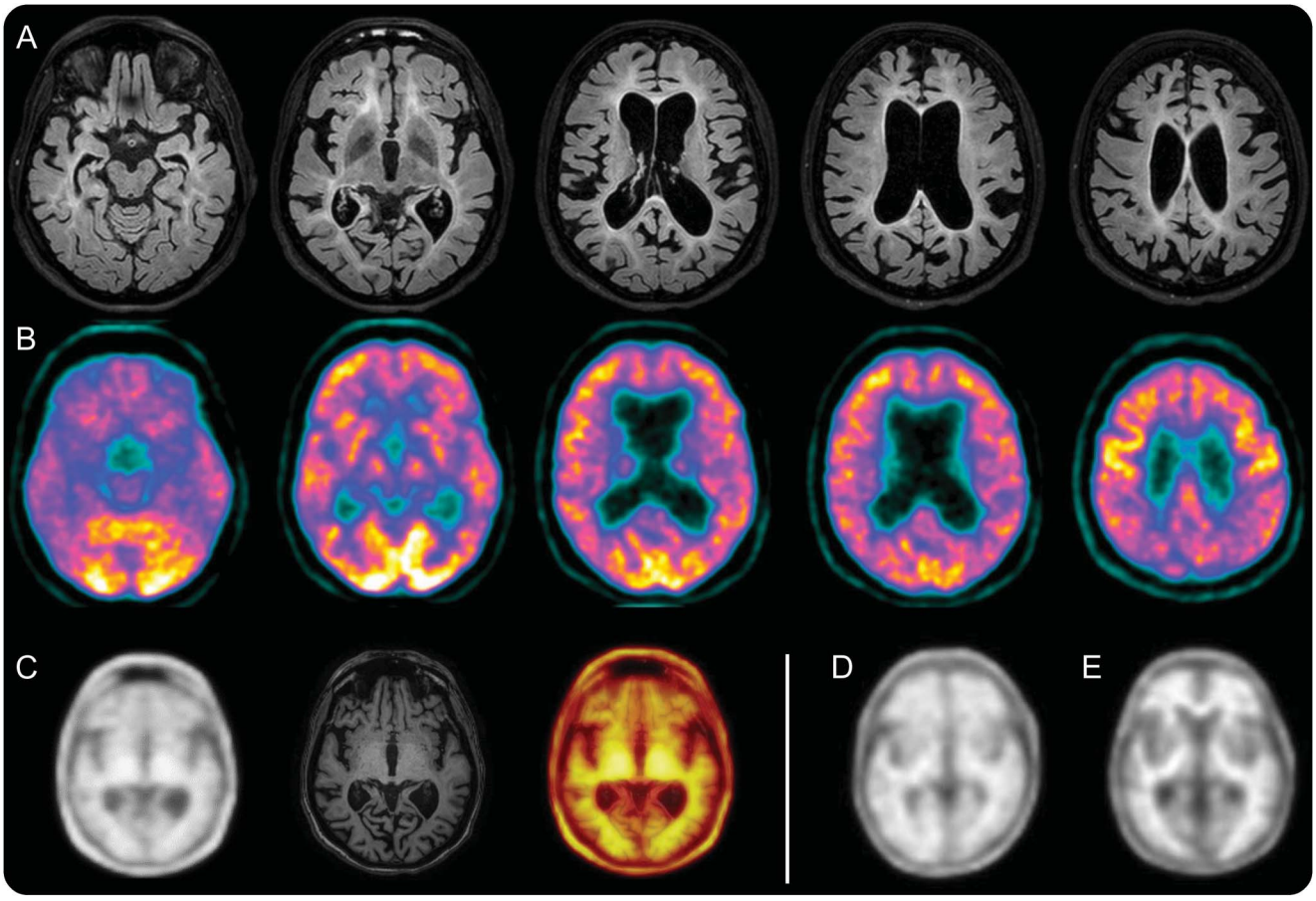
One year after diagnosis, the patient showed a severe worsening of the neurologic conditions. She is currently dependent in every activity of her life (MMSE 7/30). Notably, she developed chronic urinary retention and needed to be catheterized.

Discussion. We described a case of NHD characterized by progressive cognitive deterioration, starting in the fourth decade of life and presenting with memory impairment and behavioral disturbances. The neurologic presentation was in line with the other Italian cases previously reported.[1,2] Nevertheless, despite X-rays outlining multiple cystic bone lesions in hands and feet, the patient remained asymptomatic for bone involvement, in agreement with previous reports.[3]

Next-generation sequencing revealed the Q33X loss-of-function mutation in *TREM2*. The Q33X mutation has been described in homozygosity in a proband of a Turkish family with a frontotemporal dementia–like syndrome without bone involvement[4] and in heterozygosity as a possible risk factor for Alzheimer disease (AD).[5,6]

Notably, both the patient and her parents showed amyloid deposition at florbetapir-PET, suggesting the

Supplemental data at Neurology.org



(A) Fluid-attenuated inversion recovery MRI brain sequences show global atrophy and diffuse white matter hyperintensities. (B) Patient ^{18}F-fluorodeoxyglucose-PET reveals hypometabolism in the temporal and parietal areas and in the dorsal cingulus and in the precuneus. (C) Patient florbetapir-PET-amyloid scan (left), T1 3D-MRI scan (middle), and PET/T1 3D-MRI fusion scan (right), after coregistration by Statistical Parametric Mapping software: β-amyloid (Aβ) deposition appears as a heavily lightened increased signal throughout the cerebral cortex of the inferior frontal and occipital lobes. (D, E) Parents' florbetapir-PET-amyloid scans show a whitened increased signal due to Aβ deposition in the gray matter of the frontal, occipital, and lateral temporal lobes, cingulate cortex, and precuneus of the father (D) and of the frontal, occipital, and posterior temporal lobes and cingulate cortex of the mother (E).

existence of common mechanisms between NHD and AD pathogenesis and the potential involvement of microglia in both formation and clearance of Aβ.[7] Nevertheless, the allelic dosage difference (1 mutated allele in AD and 2 in NHD) may account for the differences observed in clinical phenotypes. Moreover, the latter requires the presence of a mutation in *TREM2* in homozygosis, whereas the former may be sporadic. Regarding the bone pathology, the absence of cystic bone lesions of hands and feet in the patient's mother suggests that one allele only is not enough to cause bone pathology.

We describe a case of NHD disease with evidence of Aβ deposition in the CNS, suggesting overlapping pathogenic mechanisms between NHD and AD. However, as amyloid pathology often occurs in cognitively normal adults, further studies in larger populations are needed to test whether the Q33X variant plays a role in the deposition of amyloid.

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Alzheimer's Disease Diagnosis: Discrepancy between Clinical, Neuroimaging, and Cerebrospinal Fluid Biomarkers Criteria in an Italian Cohort of Geriatric Outpatients: A Retrospective Cross-sectional Study

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Background: The role of cerebrospinal fluid (CSF) biomarkers, and neuroimaging in the diagnostic process of Alzheimer's disease (AD) is not clear, in particular in the older patients.

Objective: The aim of this study was to compare the clinical diagnosis of AD with CSF biomarkers and with cerebrovascular damage at neuroimaging in a cohort of geriatric patients.

Methods: Retrospective analysis of medical records of ≥65-year-old patients with cognitive impairment referred to an Italian geriatric outpatient clinic, for whom the CSF concentration of amyloid-β (Aβ), total Tau (Tau), and phosphorylated Tau (p-Tau) was available. Clinical diagnosis (no dementia, possible and probable AD) was based on the following two sets of criteria: (1) the *Diagnostic Statistical Manual of Mental Disorders* (*DSM*-IV) plus the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) and (2) the National Institute on Aging-Alzheimer's Association (NIA-AA). The Fazekas visual scale was applied when a magnetic resonance imaging scan was available.

Results: We included 94 patients, mean age 77.7 years, mean Mini Mental State Examination score 23.9. The concordance (kappa coefficient) between the two sets of clinical criteria was 70%. The mean CSF concentration (pg/ml) (±SD) of biomarkers was as follows: Aβ 687 (±318), Tau 492 (±515), and p-Tau 63 (±56). There was a trend for lower Aβ and higher Tau levels from the no dementia to the probable AD group. The

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Original Article

ORIGINAL ARTICLE

Autologous intramuscular transplantation of engineered satellite cells induces exosome-mediated systemic expression of Fukutin-related protein and rescues disease phenotype in a murine model of limb-girdle muscular dystrophy type 2I

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Abstract

α-Dystroglycanopathies are a group of muscular dystrophies characterized by α-DG hypoglycosylation and reduced extracellular ligand-binding affinity. Among other genes involved in the α-DG glycosylation process, fukutin related protein (FKRP) gene mutations generate a wide range of pathologies from mild limb girdle muscular dystrophy 2I (LGMD2I), severe congenital muscular dystrophy 1C (MDC1C), to Walker-Warburg Syndrome and Muscle-Eye-Brain disease. FKRP gene encodes for a glycosyltransferase that *in vivo* transfers a ribitol phosphate group from a CDP –ribitol present in muscles to α-DG, while in vitro it can be secreted as monomer of 60kDa. Consistently, new evidences reported glycosyltransferases in the blood, freely circulating or wrapped within vesicles. Although the physiological function of blood stream glycosyltransferases remains unclear, they are likely released from blood borne or distant cells. Thus, we hypothesized that freely or wrapped FKRP might circulate as an extracellular glycosyltransferase, able to exert a "glycan remodelling" process, even at distal compartments. Interestingly, we firstly demonstrated a successful transduction of MDC1C blood-derived CD133+ cells and FKRP L276I^{KI} mouse derived satellite cells by a lentiviral vector expressing the wild-type of human FKRP gene. Moreover, we showed that LV-FKRP cells were driven to release exosomes carrying FKRP. Similarly, we observed the presence of FKRP positive exosomes in the plasma of FKRP L276I^{KI} mice intramuscularly injected with engineered satellite cells. The distribution of

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ORIGINAL ARTICLE

AJT

Immunoisolation of murine islet allografts in vascularized sites through conformal coating with polyethylene glycol

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Islet encapsulation may allow transplantation without immunosuppression, but thus far islets in large microcapsules transplanted in the peritoneal cavity have failed to reverse diabetes in humans. We showed that islet transplantation in confined well-vascularized sites like the epididymal fat pad (EFP) improved graft outcomes, but only conformal coated (CC) islets can be implanted in these sites in curative doses. Here, we showed that CC using polyethylene glycol (PEG) and alginate (ALG) was not immunoisolating because of its high permselectivity and strong allogeneic T cell responses. We refined the CC composition and explored PEG and islet-like extracellular matrix (Matrigel; MG) islet encapsulation (PEG MG) to improve capsule immunoisolation by decreasing its permselectivity and immunogenicity while allowing physiological islet function. Although the efficiency of diabetes reversal of allogeneic but not syngeneic CC islets was lower than that of naked islets, we showed that CC (PEG MG) islets from fully MHC-mismatched Balb/c mice supported long-term (>100 days) survival after transplantation into diabetic C57BL/6 recipients in the EFP site (750-1000 islet equivalents/mouse) in the absence of immunosuppression. Lack of immune cell penetration and T cell allogeneic priming was observed. These studies support the use of CC (PEG MG) for islet encapsulation and transplantation in clinically relevant sites without chronic immunosuppression.

KEYWORDS
animal models: murine, basic (laboratory) research/science, bioengineering, diabetes, encapsulation, islet transplantation, islets of Langerhans, regenerative medicine, translational research/science

Abbreviations: ALG, alginate; BL, blood; CC, conformal coated; DTT, dithiothreitol; dVS, divinyl sulfone; ECM, extracellular matrix; EFP, epididymal ft pad; GSIR, glucose-stimulated insulin release; H&E, hematoxilin & eosin; IFNgamma, interferon gamma; IP, intraperitoneal cavity; KD, kidney subcapsular space; MAL, maleimide; MG, Matrigel; MLR, mixed lymphocyte reaction; mTOR, mechanistic target of rapamycin; OCR, oxygen consumption rate; PBL, peripheral blood lymphocyte; PEG, polyethylene glycol; R, responders; ROS, reactive oxygen species; S, stimulators; SC, subcutaneous; SD, standard deviation; SPL, spleen; T1D, type 1 diabetes.

SCIENTIFIC REPORTS

OPEN

Metal Nanoclusters with Synergistically Engineered Optical and Buffering Activity of Intracellular Reactive Oxygen Species by Compositional and Supramolecular Design

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Metal nanoclusters featuring tunable luminescence and high biocompatibility are receiving attention as fluorescent markers for cellular imaging. The recently discovered ability of gold clusters to scavenge cytotoxic reactive oxygen species (ROS) from the intracellular environment extends their applicability to biomedical theranostics and provides a novel platform for realizing multifunctional luminescent probes with engineered anti-cytotoxic activity for applications in bio-diagnostics and conceivably cellular therapy. This goal could be achieved by using clusters of strongly reactive metals such as silver, provided that strategies are found to enhance their luminescence while simultaneously enabling direct interaction between the metal atoms and the chemical surroundings. In this work, we demonstrate a synergic approach for realizing multifunctional metal clusters combining enhanced luminescence with strong and lasting ROS scavenging activity, based on the fabrication and *in situ* protection of Ag nanoclusters with a supramolecular mantle of thiolated-Au atoms (Ag/Au-t). Confocal imaging and viability measurements highlight the biocompatibility of Ag/Au-t and their suitability as fluorescent bio-markers. ROS concentration tests reveal the remarkable scavenging activity of Ag-based clusters. Proliferation tests of cells in artificially stressed culture conditions point out their prolonged anti-cytotoxic effect with respect to gold systems, ensuring positive cell proliferation rates even for long incubation time.

Metal nanoclusters, owing to their size- and shape-tunable electronic properties[1], ultra-large surface-to-volume ratios, low toxicity[2] and to the flexibility of their physical properties *via* surface functionalization[3–7], are receiving growing attention in several technological areas, spanning from solid state lighting[8], solar cells[9] and sensors[10, 11] to photo-catalysis[12, 13] and biomedical applications[10, 14–20]. The archetype metal nanoclusters are gold-based systems, whose luminescence properties can be controlled through a variety of approaches including, quantum confinement effects[1], ligand-to-metal electron transfer[3, 21, 22], controlled surface complexation[4, 23], ligand-controlled formation of super-cluster architectures[24] and through so-called aggregation induced emission between thiolate-protected clusters[25]. In addition to this, Au clusters have recently been demonstrated to scavenge intracellular reactive oxygen species (ROS), which are highly reactive compounds that are typically formed as a by-product of the cellular oxygen metabolism and play important roles in cell signalling and homeostasis[26].

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Dopamine receptor type 2 (DRD2) and somatostatin receptor type 2 (SSTR2) agonists are effective in inhibiting proliferation of progenitor/stem-like cells isolated from nonfunctioning pituitary tumors

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The role of progenitor/stem cells in pituitary tumorigenesis, resistance to pharmacological treatments and tumor recurrence is still unclear. This study investigated the presence of progenitor/stem cells in non-functioning pituitary tumors (NFPTs) and tested the efficacy of dopamine receptor type 2 (DRD2) and somatostatin receptor type 2 (SSTR2) agonists to inhibit *in vitro* proliferation. They found that 70% of 46 NFPTs formed spheres co-expressing stem cell markers, transcription factors (DAX1, SF1, ERG1) and gonadotropins. Analysis of tumor behavior showed that spheres formation was associated with tumor invasiveness (OR = 3,96; IC: 1.05–14.88, $p = 0.036$). The *in vitro* reduction of cell proliferation by DRD2 and SSTR2 agonists (31 ± 17% and 35 ± 13% inhibition, respectively, $p < 0.01$ vs. basal) occurring in about a half of NFPTs cells was conserved in the corresponding spheres. Accordingly, these drugs increased cyclin-dependent kinase inhibitor p27 and decreased cyclin D3 expression in spheres. In conclusion, they provided further evidence for the existence of cells with a progenitor/stem cells-like phenotype in the majority of NFPTs, particularly in those with invasive behavior, and demonstrated that the antiproliferative effects of dopaminergic and somatostatinergic drugs were maintained in progenitor/stem-like cells.

Key words: tumor stem cells, pituitary adenomas, dopamine, somatostatin, drug resistance
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Introduction

Recently, a subpopulation of progenitor/stem cells has been demonstrated to exist in pituitary tumors. In particular, these cells were characterized by the expression of stem cells markers, the ability to grow as rounded cell spheres and to self-renew.[1–4] Based on the cancer stem cell hypothesis, tumors develop from a small subpopulation of self-renewing cells, that are able to differentiate into the heterogeneous lineages of the cancer cells and to regenerate the tumor, and are resistant to conventional chemotherapeutic agents. To date the tumor-initiating and tumor-driving role of progenitor/stem cells identified in pituitary tumors has yet to be proven. Neurosurgery by the transphenoidal approach is the treatment of choice for clinically non-functioning pituitary tumors

The Italian LGMD registry: relative frequency, clinical features, and differential diagnosis

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ORIGINAL COMMUNICATION

Revisiting mitochondrial ocular myopathies: a study from the Italian Network

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Abstract Ocular myopathy, typically manifesting as progressive external ophthalmoplegia (PEO), is among the most common mitochondrial phenotypes. The purpose of this study is to better define the clinical phenotypes associated with ocular myopathy. This is a retrospective study on a large cohort from the database of the "Nation-wide Italian Collaborative Network of Mitochondrial Diseases". We distinguished patients with ocular myopathy as part of a multisystem mitochondrial encephalomyopathy (PEO-encephalomyopathy), and then PEO with isolated ocular myopathy from PEO-plus when PEO was associated with additional features of multisystemic involvement. Ocular myopathy was the most common feature in our cohort of mitochondrial patients. Among the 722 patients with a definite genetic diagnosis, ocular myopathy was observed in 399 subjects (55.3%) and was positively associated with mtDNA single deletions and *POLG* mutations. Ocular myopathy as manifestation of a multisystem mitochondrial encephalomyopathy (PEO-encephalomyopathy, $n = 131$) was linked to the m.3243A>G mutation, whereas the other "PEO" patients ($n = 268$) were associated with mtDNA single deletion and *Twinkle* mutations. Increased lactate was associated with central neurological involvement. We then defined, among the PEO group, as "pure PEO" the patients with isolated ocular myopathy and "PEO-plus" those with ocular myopathy and other features of neuromuscular and multisystem involvement, excluding central nervous system. The male proportion was significantly lower in pure PEO than PEO-plus. This study reinforces

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RESPIRATORY CARE Paper in Press. Published on August 14, 2017 as DOI: 10.4187/respcare.05031

Use of Noninvasive Ventilation During Feeding Tube Placement

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Parenteral nutrition is indicated in amyotrophic lateral sclerosis (ALS) when dysphagia, loss of appetite, and difficulty protecting the airways cause malnutrition, severe weight loss, dehydration, and increased risk of aspiration pneumonia. The aim of this review is to compare percutaneous endoscopic gastrostomy (PEG), radiologically inserted G-tube (RIG), and percutaneous radiologic gastrostomy (PRG) in patients with ALS, performed with or without noninvasive ventilation (NIV). We searched PubMed, MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), the EBSCO Online Research Database, and Scopus up to December 2015. A priori selection included all randomized controlled trials (RCTs), quasi-randomized trials, and prospective and retrospective studies. The primary outcome was 30-d survival. We found no RCTs or quasi-RCTs. Seven studies about the implementation of the PEG/RIG procedure during the use of NIV and 5 studies without NIV were included. In another study of 59 subjects undergoing open gastrostomy, all with vital capacity < 30% of normal, 18 of whom were dependent on continuous NIV at full ventilatory support settings, there were no respiratory complications. Thus, the use of NIV during the implementation of these procedures, especially when used at full ventilatory support settings of pressure preset 18–25 cm H_2O, can support alveolar ventilation before, during, and after the procedures and prevent respiratory complications. The procedures investigated appear equivalent, but the methodological quality of the studies could be improved. Possible benefits with regard to nutrition parameters, quality of life, and psychological features need to be further investigated. *Key words: amyotrophic lateral sclerosis (ALS); noninvasive ventilation (NIV); gastrostomy; clinical effectiveness; quality of life (QOL); systematic review.* [Respir Care 0;0(0):1–•. © 0 Daedalus Enterprises]

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Introduction

Amyotrophic lateral sclerosis (ALS) is a neurological disorder characterized by a progressive degeneration of the motor neurons. Bulbar onset affects 25–30% of all patients, but all patients surviving long enough eventually develop severe bulbar-innervated muscle impairment that causes dysphagia, aspiration of food and saliva, and severe dysarthria.[1,2] Dysphagia causes malnutrition, dehydration, weight loss, and an increased risk of aspiration pneumonia[3] and is an important negative prognostic factor in ALS.[4] Moreover, poor appetite due to depression, reduced ability to feed oneself, and hypermetabolism can also lead to decreased oral feeding and subsequent malnutrition/dehydration.[5] Malnutrition increases muscle weakness, increases fatigue,[6] and decreases respiratory capacity.[7] This situation creates a vicious cycle, leading to the development of depression and decreasing quality of life (QOL).[8] Dietary changes are thus necessary to maintain proper caloric intake and prevent aspiration.[9,10] When oral feeding becomes insufficient, enteral nutrition in patients with ALS can be guaranteed through gastrostomy placement. The procedures include percutaneous endoscopic gastrostomy (PEG), radiologically inserted G-tube (RIG), percutaneous radiologic gastrostomy (PRG), and open gastrostomy.[11,12] Although percutaneous gastrostomy procedures are more frequently employed than those requiring general anesthesia,[13] the frequency of PEG/RIG/PRG insertion varies widely across different countries and studies.[9] To prevent and manage respiratory symptoms, the use of noninvasive ventilation (NIV), which has become synonymous with CPAP and low span (< 10 cm H_2O) bi-level PAP, is being used during the insertion of feeding tubes for many patients with FVC $< 50\%$ of predicted normal.[14] It should be noted, however, that in many centers, patients become dependent on continuous NIV at full ventilatory support settings. They require either high span (15–25 cm H_2O) bi-level PAP or intermittent positive-pressure ventilation at full ventilatory support settings, delivered via noninvasive oral, nasal, or oronasal interfaces. Generally, portable ventilators are used with active circuits on volume control mode with exhaled tidal volume > 800 mL or pressure preset at 17–25 cm H_2O.[15] Many of these patients do not undergo gastrostomy until their vital capacities (VCs) are $< 10\%$ of predicted normal.[15]

There is no consistent evidence about which of the procedures is the safest and most effective in ALS. Although literature concerning RIG/PRG and open gastrotomies is scarce, frequency of PEG in patients with ALS, which was only around 2.7% in the early 1970s,[16] has more recently increased.[12] Indeed, it has been performed on 13–40% of patients with ALS in the United States,[17,18] 14–38% in the United Kingdom,[19,20] 11–24% in Italy,[21–23] 21–60% in Japan,[9,24] and 20% in Canada.[25] Meanwhile, the apparent increasing demand for RIG/PRGs and their possible advantages and disadvantages compared with PEGs in maintaining adequate nutrition and weight stabilization have not been assessed systematically and remain unclear.[14,26] To the best of our knowledge, there has been only a single attempt to provide the best evidence to support procedures for parenteral nutrition, and it only compared PEG tube feeding with oral feeding for patients with ALS.[9] The main aim of this review is to compare PEG, RIG, and PRG, with and without NIV use, for efficacy and safety.

Methods

Literature Search Strategy

The primary literature search method employed PubMed, MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL), the EBSCO Online Research Database, and Scopus. The search strategy used a combination of subject heading terms appropriate for each database and key words such as "amyotrophic lateral sclerosis," "ALS," "noninvasive ventilation," "NIV," "gastrostomy," "feeding tube," "procedure," "placement," "percutaneous endoscopic gastrostomy," "PEG," "percutaneous radiologic gastrostomy," "PRG," "radiologically inserted G-tube," and "RIG" with Boolean terms such as AND and OR. These words were searched for in the title, abstract, key words, and MeSH (medical subject headings) terms. The reference lists of all eligible trials were checked, and the Cited By research tool was used. Findings were limited to English language and to human studies between 1980 and 2015. No unpublished studies or gray literature were considered (Fig. 1).

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Journal of Neuroinflammation

RESEARCH Open Access

Adiponectin levels in the serum and cerebrospinal fluid of amyotrophic lateral sclerosis patients: possible influence on neuroinflammation?

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Abstract

Background: Adiponectin (APN) is a key player in energy homeostasis strictly associated with cerebrovascular and neurodegenerative diseases. Since APN also belongs to anti-inflammatory-acting adipokines and may influence both neuroinflammation and neurodegenerative processes, we decided to study the APN levels in amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases.

Methods: We assessed APN levels by ELISA immunoassay in both the serum and cerebrospinal fluid of a cohort of familial and sporadic ALS patients, characterized by normal body mass index and absence of dysautonomic symptoms. The screening of serum APN levels was also performed in patients affected by other neurological disorders, including fronto-temporal dementia (FTD) patients. Means were compared using the non-parametric Wilcoxon test, and Pearson's or Spearman's rho was used to assess correlations between variables.

Results: In the whole ALS group, serum APN levels were not different when compared to the age- and sex-matched control group (CTR), but a gender-specific analysis enlightened a significant opposite APN trend between ALS males, characterized by lower values (ALS 9.8 ± 5.2 vs. CTR 15 ± 9.7 μg/ml), and ALS females, showing higher amounts (ALS 26.5 ± 11.6 vs. CTR 14.6 ± 5.2 μg/ml). This sex-linked difference was significantly enhanced in familial ALS cases ($p \leq 0.01$). The APN levels in ALS cerebrospinal fluids were unrelated to serum values and not linked to sex and/or familiarity of the disease. Finally, the screening of serum APN levels in patients affected by other neurological disorders revealed the highest serum values in FTD patients.

Conclusions: Opposite serum APN levels are gender-related in ALS and altered in several neurological disorders, with the highest values in FTD, which shares with ALS several overlapping and neuropathological features. Further investigations are needed to clarify the possible involvement of APN in neuroinflammation and neurodegeneration.

Keywords: Adiponectin, Adipokine, Neurodegeneration, Neuroinflammation, Motor neuron disease, Fronto-temporal dementia

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Interestingly, the direct significant correlation between APN levels and HDL values suggests that this parameter may be predictive of APN quantification in ALS patients. Conversely, APN levels were independent of BMI and systemic inflammatory status in ALS patients, according to previous literature [32].

The association between peripheral and CNS levels is still controversial [3, 43–45], but our data reveal independent amounts within these districts. A similar difference between plasma and CSF was demonstrated for progranulin protein [46], thus suggesting no main alterations of BBB or, alternatively, a compromised modulation of APN in the ALS CNS. The observed APN levels in the CSF of ALS patients are in line with literature data for healthy controls [44].

Adiponectin alterations were observed in all neurological diseases considered, with FTD patients displaying the highest amounts. Although FTD and AD patients were significantly older than the other categories, the appropriate age adjustment maintained the significance, thus suggesting a reduced effect of ageing. In the studied cohort of female ALS, APN levels were extremely elevated and not associated with dementia diagnosis. As already demonstrated in women [47], we cannot exclude that APN increased levels may prospectively act as a "prodromic factor" for dementia/cognitive impairment in ALS. Levels of APN may also be translatable in selective biomarkers for cognitive impairment among two closely related conditions with overlapping clinical features, such as ALS and FTD [13, 22, 23]. However, further investigations are required to better explain the correlation between APN and neuroinflammation.

Conclusions

Our data demonstrate circulating APN alterations in several neurodegenerative diseases characterized by neuroinflammation. We report a gender-related opposite APN trend in ALS patient sera, but normal levels in their CSF. In addition, in FTD patients sharing some overlapping clinical features with ALS, the highest serum APN levels were observed.

Abbreviations

AD: Alzheimer's disease; ALS: Amyotrophic lateral sclerosis; ALT: glutamate-pyruvate transaminase 1/alanine aminotransferase; APN: Adiponectin; AST: glutamic oxaloacetic transaminase/aspartate aminotransferase; CSF: Cerebrospinal fluid; DN: Dysimmuno-neuropathy; FTD: Fronto-temporal dementia; OB: Obese; OND: Other neurological diseases

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Availability of data and materials

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

Authors' contributions

RC, PB and LC designed the study, performed the experiments and analysed the data. CM and AD selected the patients and collected the samples and all the required clinical data. VS supervised the study. RC, PB and LC wrote the manuscript. All authors were critically involved in the data analysis, discussion and revision of the paper and had final responsibility for the decision to submit the present form of the paper to this journal. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Ethical approval for the study was obtained from the IRCCS Istituto Auxologico Italiano Ethics Committee in accordance with specific Italian and European laws. The study was conducted in conformity to the principles set out in the Declaration of Helsinki, and all participants provided written informed consent.

Publisher's Note

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Review Article

Brain-Computer Interface for Clinical Purposes: Cognitive Assessment and Rehabilitation

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Alongside the best-known applications of brain-computer interface (BCI) technology for restoring communication abilities and controlling external devices, we present the state of the art of BCI use for cognitive assessment and training purposes. We first describe some preliminary attempts to develop verbal-motor free BCI-based tests for evaluating specific or multiple cognitive domains in patients with Amyotrophic Lateral Sclerosis, disorders of consciousness, and other neurological diseases. Then we present the more heterogeneous and advanced field of BCI-based cognitive training, which has its roots in the context of neurofeedback therapy and addresses patients with neurological developmental disorders (autism spectrum disorder and attention-deficit/hyperactivity disorder), stroke patients, and elderly subjects. We discuss some advantages of BCI for both assessment and training purposes, the former concerning the possibility of longitudinally and reliably evaluating cognitive functions in patients with severe motor disabilities, the latter regarding the possibility of enhancing patients' motivation and engagement for improving neural plasticity. Finally, we discuss some present and future challenges in the BCI use for the described purposes.

1. Introduction

BCIs have been studied with the primary motivation of providing assistive technologies for people with severe motor disabilities, particularly locked-in syndrome (LIS) caused by neurodegenerative disease such as Amyotrophic Lateral Sclerosis (ALS) or by stroke [1]. Such approach involves the use of suitable cortical signals as input to control external devices or for Augmentative and Alternative Communication purposes in patients suffering from central nervous system injury. BCI has been studied for more than 25 years and has been extensively validated, even if with still heterogeneous results according to both the method employed and the populations involved [2, 3]. A review of BCI studies is not within the objective of the present work [4].

A newly emerging field of research concerns the use of BCIs to enhance motor and cognitive recovery within neurorehabilitation settings. In fact, most of common rehabilitation tools require a minimal level of motor control to perform the therapeutic tasks; therefore, patients with severe motor deficits are not allowed to accomplish traditional rehabilitation training. Some recent reviews have presented and discussed main advances in the use of BCIs for rehabilitation purposes [5–7]. A further work has discussed the current status of BCI as a rehabilitation strategy in stroke patients [8]. In addition to the use of BCI to restore motor function

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Safety and Efficacy of the New Micromesh-Covered Stent CGuard in Patients Undergoing Carotid Artery Stenting: Early Experience From a Single Centre

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WHAT THIS PAPER ADDS

According to the existing literature, CAS is associated with a significantly higher stroke rate compared with CEA in the 30 day post-operative period. Beyond this, long-term outcomes for CAS and CEA seem comparable. Technical advances in stent design may improve CAS outcomes both in terms of procedural stroke and rate of new DW-MRI cerebral lesions. The present single centre study evaluated the safety and efficacy of the new nitinol stent covered by a micromesh CGuard in preventing embolic events during the CAS procedure and during the stent healing period.

Objective/Background: Plaque protrusion through stent struts represents one of the principal causes of cerebral embolisation during carotid artery stenting (CAS) and the stent healing period. The aim of this study was to evaluate the safety (technical success) and efficacy (clinical success) of the CGuard stent system – a new nitinol stent covered by a closed-cell polyethylene terephthalate mesh designed to prevent embolic events.

Methods: Eighty-two consecutive patients who underwent CAS with CGuard from June 2015 were included in this study. The same surgeon performed all procedures. Primary endpoints included technical and clinical success. Clinical success was considered to be absence of death, major or minor stroke. The incidence of new ischaemic brain lesions was also evaluated by diffusion weighted magnetic resonance imaging (DW-MRI) in a subgroup of patients as a secondary endpoint.

Results: In this study, 82 patients (73.8 ± 8.5 years, 75% male, 19% symptomatic) underwent CAS procedures. Immediate technical success was 100%, with the stenosis diameter reduced from 81.4 ± 4.9% to 11.0 ± 3.5%. There was peri-operative technical and clinical success in 100% of symptomatic patients, and in 98.5% of asymptomatic patients, because of the occurrence of one acute stent thrombosis 4 hours post-CAS followed by a minor stroke. In the post-operative period (30 days), no new events were registered. The most recent 21 patients (24%) underwent DW-MRI in the peri-operative period: new ischaemic brain lesions were recorded in 23.8% of patients and the average lesion volume per patients was 0.039 ± 0.025 cm^3.

Conclusions: The technical and clinical outcomes of this single centre study suggest that the CGuard may be a safe and effective device for endovascular treatment of symptomatic and asymptomatic subjects, independent of aortic arch anatomy. Further larger comparative studies are needed to confirm these benefits.

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Keywords: Carotid artery stenting, Carotid revascularisation, Mesh-covered stent

INTRODUCTION

Carotid artery stenosis is thought to cause up to 20% of ischaemic strokes,[1] mainly caused by atheromatous narrowing at the carotid bifurcation. Carotid endarterectomy (CEA) is the current gold standard therapy.[2,3] In recent years, with the advent of minimally invasive techniques, carotid artery stenting (CAS) has emerged as a treatment option, especially for subjects at increased surgical risk.

The progressive reduction of peri-procedural complications associated with the CAS procedure may be because of technical advances in stenting, including stent design, the use of embolic protection devices and increased operator experience. Despite embolic protection device usage,

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BMJ Open

Protein misfolding, amyotrophic lateral sclerosis and guanabenz: protocol for a phase II RCT with futility design (ProMISe trial)

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ABSTRACT

Introduction Recent studies suggest that endoplasmic reticulum stress may play a critical role in the pathogenesis of amyotrophic lateral sclerosis (ALS) through an altered regulation of the proteostasis, the cellular pathway-balancing protein synthesis and degradation. A key mechanism is thought to be the dephosphorylation of eIF2α, a factor involved in the initiation of protein translation. Guanabenz is an alpha-2-adrenergic receptor agonist safely used in past to treat mild hypertension and is now an orphan drug. A pharmacological action recently discovered is its ability to modulate the synthesis of proteins by the activation of translational factors preventing misfolded protein accumulation and endoplasmic reticulum overload. Guanabenz proved to rescue motoneurons from misfolding protein stress both in in vitro and in vivo ALS models, making it a potential disease-modifying drug in patients. It is conceivable investigating whether its neuroprotective effects based on the inhibition of eIF2α dephosphorylation can change the progression of ALS.

Methods and analyses Protocolised Management In Sepsis is a multicentre, randomised, double-blind, placebo-controlled phase II clinical trial with futility design. We will investigate clinical outcomes, safety, tolerability and biomarkers of neurodegeneration in patients with ALS treated with guanabenz or riluzole alone for 6 months. The primary aim is to test if guanabenz can reduce the proportion of patients progressed to a higher stage of disease at 6 months compared with their baseline stage as measured by the ALS Milano-Torino Staging (ALS-MITOS) system and to the placebo group. Secondary aims are safety, tolerability and change in at least one biomarker of neurodegeneration in the guanabenz arm compared with the placebo group. Findings will provide reliable data on the likelihood that guanabenz can slow the course of ALS in a phase III trial.

Ethics and dissemination The study protocol was approved by the Ethics Committee of IRCCS 'Carlo Besta Foundation' of Milan (Eudract no. 2014-005367-32 Pre-results) based on the Helsinki declaration.

Strengths and limitations of this study

- Amyotrophic lateral sclerosis (ALS) is a rare disease and randomised controlled trial (RCT) striving to obtain reliable data on the efficacy of candidate neuroprotective molecules should be performed with a multicentre design. Our consortium, including 25 ALS centres in Italy, satisfies the criteria of complementarily and synergy needed for a multicentre RCT. The coordinating centre is experienced in leading RCTs and the participating centres have a long-standing collaboration in the field of ALS, both in the clinical management of patients and clinical research. This will guarantee a standardised approach in all the patients.
- The study drug, guanabenz, has a mechanism of action close to pathogenic changes currently considered central to the pathogenesis of ALS, and preclinical studies are most promising.
- The futility design is an original and innovative methodological approach to neurodegenerative disease clinical trials. It allows optimising time and resources for a larger III phase study, but the a priori cut-off may be challenging.
- The primary outcome is a change in patients' function. It has been chosen to overcome the internal validity limitations of the ALS Functional Rating Score-Revised (ALSFRS-R) and to provide clinically meaningful results reflecting a concrete, though potential, advantage to patients. However, it may not be able to capture mild changes.

INTRODUCTION

Background and rationale

Recent evidence highlighted the crucial role that accumulation of misfolded protein aggregates in neurons and glial cells and failure of clearance mechanisms have in the

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Contributors IT, EDB and GL: study concept and final design. All the coauthors of the PROMISE trial study group (namely ACò, CC, NR, GM, CL, VS, JM, FG, EG, RE, GA, GB, MF, MC, PV, MRMò, GSù, LM, RR, GS, VLB, MC, IS, SM) have contributed to the design of the protocol.

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Phosphorylated Neurofilament Heavy Chain: A Biomarker of Survival for *C9ORF72*-Associated Amyotrophic Lateral Sclerosis

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As potential treatments for *C9ORF72*-associated amyotrophic lateral sclerosis (c9ALS) approach clinical trials, the identification of prognostic biomarkers for c9ALS becomes a priority. We show that levels of phosphorylated neurofilament heavy chain (pNFH) in cerebrospinal fluid (CSF) predict disease status and survival in c9ALS patients, and are largely stable over time. Moreover, c9ALS patients exhibit higher pNFH levels, more rapid disease progression, and shorter survival after disease onset than ALS patients without *C9ORF72* expansions. These data support the use of CSF pNFH as a prognostic biomarker for clinical trials, which will increase the likelihood of successfully developing a treatment for c9ALS.

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Despite more than 50 large clinical trials in the past half-century, there is only 1 minimally effective treatment for amyotrophic lateral sclerosis (ALS), a devastating motor neuron disease. Nevertheless, since the discovery of *C9ORF72* G_4C_2 repeat expansions as the most common genetic cause of ALS,[1,2] significant advances have been made toward elucidating the mechanisms by which this mutation causes *C9ORF72*-associated ALS (c9ALS), and devising therapeutic strategies to combat them. Multiple lines of evidence place G_4C_2 repeat RNA and dipeptide repeat (DPR) proteins synthesized from these transcripts at the crux of c9ALS. Therapeutic strategies that target G_4C_2 RNA, such as antisense oligonucleotides (ASOs) and small molecules, reduce DPR protein levels, and mitigate other abnormalities caused by G_4C_2 transcripts in c9ALS models.[3–6]

As therapeutics for c9ALS are sought, we must address barriers in moving a treatment from bench to bedside, such as the lack of biomarkers to forecast disease progression and confirm target engagement in clinical trials. We recently established poly(GP) DPR proteins as a promising pharmacodynamic biomarker for G_4C_2 RNA-

Additional Supporting Information may be found in the online version of this article.

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targeting ASOs (c9ASOs),[6] but forthcoming clinical trials for c9ASOs will also benefit from biomarkers that predict disease course. Phosphorylated neurofilament heavy chain (pNFH) and neurofilament light chain, which are released into the interstitial fluid during axonal injury and neurodegeneration, have emerged as putative prognostic biomarkers.[7] Levels of pNFH are elevated in cerebrospinal fluid (CSF) and blood from patients with ALS,[8–14] and some studies show that pNFH levels are associated with survival and/or indicators of disease progression.[8,9,11–13,15] The prognostic potential of pNFH, however, has yet to be specifically evaluated in c9ALS patients, a population that differs clinically and pathophysiologically from ALS patients without a *C9ORF72* repeat expansion.[16–19] We thus investigated pNFH as an urgently needed prognostic biomarker for c9ALS.

Subjects and Methods

Participants

An international sampling of CSF from *C9ORF72* expansion carriers (n = 135) and noncarriers with no known ALS- or frontotemporal dementia (FTD)-linked mutation (n = 107) was used. Samples were obtained from asymptomatic *C9ORF72* expansion carriers, healthy individuals, and clinically symptomatic patients diagnosed with ALS, ALS with comorbid FTD (ALS-FTD), or FTD (Table). ALS patients met El Escorial criteria for this diagnosis.[20] Diagnosis of FTD was obtained through established guidelines[21–24] and supported by neuropsychological testing and, in autopsied patients, by pathologically verified frontotemporal lobar degeneration. Our cohort comprised a collection of existing samples from multiple biobanks and included samples collected specifically for studies on neurofilaments. Longitudinally collected CSF was available from 37 *C9ORF72* expansion carriers and 17 noncarriers. Written informed consent was obtained from all participants or their legal next of kin if they were unable to give written consent, and biological samples were obtained with ethics committee approval.

Sample Collection

The standard operating procedures for the collection, processing, and storage of CSF were generally consistent among sites. In brief, CSF was collected in polypropylene tubes by lumbar puncture (LP) and immediately placed on ice. With the exception of samples from 3 groups, the CSF was spun at low speed at 4°C within 30 minutes of collection to pellet any cellular debris. Samples were aliquoted before storing at −80°C.

pNFH Analysis

The previously described Meso Scale Discovery immunoassay used for this study employs a mouse antihuman pNFH antibody and a sulfo-tagged polyclonal anti-pNFH antibody as the capture and detection antibodies, respectively, and a purified bovine pNFH calibrator.[25] The assay has been analytically validated as a laboratory-developed test in the Iron Horse Clinical Laboratory Improvement Amendments–certified laboratory. Samples tested in duplicate have a coefficient of variation < 10%. The intra- and interday precision of the assay is also <10%. Reagents and quality control samples were transferred to Mayo Clinic Jacksonville, where all CSF samples were tested at an 8-fold dilution, to establish commutability between the two sites.

Statistical Analysis

ALS patient functional status was determined using the Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R). For cross-sectional studies, the disease progression score was calculated using the equation: (48 − ALSFRS-R score at baseline)/disease duration in months from disease onset to baseline LP.[8] For longitudinal studies, we used: (48 − ALSFRS-R score at last LP)/disease duration in months from disease onset to last LP.

Comparisons of pNFH levels across disease groups, and associations of pNFH with disease progression score or survival since disease onset, were conducted separately for *C9ORF72* expansion carriers and noncarriers, as described below. Our primary analyses also included comparing pNFH levels, disease progression scores, and survival after disease onset between *C9ORF72* expansion carriers and noncarriers. Given our 9 primary analyses, a Bonferroni adjustment was made and $p \leq 0.0056$ was considered statistically significant.

For regression analyses, pNFH values were log-scaled, and a square root transformation was applied to the disease progression scores due to their skewed distributions.

pNFH levels were compared among disease groups (asymptomatic/healthy, ALS/ALS-FTD, FTD) using multivariate linear regression (MLR) models adjusted for age at LP and gender. Given a statistically significant ($p \leq 0.0056$) difference among groups, post hoc pairwise comparisons were made, with $p \leq 0.0167$ considered significant after Bonferroni correction. The ability of pNFH to discriminate between disease groups was examined by estimating the area under the receiver operating characteristic (ROC) curve.

Associations of pNFH levels in ALS/ALS-FTD patients with disease progression scores were evaluated using MLR models adjusted for age at disease onset, gender, and onset site. Additional adjustment for disease group (ALS or ALS-FTD) was made only in *C9ORF72* mutation carriers, as only 2 noncarriers had ALS-FTD.

Associations of pNFH levels in ALS/ALS-FTD patients with survival after disease onset were examined using multivariate Cox proportional hazards regression models. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated, and censoring occurred at the date of last follow-up. The multivariate model for *C9ORF72* mutation carriers was adjusted for age at disease onset, gender, disease group, and onset site. For non–mutation carriers, we adjusted only for age at disease onset and onset site due to the smaller number of deaths in this subgroup.[26] We additionally estimated the concordance index (c-index) with and without pNFH in a given Cox model to provide an alternative measure of the predictive ability of pNFH; a c-index of 0.5 indicates

AMYOTROPHIC LATERAL SCLEROSIS

Poly(GP) proteins are a useful pharmacodynamic marker for *C9ORF72*-associated amyotrophic lateral sclerosis

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There is no effective treatment for amyotrophic lateral sclerosis (ALS), a devastating motor neuron disease. However, discovery of a G_4C_2 repeat expansion in the *C9ORF72* gene as the most common genetic cause of ALS has opened up new avenues for therapeutic intervention for this form of ALS. G_4C_2 repeat expansion RNAs and proteins of repeating dipeptides synthesized from these transcripts are believed to play a key role in *C9ORF72*-associated ALS (c9ALS). Therapeutics that target G_4C_2 RNA, such as antisense oligonucleotides (ASOs) and small molecules, are thus being actively investigated. A limitation in moving such treatments from bench to bedside is a lack of pharmacodynamic markers for use in clinical trials. We explored whether poly(GP) proteins translated from G_4C_2 RNA could serve such a purpose. Poly(GP) proteins were detected in cerebrospinal fluid (CSF) and in peripheral blood mononuclear cells from c9ALS patients and, notably, from asymptomatic *C9ORF72* mutation carriers. Moreover, CSF poly(GP) proteins remained relatively constant over time, boding well for their use in gauging biochemical responses to potential treatments. Treating c9ALS patient cells or a mouse model of c9ALS with ASOs that target G_4C_2 RNA resulted in decreased intracellular and extracellular poly(GP) proteins. This decrease paralleled reductions in G_4C_2 RNA and downstream G_4C_2 RNA–mediated events. These findings indicate that tracking poly(GP) proteins in CSF could provide a means to assess target engagement of G_4C_2 RNA–based therapies in symptomatic *C9ORF72* repeat expansion carriers and presymptomatic individuals who are expected to benefit from early therapeutic intervention.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive motor neuron disease that typically results in muscle atrophy, paralysis, and eventually death within 5 years of onset. Up to 50% of ALS patients develop cognitive and behavioral impairments, and ~15% fulfill the criteria for frontotemporal dementia (FTD), which is characterized by changes in personality, behavior, and language (*1*).

Only one minimally effective drug, riluzole, is approved for ALS despite more than 30 clinical trials conducted since 1995. The dearth of ALS therapeutics stems partly from an incomplete understanding of the causative pathomechanisms. However, discovery of a G_4C_2 repeat expansion in the *C9ORF72* gene as the most common genetic cause of ALS and FTD (*2*, *3*) has resulted in impressive efforts toward elucidating how this mutation causes c9ALS (*C9ORF72*-associated ALS) or c9FTD, collectively referred to as c9ALS/FTD. Although the normal number of *C9ORF72* G_4C_2 repeats is lower than 30, c9ALS/FTD patients have several hundred to several thousand (*4*). Putative pathomechanisms associated with G_4C_2 repeat expansions include loss of C9ORF72 function as well as toxicity stemming from the accumulation of sense and antisense transcripts of the expanded repeats. These RNA transcripts assemble into structures called foci, aberrantly interact with RNA binding proteins, and cause defects in nucleocytoplasmic transport (*5*, *6*). They additionally serve as templates for the synthesis of proteins of repeating dipeptides through repeat associated non-ATG (RAN) translation (*7*–*11*). Poly(GP), poly(GA), and poly(GR) proteins are produced from sense G_4C_2-containing transcripts, whereas poly(GP), poly(PA), and poly(PR) proteins are produced from antisense G_2C_4-containing transcripts. Neuronal inclusions of these so-called c9RAN

proteins are pathognomonic to c9ALS/FTD, and studies show that certain c9RAN proteins, such as poly(GA), poly(GR), and poly(PR), are toxic in in vitro and in vivo overexpression models (*5*, *6*). Potential mechanisms of toxicity associated with c9RAN proteins include nucleolar stress, impaired proteasomal function, and, as with G_4C_2 repeat RNA, impaired nucleocytoplasmic transport (*5*, *6*).

On the basis of the expanding body of evidence supporting the role of G_4C_2 repeat RNA and c9RAN proteins in c9ALS/FTD pathogenesis, therapeutic approaches that target G_4C_2 RNA are being actively pursued. For example, antisense oligonucleotides (ASOs) complementary to G_4C_2 RNA or *C9ORF72* transcripts (c9ASOs) decrease G_4C_2-containing RNA and consequently decrease the number of cells with RNA foci, as well as mitigate abnormalities in gene expression and nucleocytoplasmic transport in neurons differentiated from c9ALS patient–derived induced pluripotent stem cells (iPSCs) (*12*–*14*). In primary neurons and brain tissues from c9BAC mice expressing expanded G_4C_2 repeats, c9ASOs decrease G_4C_2 repeat–containing RNA, foci formation, and production of poly(GP) proteins (*15*, *16*). Moreover, small-molecule binders of G_4C_2 RNA inhibit foci formation and RAN translation in patient-derived cell models (*17*).

Because possible therapeutics for c9ALS/FTD are being developed for clinical trials, it is paramount to address barriers in moving a treatment from bench to bedside. Chief among these is the lack of markers capable of predicting disease progression, monitoring the response to therapy, and confirming target engagement. Given that c9RAN proteins are synthesized from G_4C_2 repeat RNA, the target of therapeutic interventions under investigation, we anticipate that c9RAN proteins in cerebrospinal fluid (CSF) will reflect target engagement and biochemical responses to treatment. Although three c9RAN proteins are produced from G_4C_2 RNA, namely, poly(GP), poly(GA), and poly(GR), we believe that poly(GP) may be an especially suitable marker candidate. Both poly(GP) and poly(GA) are more highly expressed in the central nervous system (CNS) of c9ALS/FTD patients than poly(GR) (*18*). However, poly(GP) is more likely to be accurately measured in biospecimens because it is more soluble than poly(GA) (*19*). Indeed, in a small cohort of c9ALS patients, we established that poly(GP) can be detected in CSF (*17*). To prepare for upcoming clinical trials for c9ALS, the present study used patient CSF and several preclinical models to investigate the hypothesis that poly(GP) proteins could serve as an urgently needed pharmacodynamic marker for developing and testing therapies for treating c9ALS.

RESULTS

Poly(GP) is detected in the CSF of asymptomatic and symptomatic *C9ORF72* repeat expansion carriers

To test our hypothesis, we used an international sampling of subjects (table S1) to (i) replicate our finding that poly(GP) is present in CSF from *C9ORF72* mutation carriers (*17*), (ii) compare poly(GP) proteins in CSF between asymptomatic and symptomatic carriers, and (iii) examine the longitudinal profile of CSF poly(GP). Given these three primary analyses, $P \leq 0.017$ was considered significant after Bonferroni adjustment.

Our CSF series comprised samples from 83 c9ALS patients [71 with c9ALS alone and 12 with comorbid FTD (c9ALS-FTD)] and 27 asymptomatic *C9ORF72* repeat expansion carriers. CSF collected longitudinally was available for 33 of these subjects. Also included were samples from 24 *C9ORF72* repeat expansion carriers clinically diagnosed with diseases other than c9ALS or c9ALS-FTD [c9FTD ($n = 20$), Alzheimer's disease ($n = 2$), bipolar disease ($n = 1$), and dementia with Lewy bodies ($n = 1$)] and from 120 individuals without the *C9ORF72* mutation. The latter encompassed patients with ALS ($n = 57$) or other neurological diseases [FTD ($n = 4$), Alzheimer's disease ($n = 10$), and primary lateral sclerosis ($n = 1$)], as well as healthy controls ($n = 48$). Subject characteristics are provided in Table 1.

Poly(GP) in CSF was measured in a blinded manner using our previously described Meso Scale Discovery–based immunoassay (*17*, *19*). We have validated that measures of poly(GP) in CSF determined using this assay significantly correlate with measures of poly(GP) assessed using a different antibody pair (Spearman's $r = 0.99$, $P < 0.0001$, $n = 14$; fig. S1A) or using a different immunoassay platform, the Simoa HD-1 Analyzer (Spearman's $r = 0.98$, $P < 0.0001$, $n = 14$; fig. S1B).

As anticipated on the basis of our previous study of 14 c9ALS patients (*17*), poly(GP) was detected in CSF from *C9ORF72* mutation carriers in the large sample series used in the present study (Fig. 1A, Table 1, and tables S2 to S4). Poly(GP) proteins were significantly higher in individuals with the expansion ($n = 134$) than in those without ($n = 120$) in unadjusted analysis ($P < 0.0001$) and analyses adjusted for age at CSF collection, gender, and disease group ($P < 0.0001$). Notably, poly(GP) was detected in CSF from both asymptomatic and symptomatic *C9ORF72* mutation carriers (Fig. 1B, Table 1, and table S2). In comparing asymptomatic individuals ($n = 27$) and patients with c9ALS or c9ALS-FTD ($n = 83$), there was nominal

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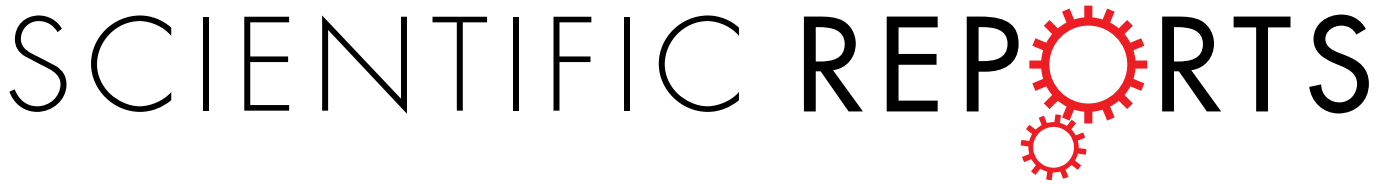

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Inefficient skeletal muscle oxidative function flanks impaired motor neuron recruitment in Amyotrophic Lateral Sclerosis during exercise

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This study aimed to evaluate muscle oxidative function during exercise in amyotrophic lateral sclerosis patients (pALS) with non-invasive methods in order to assess if determinants of reduced exercise tolerance might match ALS clinical heterogeneity. 17 pALS, who were followed for 4 months, were compared with 13 healthy controls (CTRL). Exercise tolerance was assessed by an incremental exercise test on cycle ergometer measuring peak O_2 uptake ($\dot{V}O_{2peak}$), vastus lateralis oxidative function by near infrared spectroscopy (NIRS) and breathing pattern ($\dot{V}E_{peak}$). pALS displayed: (1) 44% lower $\dot{V}O_{2peak}$ *vs.* CTRL ($p < 0.0001$), paralleled by a 43% decreased peak skeletal muscle oxidative function ($p < 0.01$), with a linear regression between these two variables ($r^2 = 0.64$, $p < 0.0001$); (2) 46% reduced $\dot{V}E_{peak}$ *vs.* CTRL ($p < 0.0001$), achieved by using an inefficient breathing pattern (increasing respiratory frequency) from the onset until the end of exercise. Inefficient skeletal muscle O_2 function, when flanking the impaired motor units recruitment, is a major determinant of pALS clinical heterogeneity and working capacity exercise tolerance. CPET and NIRS are useful tools for detecting early stages of oxidative deficiency in skeletal muscles, disclosing individual impairments in the O_2 transport and utilization chain.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder involving alpha motor neurons and abnormal recruitment of motor units, due to lesions in the corticospinal pathways. The resulting clinical manifestations of asthenia, spasticity, and amyotrophy harshly impair functional independence of patients and their quality of life. ALS patients (pALS) have a heterogeneous onset, with increasing fatigability that may begin with impaired activation of limbs, or with dysphagia or dysarthria when the bulbar district is affected first, and with a final failure of respiratory muscles. The appearance of ALS, from the earliest phases of the disease, also typically consists of reduced exercise tolerance until there is complete restriction of activities of daily living[1–3]. The characteristic heterogeneity in exercise tolerance of pALS is related to both the pathologic pattern of motor unit recruitment, and muscle impairment due to disuse of potentially healthy muscles[3,4].

The reduced exercise tolerance in pALS (*i.e.*, the capacity to maintain workloads ranging from habitual activities to rehabilitation exercises) has been associated with mitochondrial dysfunction, both as a direct pathogenic mechanism and as a factor contributing to the exercise limitation[5,6]. Furthermore, the degree of exercise intolerance in pALS might correlate with the reduction in the number and effectiveness of functional mitochondria able to guarantee an adequate O_2 extraction at the skeletal muscles[5]. During the early stages (less than 9 months from disease onset), pALS show no evidence of mitochondrial dysfunction. However, this is clearly present with increasing severity and when the disease is finally identified by clinical disability scales[7]. Nevertheless, a

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Pyrimethamine Significantly Lowers Cerebrospinal Fluid Cu/Zn Superoxide Dismutase in Amyotrophic Lateral Sclerosis Patients With *SOD1* Mutations

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Objective: Cu/Zn superoxide dismutase (SOD1) reduction prolongs survival in SOD1-transgenic animal models. Pyrimethamine produces dose-dependent SOD1 reduction in cell culture systems. A previous phase 1 trial showed pyrimethamine lowers SOD1 levels in leukocytes in patients with *SOD1* mutations. This study investigated whether pyrimethamine lowered SOD1 levels in the cerebrospinal fluid (CSF) in patients carrying *SOD1* mutations linked to familial amyotrophic lateral sclerosis (fALS/SOD1).
Methods: A multicenter (5 sites), open-label, 9-month-duration, dose-ranging study was undertaken to determine the safety and efficacy of pyrimethamine to lower SOD1 levels in the CSF in fALS/SOD1. All participants underwent 3 lumbar punctures, blood draw, clinical assessment of strength, motor function, quality of life, and adverse effect assessments. SOD1 levels were measured in erythrocytes and CSF. Pyrimethamine was measured in plasma and CSF. Appel ALS score, ALS Functional Rating Scale–Revised, and McGill Quality of Life Single-Item Scale were measured at screening, visit 6, and visit 9.
Results: We enrolled 32 patients; 24 completed 6 visits (18 weeks), and 21 completed all study visits. A linear mixed effects model showed a significant reduction in CSF SOD1 at visit 6 ($p < 0.001$) with a mean reduction of 13.5% (95% confidence interval [CI] = 8.4–18.5) and at visit 9 ($p < 0.001$) with a mean reduction of 10.5% (95% CI = 5.2–15.8).
Interpretation: Pyrimethamine is safe and well tolerated in ALS. Pyrimethamine is capable of producing a significant reduction in total CSF SOD1 protein content in patients with ALS caused by different *SOD1* mutations. Further long-term studies are warranted to assess clinical efficacy.

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Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative disease of upper and lower motor neurons causing progressive weakness of limbs, swallowing, and breathing, resulting in death within 3 to 5 years.[1] The cause is uncertain in most patients but in approximately 10% of patients, the disease is familial.[2] Since 1993, mutations in > 36 genes have been associated with causing ALS.[3] Mutations in

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the cytoplasmic free radical scavenging enzyme Cu/Zn superoxide dismutase (SOD1) account for 3 to 23% of familial cases (familial ALS [fALS]) and 1 to 3% of sporadic cases.[2–4] Transgenic mice overexpressing mutant human SOD1 develop a progressive motor neuron degenerative disease mimicking human ALS, whereas knockout of the murine *SOD1* gene does not result in a similar phenotype.[5] These findings combined with the observation that there is no relationship between the level of SOD1 activity and patient prognosis suggest that there is a toxic gain of function for the SOD1 mutant molecule with a predilection for the motor system.[3] Reducing the content of mutant SOD1 attenuates disease progression proportionate to the suppression of mutant protein using interfering RNA.[6] Collective evidence supports the hypothesis that lowering the total SOD1 protein content may be beneficial and influence the disease course in ALS. Attempts to lower SOD1 expression are currently being pursued using antisense oligonucleotides[7] and by increasing consumption of SOD1 by activating heat shock proteins[8] via the drug arimoclomol. Using U.S. Food and Drug Administration (FDA)-approved drugs that also have the ability to lower SOD1 content is another approach.[9] Monitoring the CSF SOD1 protein level has been identified as a reliable biomarker for SOD1 reduction within the anterior horn cell in transgenic rats with *SOD1*-mediated ALS.[7] In humans with ALS and *SOD1* mutations, CSF SOD1 shows minimal variability and is a reliable biomarker for *SOD1*-mediated fALS.[10] We have previously reported that oral treatment with pyrimethamine in ALS patients with a mutation in *SOD1* resulted in a reduction of SOD1 levels in peripheral blood leukocytes and, in both patients studied, a reduction in SOD1 protein content and activity in the cerebrospinal fluid (CSF).[9] We now report a phase 1/2 study whose primary aim was to determine whether pyrimethamine lowers SOD1 in the CSF in ALS patients with a wide variety of *SOD1* mutations and over a longer period of time, with a secondary aim to establish safety and tolerability.

Patients and Methods

The institutional review board at Weill Cornell Medicine approved this study, followed by approval by the relevant institutional or national ethical review boards at the participating institutions in the USA, Italy, Germany, and Sweden, following FDA and European Medicines Agency regulations and adhering to the Principles of the Declaration of Helsinki (World Medical Association, 1964). The study was registered at www.clinicaltrials.gov as NCT01083667. This was a single-arm, open-label study with the primary endpoint of determining whether oral medication with pyrimethamine results in a reduction of CSF SOD1 levels in ALS patients with different types of *SOD1* mutations. Based on our earlier study,[9] the target dose was set at 75mg per day supplemented with 10mg of leucovorin. However, different dosing was achieved due to reductions required to maintain tolerability. We enrolled 32 patients (Table 1).

Inclusion criteria were: the presence of objective weakness in at least 1 neural segment and a pathogenic mutation in *SOD1*, (El Escorial Definite ALS revised[11]), age of 18 years or older, capable of providing written informed consent and complying with trial procedures, not taking riluzole or on a stable dose for 30 days or more, and not taking coenzyme Q10 or on a stable dose and brand for 30 days or more. Exclusion criteria were: history of malabsorption syndrome, exposure to any other agents considered a therapeutic target for ALS within 30 days of entry into this study, women who were pregnant or planning on becoming pregnant, women who were breastfeeding, alcoholism, taking phenytoin or other medications that may interfere with folate levels, seizures, megaloblastic anemia, folate deficiency, cardiac rhythm disorders, impaired renal or liver function, tracheostomy, mechanical ventilation, and use of any of the following medications: cytosine arabinoside, methotrexate, daunorubicin, sulfonamides, zidovudine, lorazepam, warfarin, sulfamethoxazole, trimethoprim, and lithium.

There were 10 visits: screen/baseline (week 0) and weeks 3, 6, 9, 12, 15, 18, 24, 30, and 36. Visits at weeks 0, 18, and 36 were critical visits for data acquisition. At all visits, weight, vital signs, and concomitant medication screen combined with adverse effect assessment occurred. Blood for SOD1 and pyrimethamine levels was obtained. At weeks 0, 18, and 36, a lumbar puncture (LP) was performed. At weeks 0, 6, 18, and 36, we measured the ALS Functional Rating Scale–Revised (ALSFRS-R), Appel ALS (AALS), and the McGill Quality of Life Single-Item Scale (MQOL-SIS) scores. The ALSFRS-R score is a questionnaire-based assessment of motor function that has been validated in natural history studies of ALS and therapeutic trials.[12] The AALS score is an objective measure of global motor function that has been validated in natural history studies and therapeutic trials.[13] The MQOL-SIS is a single question in which the patient rates their overall quality of life (QOL) on a scale from 1 to 10 (10 being the best possible and 0 being the worst possible) for the past 48 hours. MQOL-SIS has good correlation with ALS QOL.[14]

Summary of Dose Escalation and Algorithm for Reduction

Pyrimethamine was supplied in 25mg tablets (CorePharma, Middlesex, NJ). The target dose was 75mg, based on our experience in the first study of pyrimethamine,[9] where 100mg was poorly tolerated but 75mg was deemed to be a dose that most patients could tolerate over an extended period of time. The escalation of dose was as follows. At baseline, patients started taking a 25mg tablet daily together with 5mg leucovorin twice daily. The leucovorin remained at the same dose for the duration of the study. Pyrimethamine dose increased to 37.5mg at 3

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ORIGINAL RESEARCH
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Cognitive-constructivist Approach in Medical Settings: The Use of Personal Meaning Questionnaire for Neurological Patients' Personality Investigation

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Objective: The cognitive-constructivist psychotherapy approach considers the self as a continuous regulation process between present and past experience, in which attributions of meaning is characterized by the use of internal rules. In this conception, everyone would be driven by a specific inner coherence called Personal Meaning Organization (PMO). Such approach has never been applied to neurological patients by means of *ad hoc* developed tools. We performed an explorative study aimed to characterize personality styles in different neurological conditions within the theoretical framework of cognitive-constructivist model.

Materials and Methods: Three groups of neurological patients (Amyotrophic Lateral Sclerosis, Multiple Sclerosis, Primary Headache) and a sample of healthy participants, each composed by 15 participants, for a total of 60 participants, were recruited. The Personal Meaning Questionnaire (PMQ), an Italian questionnaire assessing PMOs construct, and other clinical tools for psychological and quality of life assessment were administered to all subjects.

Results: The main finding concerned the detection, across all clinical conditions, of a higher prevalence of phobic personality style, with Amyotrophic Lateral Sclerosis showing a relevant prevalence of such PMO with respect to all other neurological conditions and controls. However, with respect to controls, in all clinical conditions, PMQ highlighted a tendency, even if not statistically significant, to codify experience by means of specific cognitive and emotional patterns.

Conclusion: Our findings represent the first contribution towards understanding the personality profiles of patients affected by neurological conditions according to cognitive-constructivist theory.

Keywords: Amyotrophic Lateral Sclerosis, cognitive-constructivist psychotherapy, multiple sclerosis, personality, personal meaning questionnaire, primary headache

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ORIGINAL COMMUNICATION

An eye-tracker controlled cognitive battery: overcoming verbal-motor limitations in ALS

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Abstract We assessed language, attention, executive, and social cognition abilities in a sample of patients with Amyotrophic Lateral Sclerosis (ALS) by means of a recently developed cognitive battery based on oculomotor control with eye-tracking (ET) technology. Twenty-one ALS patients and 21 age- and education-matched healthy subjects underwent the ET-based cognitive assessment, together with the standard cognitive screening tools [Frontal Assessment Battery (FAB); Montreal Cognitive Assessment (MoCA); and Digit Sequencing Task]. Psychological measures of anxiety (State-Trait Anxiety Inventory-Y) and depression (Beck Depression Inventory) were also collected, and an ET usability questionnaire was administered. For patients, clinical and respiratory examinations were also performed, together with behavioural assessment (Frontal Behavioural Inventory). The developed battery discriminated among patients and controls with regard to measures of verbal fluency, frontal abilities, and social cognition. Measures of diagnostic utility confirmed a higher diagnostic accuracy of such ET-based tests with respect to FAB; similar diagnostic accuracy emerged when comparing them to the other standard cognitive tools (MoCA, WM). Usability ratings about the ET tests were comparable among the two groups. The ET-based neuropsychological battery demonstrated good levels of diagnostic accuracy and usability in a clinical population of non-demented ALS patients, compared to matched healthy controls. Future studies will be aimed at further investigate validity and usability components by recruiting larger sample of patients, both in moderate-to-severe stages of the disease and affected by more severe cognitive impairment.

Keywords Amyotrophic lateral sclerosis · Eye tracker · Cognitive assessment · Behavioural assessment · Oculomotor control · Verbal-motor limitations

Barbara Poletti and Laura Carelli contributed equally to this work.

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Introduction

Cognitive and behavioural changes in patients with amyotrophic lateral sclerosis (ALS) have been increasingly recognized as an integral feature of the disease, with the most commonly reported alterations regarding executive functions [1, 2]. Recent studies have depicted cognitive impairment in ALS as a heterogeneous feature, with changes involving a range of cognitive functions beyond

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ORIGINAL ARTICLE

An eye-tracking controlled neuropsychological battery for cognitive assessment in neurological diseases

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Abstract Traditional cognitive assessment in neurological conditions involving physical disability is often prevented by the presence of verbal–motor impairment; to date, an extensive motor–verbal-free neuropsychological battery is not available for such purposes. We adapted a set of neuropsychological tests, assessing language, attentional abilities, executive functions and social cognition, for eye-tracking (ET) control, and explored its feasibility in a sample of healthy participants. Thirty healthy subjects performed a neuropsychological assessment, using an ET-based neuropsychological battery, together with standard "paper and pencil" cognitive measures for frontal (Frontal Assessment Battery—FAB) and working memory abilities (Digit Sequencing Task) and for global cognitive efficiency (Montreal Cognitive Assessment—MoCA). Psychological measures of anxiety (State-Trait Anxiety Inventory-Y—STAI-Y) and depression (Beck Depression Inventory—BDI) were also collected, and a usability questionnaire was administered. Significant correlations were observed between the "paper and pencil" screening of working memory abilities and the ET-based neuropsychological measures. The ET-based battery also correlated with the MoCA, while poor correlations were observed with the FAB. Usability aspects were found to be influenced by both working memory abilities and psychological components. The ET-based neuropsychological battery developed could provide an extensive assessment of cognitive functions, allowing participants to perform tasks independently from the integrity of motor or verbal channels. Further studies will be aimed at investigating validity and usability components in neurological populations with motor–verbal impairments.

Keywords Eye-tracking · Neuropsychological battery · Motor–verbal limitations · Neurological diseases

Introduction

Cognitive assessment in neurological diseases represents a relevant topic, due to clinical issues and ethical aspects, the latter concerning patients' decisional capacity with regard

B. Poletti and L. Carelli contributed equally to this work.

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BRAIN
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UPDATE

The synaptic function of parkin

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Loss of function mutations in the gene *PARK2*, which encodes the protein parkin, cause autosomal recessive juvenile parkinsonism, a neurodegenerative disease characterized by degeneration of the dopaminergic neurons localized in the substantia nigra pars compacta. No therapy is effective in slowing disease progression mostly because the pathogenesis of the disease is yet to be understood. From accruing evidence suggesting that the protein parkin directly regulates synapses it can be hypothesized that *PARK2* gene mutations lead to early synaptic damage that results in dopaminergic neuron loss over time. We review evidence that supports the role of parkin in modulating excitatory and dopaminergic synapse functions. We also discuss how these findings underpin the concept that autosomal recessive juvenile parkinsonism can be primarily a synaptopathy. Investigation into the molecular interactions between parkin and synaptic proteins may yield novel targets for pharmacologic interventions.

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Keywords: parkin; Parkinson's disease; synapse; dopamine; glutamate

Abbreviations: AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; KAR = kainic acid receptor; NMDAR = *N*-methyl-D-aspartate receptor; PDZ = postsynaptic density-95, disc large, zona occludens; PSD = postsynaptic density; SNc = substantia nigra pars compacta

Introduction

Parkin (encoded by *PARK2*) is a ubiquitin-ligase enzyme expressed in the CNS and in peripheral tissues (Sunada *et al.*, 1998; Picchio *et al.*, 2004; Serdaroglu *et al.*, 2005; Fujiwara *et al.*, 2008; Kasap *et al.*, 2009; Auburger *et al.*, 2012). At the intracellular level, it catalyses the transfer of ubiquitin from ubiquitin-carrier enzymes to protein substrates and regulates their trafficking and turnover (Cookson, 2003; Houlden and Singleton, 2012; Zhang *et al.*, 2015). Numerous substrates for parkin have been identified, indicating that it is a multifunctional protein involved in many intracellular processes, including the control of mitochondrial integrity and the regulation of apoptosis and transcription (Scarffe *et al.*, 2014; Charan and LaVoie, 2015). By ubiquitinating these proteins in various tissues, wild-type parkin may modulate cardiac health (Piquereau *et al.*, 2013), the risk of cancer (Veeriah *et al.*, 2009; Hu *et al.*, 2016) and disorders such as Alzheimer's disease (Burns *et al.*, 2009), autism (Glessner *et al.*, 2009),

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AMYOTROPHIC LATERAL SCLEROSIS

Mutations in the vesicular trafficking protein annexin A11 are associated with amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. We screened 751 familial ALS patient whole-exome sequences and identified six mutations including p.D40G in the *ANXA11* gene in 13 individuals. The p.D40G mutation was absent from 70,000 control whole-exome sequences. This mutation segregated with disease in two kindreds and was present in another two unrelated cases ($P = 0.0102$), and all mutation carriers shared a common founder haplotype. Annexin A11–positive protein aggregates were abundant in spinal cord motor neurons and hippocampal neuronal axons in an ALS patient carrying the p.D40G mutation. Transfected human embryonic kidney cells expressing *ANXA11* with the p.D40G mutation and other N-terminal mutations showed altered binding to calcyclin, and the p.R235Q mutant protein formed insoluble aggregates. We conclude that mutations in *ANXA11* are associated with ALS and implicate defective intracellular protein trafficking in disease pathogenesis.

INTRODUCTION

Gene hunting in rare Mendelian disorders has been transformed by exome sequencing. This approach is particularly attractive for late-onset autosomal dominant syndromes with short disease durations, such as amyotrophic lateral sclerosis (ALS), where DNA is rarely available from multiple affected individuals in the same kindred to support traditional linkage analyses. ALS has a lifetime risk of 1 in 400 and is characterized by degeneration of brain and spinal cord motor neurons resulting in progressive paralysis and death within ~3 years (*1*). Ten percent of ALS cases are familial (FALS), and a causative gene mutation can be identified in ~60% of European kindreds (*2*). Mutations in the same genes account for ~10% of sporadic ALS cases (SALS), reflecting incomplete penetrance. Nonsynonymous mutations in the *SOD1*, *TARDBP*, and *FUS* genes and an intronic hexanucleotide repeat expansion in *C9orf72* together account for ~20% of all ALS cases, and other rarer genes account for another ~1 to 3% of cases (*3*, *4*). Whole-genome or whole-exome sequencing (WGS/WES) has enabled identification of nine ALS genes through either shared variant segregation analysis in ALS kindreds (*VCP*, *PFN1*, *MATR3*, *CHCHD10*, and *CCNF*) or rare variant burden analysis (*TUBA4A*, *TBK1*, *NEK1*, and *C21orf2*) (*5*–*15*). Here, we analyzed whole-exome sequences from patients with FALS and identified a nonsynonymous founder mutation in the *ANXA11* gene that is present in all affected family members tested and is also found in multiple unrelated index cases. Annexin A11 is a widely expressed calcium-dependent phospholipid-binding protein (505 amino acids, 56 kDa) that belongs to the larger human annexin protein family of 12 members (*16*). Each family member has four highly conserved annexin domains, which can form complexes with calcium ions facilitating binding to anionic cell membranes. Unique to the annexin family, annexin A11 has the longest N terminus (~196 amino acids), which is hydrophobic and disordered and binds to several interacting partners, the best characterized being calcyclin (encoded by *S100A6*) (*17*). Annexin A11 is associated with autoimmune disorders such as systemic lupus erythematosus, and case-control studies have found a genetic association between the p.R230C single-nucleotide polymorphism (SNP) with the multisystem autoimmune disease sarcoidosis (*18*, *19*). Additionally, increased annexin A11 expression is also found in breast cancer and other acquired malignancies (*18*). Here, we present a new role for annexin A11 in a rare neurodegenerative Mendelian disorder, ALS.

RESULTS

Exome sequencing detects missense *ANXA11* mutations in ALS cases

From our cohort of 751 European FALS patients (negative for *C9orf72* GGGGCC expansions), we obtained exome sequencing data for two or more affected relatives from only 50 families (average, 2.14 individuals per family), highlighting the difficulty in obtaining DNA samples from extended kindreds for this late-onset disorder. Families ranged from a simple pair of siblings (sharing an estimated 50% of their variants) to an index case, parent, and a second cousin (sharing an estimated 1.5% of their variants). On average, 84% (range, 58 to 97%) of the protein-coding bases contained in reference sequence (RefSeq) transcripts were sequenced in all family members to a depth of ≥10 reads, a cutoff threshold in line with that used by the Exome Aggregation Consortium (ExAC)

for defining high-quality variants (*20*). Our filtering strategy was to detect new high-quality, coding and splicing variants that are absent from the 1000 genomes, UK10K, Exome Variant Server (EVS), and ExAC databases (n > 72,000). This produced an average of ~10 candidates per family (range, 0 to 27) (table S1). As proof of principle, the analysis identified mutations shared in single kindreds from several known ALS genes, including *SOD1*, *TARDBP*, *FUS*, *DCTN1*, *and TUBA4A* (*9*, *21*–*24*). It was immediately apparent that only two variants appeared in the list of candidates for more than one family: The well-characterized pathogenic p.M337V mutation in *TARDBP* (*25*) was found in two North American families, and a new p.D40G variant in *ANXA11* (Refseq ID NM_145869) was found in two British families (an uncle-niece pair and two cousins). (The full list of candidate variants found in both U.K. *ANXA11* p.D40G families are listed in table S2.) One additional Italian proband from the extended FALS exome cohort also carried the same *ANXA11* p.D40G variant. We then extended the analysis to include 694 unrelated European FALS probands (including the 50 probands from our multiplex families) and sought new protein-changing variants that were shared by three or more probands. This approach also identified the *ANXA11* p.D40G variant and the following well-characterized pathogenic ALS mutations: 10× *SOD1* p.I114T (*26*), 6× *TARDBP* p.A382T (*22*), 5× *SOD1* p.A5V (*27*), 4× *FUS* p.R521C (*23*), 3× *TARDBP* p.M337V, 3× *SOD1* p.G94D (*28*), and 3× *FUS* p.P525L (*29*).

We then used Sanger sequencing to sequence the coding exons of *ANXA11* in a separate set of 180 British apparent SALS cases and identified one further heterozygous p.D40G carrier, bringing the total number to 4 out of a combined cohort of 874 probands. Because the two U.K. families carrying the p.D40G mutation were not sufficiently powered to conduct a linkage analysis [simulated lod (logarithm of the odds ratio for linkage) score of 0.63 using MERLIN] (*30*), we sought an alternate method to ascertain the significance of the p.D40G mutation. It is possible that a variant could be present four times in a sample of 874 cases and absent from 72,000 other individuals yet still be unrelated to the disease. We therefore tested the null hypothesis that any equal-sized cohort of Europeans could also contain a new variant shared by at least four people. We achieved this by running simulation studies using the aggregated variant call counts from the non-Finnish European (NFE) subset of the ExAC database (n = 33,370). Briefly, all ExAC NFE variants were randomly distributed across 33,370 individuals, and random cohorts of 874 people were extracted. The cohort was deemed to have "passed" if it contained at least one protein-changing variant found four or more times within the cohort, but absent from the remainder of ExAC, UK10K, EVS, and 1000 genomes databases. After 250,000 iterations, only 2550 simulated cohorts contained such a variant, which demonstrated that the presence of the p.D40G variant was statistically significant (P = 0.0102). Although not population-matched to our cohort, we consider ExAC to be suitable for this purpose because it contains a high proportion of Swedes who are on average more genetically homogeneous than the U.K. population (table S3) (*31*). Therefore, ExAC would be expected to produce more shared nonpathogenic variants than our cohort, and so, we expect this estimate of significance to be a conservative one. In conjunction with the family-based study, the simulation analysis provides additional evidence that the *ANXA11* p.D40G mutation is associated with ALS.

The p.D40G mutation has a common European founder

Sanger sequencing of DNA from 17 family members across the two multigenerational British kindreds confirmed the presence of the p.D40G mutation in all four affected individuals identified from the exome capture data (Fig. 1, A and B). Four unaffected individuals also carried the mutation, but incomplete penetrance of ALS mutations is well recognized and three of these individuals were in their 40s, whereas the average age of disease onset in *ANXA11* p.D40G affected carriers is 72 years of age. DNA was available from five British p.D40G carriers who share a common haplotype on the disease allele defined by four exonic SNPs and two polymorphic microsatellites spanning the locus with phase determined by a cluster of carriers in U.K. Family 2, confirming a common founder (fig. S1; primers are listed in table S4). The minimal haplotype is defined by a physical stretch of 2.5 megabases of genomic DNA spanning the *ANXA11* locus, common to all p.D40G carriers. The core four-SNP haplotype, located in exons of genes flanking *ANXA11*, is present in ~5% of our extended FALS cohort (n = 787, including *C9orf72* expansion–positive cases) and ~5% of Europeans from the 1000 genomes database (n = 514). This suggests that the mutation arose on a European background. The maximal recombination region defining the limits of the p.D40G locus is 7.1 megabases and contains 23 genes (fig. S2). Interrogation of exome sequencing data found that no p.D40G carriers shared any additional protein-changing variants in the 23 genes within this region. Of the coding bases, 70.3 to 97.2% were covered to a read depth of ≥10 for each sample, and 97.9% were covered by ≥10 reads in at least one sample (table S5). This evidence indicates that p.D40G is the sole causal exonic variant within this locus.

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Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria

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RESEARCH ARTICLE

Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria

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Abstract

This article presents the revised consensus criteria for the diagnosis of frontotemporal dysfunction in amyotrophic lateral sclerosis (ALS) based on an international research workshop on frontotemporal dementia (FTD) and ALS held in London, Canada in June 2015. Since the publication of the Strong criteria, there have been considerable advances in the understanding of the neuropsychological profile of patients with ALS. Not only is the breadth and depth of neuropsychological findings broader than previously recognised – – including deficits in social cognition and language – but mixed deficits may also occur. Evidence now shows that the neuropsychological deficits in ALS are extremely heterogeneous, affecting over 50% of persons with ALS. When present, these deficits significantly and adversely impact patient survival. It is the recognition of this clinical heterogeneity in association with neuroimaging, genetic and neuropathological advances that has led to the current re-conceptualisation that neuropsychological deficits in ALS fall along a spectrum. These revised consensus criteria expand upon those of 2009 and embrace the concept of the frontotemporal spectrum disorder of ALS (ALS-FTSD).

KEYWORDS: *Amyotrophic lateral sclerosis*, *frontotemporal dementia*, *neuropsychology*, *cognition*, *behaviour*, *genetics*

Introduction

While the core feature of amyotrophic lateral sclerosis (ALS) is a relentless loss of motor function leading to paralysis and ultimately death, the awareness that it can be associated with one or more features of frontotemporal dysfunction has gained increasing acceptance (1). This in part can be traced to the development of international

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Genetic analysis of the *SOD1* and *C9ORF72* genes in Hungarian patients with amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the death of motor neurons. To date, more than 20 genes have been implicated in ALS, and of these, the 2 most frequently mutated are the *superoxide dismutase 1 (SOD1)* gene and the *chromosome 9 open reading frame 72 (C9ORF72)* gene. In this study, we aimed to investigate the contribution of these 2 Mendelian genes to the development of the disease in Hungarian ALS patients (n = 66). Direct sequencing of the *SOD1* gene revealed a novel (p.Lys91ArgfsTer8) and 3 recurrent heterozygous mutations (p.Val14Met, p.Asp90Ala, and p.Leu144Phe) in 5 patients. The novel p.Lys91ArgfsTer8 mutation led to a frameshift causing the addition of 8 new amino acids, including a premature stop codon at position 99. The GGGGCC hexanucleotide repeat expansion of the *C9ORF72* gene was present in 1 ALS patient. This study represents the first genetic analysis of 2 major ALS causative genes in a cohort of Hungarian ALS patients and contributes to the further understanding of the genetic and phenotypic diversity of ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS; ORPHA803), also known as "Lou Gehrig's disease", is a fatal, neurodegenerative disorder characterized by the death of motor neurons in the brain, brainstem, and spinal cord, resulting in fatal paralysis (Morrison and Harding, 1994). Familial forms account for about 10% of ALS cases, whereas other cases are sporadic (Hewitt et al., 2010; Strong et al., 1991). Familial forms are mainly transmitted in a Mendelian pattern of autosomal dominant inheritance (Hardiman et al., 2011). Regarding its genetic background, more than 20 genes have been implicated in the development of ALS (Amyotrophic Lateral Sclerosis Online Genetics Database, [ALSoD], http://alsod.iop.kcl.ac.uk).

Among the ALS causative genes, *superoxide dismutase 1 (SOD1)* is one of the most commonly mutated genes and accounts for approximately 12%–23% of the familial and up to 7% of the sporadic ALS forms (Andersen, 2006). *SOD1* gene encodes the Cu/Zn superoxide dismutase enzyme, which catalyzes the inactivation of superoxide into oxygen and hydrogen peroxide, providing antioxidant defense (Smirnoff, 1993). To date, more than 170 mutations have been reported for *SOD1* in the ALSoD Database (Abel et al., 2012) since the gene was firstly associated to ALS in 1993 (Rosen et al., 1993). *SOD1* mutations occur in all the 5 exons of the gene.

Another frequently mutated ALS gene is *chromosome 9 open reading frame 72 (C9ORF72)*, which—in addition to the *SOD1* mutations—is now recognized as the main cause of familial and sporadic ALS (Gijselinck et al., 2012; Majounie et al., 2012; Ratti et al., 2012; Smith et al., 2013). A hexanucleotide (GGGGCC) repeat expansion (RE) located in the noncoding region of the gene that can reach up to 4400 units (normal range: 2–23 units) has been identified in patients with ALS and/or frontotemporal dementia. The GGGGCC RE contributes to 23%–47% of familial ALS and

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Reconsidering the causality of TIA1 mutations in ALS

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LETTER TO THE EDITOR

Reconsidering the causality of TIA1 mutations in ALS

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The role of de novo mutations in the development of amyotrophic lateral sclerosis

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