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

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

COLLABORAZIONI INTERNAZIONALI

E

FRONTESPIZI

LAVORI SCIENTIFICI

2018

" CENTRO DINO FERRARI"

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

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frontiers in Neurology

CASE REPORT
published: 30 November 2018
doi: 10.3389/fneur.2018.01031

Central Nervous System Involvement in Common Variable Immunodeficiency: A Case of Acute Unilateral Optic Neuritis in a 26-Year-Old Italian Patient

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Common Variable Immunodeficiency (CVID) is a group of heterogeneous primary immunodeficiencies sharing defective B lymphocytes maturation and dysregulated immune response and resulting in impaired immunoglobulin production. Clinical picture encompasses increased susceptibility to infections, hematologic malignancies, inflammatory, and autoimmune diseases. Neurological manifestations are uncommon and optic neuritis has been previously reported only in one case with bilateral involvement. We hereby report a case of a 26-year-old man affected by CVID undergoing regular immunoglobulin supplementation, who presented with acute unilateral demyelinating optic neuritis and lymphocytic pleocytosis in the cerebrospinal fluid. A variety of infectious, inflammatory, and neoplastic conditions were excluded and a diagnosis of clinically isolated optic neuritis was made. The patient was treated with a short course of intravenous steroids with complete recovery. Overall, this case expands our current knowledge about clinical spectrum of complications in CVID and highlights the need for further research about this complex disease.

Keywords: common variable immunodeficiency, optic neuritis, optic neuropathy, clinically isolated syndrome, autoimmunity, primary immunodeficiencies

OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Multiple Sclerosis and Neuroimmunology, a section of the journal Frontiers in Neurology

Received: *11 September 2018*
Accepted: *15 November 2018*
Published: *30 November 2018*

Citation:
Abati E, Faravelli I, Magri F, Govoni A, Velardo D, Gagliardi D, Mauri E, Brusa R, Bresolin N, Fabio G, Comi GP, Carrabba M and Corti S (2018) Central Nervous System Involvement in Common Variable Immunodeficiency: A Case of Acute Unilateral Optic Neuritis in a 26-Year-Old Italian Patient. Front. Neurol. 9:1031. doi: 10.3389/fneur.2018.01031

BACKGROUND

Common Variable Immunodeficiency (CVID) is a group of primary immunodeficiencies characterized by an impairment in antibody production related to B cell intrinsic or extrinsic defects (1). CVID is defined by increased susceptibility to infection, autoimmune manifestations, granulomatous disease, and unexplained polyclonal lymphoproliferation associated to markedly reduced levels (2 SD below the mean) of serum immunoglobulin (Ig) G, as well as IgA and/or IgM, failed antibody response to natural infections or vaccine immunization and reduction of switched memory B cells, with the exclusion of secondary causes of hypogammaglobulinemia and no evidence of profound T-cell deficiency or T-cell proliferation (1–3). Clinical manifestations

Received: 21 September 2017 | Accepted: 10 January 2018

DOI: 10.1111/jcmm.13563

REVIEW

WILEY

In vitro models of multiple system atrophy from primary cells to induced pluripotent stem cells

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Funding information
Joint Programme Neurodegenerative Disease (JPND), Grant/Award Number: DAMNDPATHS

Abstract

Multiple system atrophy (MSA) is a rare neurodegenerative disease with a fatal outcome. Nowadays, only symptomatic treatment is available for MSA patients. The hallmarks of the disease are glial cytoplasmic inclusions (GCIs), proteinaceous aggregates mainly composed of alpha-synuclein, which accumulate in oligodendrocytes. However, despite the extensive research efforts, little is known about the pathogenesis of MSA. Early myelin dysfunction and alpha-synuclein deposition are thought to play a major role, but the origin of the aggregates and the causes of misfolding are obscure. One of the reasons for this is the lack of a reliable model of the disease. Recently, the development of induced pluripotent stem cell (iPSC) technology opened up the possibility of elucidating disease mechanisms in neurodegenerative diseases including MSA. Patient specific iPSC can be differentiated in glia and neurons, the cells involved in MSA, providing a useful human disease model. Here, we firstly review the progress made in MSA modelling with primary cell cultures. Subsequently, we focus on the first iPSC-based model of MSA, which showed that alpha-synuclein is expressed in oligodendrocyte progenitors, whereas its production decreases in mature oligodendrocytes. We then highlight the opportunities offered by iPSC in studying disease mechanisms and providing innovative models for testing therapeutic strategies, and we discuss the challenges connected with this technique.

KEYWORDS
in vitro models, induced pluripotent stem cells, multiple system atrophy, neurodegeneration, oligodendrocytes

1 | INTRODUCTION

Multiple-system atrophy, also known as MSA, is an adult onset severe neurodegenerative disease characterized by glial cytoplasmic inclusions (GCIs) and progressive cellular death in selected areas of central nervous system (CNS), more specifically the striatonigral, olivopontocerebellar and central autonomic pathways. The clinical presentation mirrors these alterations and comprises parkinsonism, cerebellar ataxia, pyramidal features and autonomic symptoms in various degrees.[1] Two main clinical subtypes can be identified and characterized by either a prevalence of parkinsonian symptoms (MSA-P) or a prevalence of cerebellar ataxia (MSA-C).[2]

The estimated incidence ranges from 0.1 to 2.4 cases per 100 000 person-years, the mean value being 0.6-0.7/100 000.[1,3] Prevalence has been reported to span between 1.9 and 4.9 per 100 000, according to different population studies.[4,5] MSA-P accounts for approximately two-thirds of the cases in European countries, with regional differences,[2,6,7] whereas MSA-C is far more common in Japan.[8]

Journal of the Neurological Sciences 388 (2018) 50–60

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Review Article

Pregnancy outcomes in women with spinal muscular atrophy: A review

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ARTICLE INFO

Keywords:
Spinal muscular atrophy
Pregnancy
Delivery
Anesthesia
Neurodegeneration

ABSTRACT

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by muscle weakness and atrophy resulting from progressive degeneration and loss of the anterior horn cells in the spinal cord and brain stem nuclei. The onset of weakness ranges from prenatal age to young adulthood. Thus, many female patients reach fertile age and may consider getting pregnant. However, only little information is available about outcomes and complications of pregnancy in women with SMA. In this review, we compared different studies on the subject, then we analyzed outcomes in the different stages of the pregnancy (preconceptional period, embryonal period, fetal period, delivery and post partum), with a special focus on maternal and fetal complications, prematurity, mode of delivery, anesthesiological risk, respiratory function and influence of pregnancy on the disease course. This is the first review focused exclusively on pregnancy in women affected by SMA. Our aim is to help clinicians who wish to understand the risks connected with pregnancy in SMA patients and to manage pregnancy course and delivery in an evidence-based and patient-oriented manner.

1. Introduction

Spinal muscular atrophy (SMA) is a neurodegenerative disease characterized by progressive loss of anterior horn neurons in the spinal cord and brain stem nuclei, resulting in proximal, symmetric muscle weakness and atrophy [1]. The incidence is approximately 1 in 11,000 and carrier frequency is 1 in 54 [2]. The disease is transmitted in an autosomal recessive fashion and results from homozygous mutations in the *SMN1* gene [1]. SMA has been traditionally classified into four subtypes based on clinical phenotype, and although it is now believed that its manifestations belong to a spectrum without clear delineation of subtypes, the classification remains useful for clinical purposes. Type I SMA begins in infancy and is usually fatal in < 2 years. Type 2 SMA presents between 6 and 18 months of age with developmental motor delay and inability to stand or walk independently. The lifespan varies from 2 years to the third decade, with respiratory problems accounting for most deaths. SMA Type III appears during childhood and progresses slowly, while SMA type IV appears in adulthood. In both cases, patients have normal life expectancy [1].

Thus, many women with SMA type II, III and IV reach fertile age and some of them may consider pregnancy. With the approval of the first therapy for all type of SMA, Nusinersen, an antisense oligonucleotide that modulates the expression of SMN2, and with other therapies under development [3], it is likely that the number of SMA patients that will consider pregnancy will drastically increase.

However, little data is available about the possible effects of pregnancy on SMA course and about the outcomes of pregnancies in SMA women. The scope of the present review is to give clinicians an overview of the different studies made in this field in order to facilitate counseling and clinical decisions.

2. Methods

We searched PubMed database (1950 to present) for papers matching the key words “spinal muscular atrophy” and “pregnancy” (all publication years) (Fig. 1). We limited our results only to papers written in English language. We screened the titles and abstracts of articles found during the search and retrieved any that were considered potentially relevant. We also checked the references of these articles to identify any additional possibly relevant studies. In addition, we searched the clinical trials registers of the National Institute of Health (www.clinicaltrials.gov) and of the World Health Organization (ICTRP Search portal).

We identified 23 relevant case reports and case series (20 case

Abbreviations: FOI, Fiber-optic intubation; FVC, Forced Vital Capacity; ICU, Intensive care unit; IUGR, Intrauterine growth restriction; MIP, Maximal inspiratory pressure; NIV, Non-invasive ventilation; NMD, Neuromuscular disorder; PCF, Peak cough flow; RLD, Restrictive lung disease; SMA, Spinal muscular atrophy; UTI, Urinary tract infection

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https://doi.org/10.1016/j.jns.2018.03.001
Received 24 August 2017; Received in revised form 3 February 2018; Accepted 1 March 2018
Available online 02 March 2018
0022-510X/

Molecular Neurobiology
https://doi.org/10.1007/s12035-018-1305-4

Preconditioning and Cellular Engineering to Increase the Survival of Transplanted Neural Stem Cells for Motor Neuron Disease Therapy

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Received: 13 May 2018 / Accepted: 7 August 2018

Abstract
Despite the extensive research effort that has been made in the field, motor neuron diseases, namely, amyotrophic lateral sclerosis and spinal muscular atrophies, still represent an overwhelming cause of morbidity and mortality worldwide. Exogenous neural stem cell-based transplantation approaches have been investigated as multifaceted strategies to both protect and repair upper and lower motor neurons from degeneration and inflammation. Transplanted neural stem cells (NSCs) exert their beneficial effects not only through the replacement of damaged cells but also via bystander immunomodulatory and neurotrophic actions. Notwithstanding these promising findings, the clinical translatability of such techniques is jeopardized by the limited engraftment success and survival of transplanted cells within the hostile disease microenvironment. To overcome this obstacle, different methods to enhance graft survival, stability, and therapeutic potential have been developed, including environmental stress preconditioning, biopolymers scaffolds, and genetic engineering. In this review, we discuss current engineering techniques aimed at the exploitation of the migratory, proliferative, and secretive capacity of NSCs and their relevance for the therapeutic arsenal against motor neuron disorders and other neurological disorders.

Keywords Stem cell transplantation · Stem cells · Neural stem cells · Cellular engineering · Preconditioning · Motor neuron diseases · Amyotrophic lateral sclerosis · Spinal muscular atrophy

Introduction

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophies (SMAs) are severe diseases characterized by selective motor neuron degeneration. ALS is an incurable, progressive neurodegenerative disease characterized by loss of upper and lower motor neurons leading to irreversible muscular paralysis and eventually respiratory failure and death within 3 to 5 years after onset [1]. To date, only two approved therapies, riluzole and edaravone, with a minimal impact on survival, are available for ALS, along with supportive care (e.g., neurorehabilitation) [2, 3]. SMAs are inherited degenerative disorders affecting motor neurons of the anterior horns of spinal cord gray matter. Proximal 5q SMA results from homozygous mutations in the survival motor neuron 1 (SMN1) gene. Nusinersen, an antisense oligonucleotide that modulates the expression of SMN2, was recently approved as the first therapy for SMA 5q. However, no effective treatment is available for other types of SMA [4].

Despite the variety of studies performed in this field, there are currently no valid therapeutic strategies capable of counteracting neuronal loss after its occurrence and regenerating the damaged central nervous system (CNS). Furthermore, the complex dynamics underlying the pathogenesis of motor neuron diseases (MNDs) and the relatively selective death of motor neurons remain elusive. Therefore, there exists an urgent need to cast a light over the cellular and molecular networks involved, to identify novel targets for drug development and to develop truly compelling therapeutic approaches. Clearly, to be applicable in MNDs, regenerative therapies should ultimately regulate or antagonize these complex pathways, thereby promoting the maintenance or restoration of motor neuron function [5].

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Published online: 17 August 2018

Received: 27 March 2018 | Revised: 19 June 2018 | Accepted: 20 June 2018

DOI: 10.1002/humu.23581

RESEARCH ARTICLE

WILEY HGVS HUMAN GENOME VARIATION SOCIETY

The analysis of myotonia congenita mutations discloses functional clusters of amino acids within the CBS2 domain and the C-terminal peptide of the ClC-1 channel

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Funding information
Fondazione Telethon, Grant/Award Number: GGP14096; Association Française contre les Myopathies, Grant/Award Number: 19027; Università degli Studi di Bari Aldo Moro, Grant/Award Number: Fondi Ateneo 2014

Communicated by Garry R. Cutting

This work was supported by Telethon-Italy (grant number GGP14096 to DC), Association Française contre les Myopathies (grant number 19027 to J-FD), and Fondi di Ricerca di Ateneo 2014 of University of Bari (to PI and J-FD).

Abstract

Myotonia congenita (MC) is a skeletal-muscle hyperexcitability disorder caused by loss-of-function mutations in the ClC-1 chloride channel. Mutations are scattered over the entire sequence of the channel protein, with more than 30 mutations located in the poorly characterized cytosolic C-terminal domain. In this study, we characterized, through patch clamp, seven ClC-1 mutations identified in patients affected by MC of various severities and located in the C-terminal region. The p.Val829Met, p.Thr832Ile, p.Val851Met, p.Gly859Val, and p.Leu861Pro mutations reside in the CBS2 domain, while p.Pro883Thr and p.Val947Glu are in the C-terminal peptide. We showed that the functional properties of mutant channels correlated with the clinical phenotypes of affected individuals. In addition, we defined clusters of ClC-1 mutations within CBS2 and C-terminal peptide subdomains that share the same functional defect: mutations between 829 and 835 residues and in residue 883 induced an alteration of voltage dependence, mutations between 851 and 859 residues, and in residue 947 induced a reduction of chloride currents, whereas mutations on 861 residue showed no obvious change in ClC-1 function. This study improves our understanding of the mechanisms underlying MC, sheds light on the role of the C-terminal region in ClC-1 function, and provides information to develop new antimyotonic drugs.

KEYWORDS

C-terminal, ClC-1, myotonia congenita, patch clamp

Journal of Neurology (2018) 265:2934–2943
https://doi.org/10.1007/s00415-018-9072-8

ORIGINAL COMMUNICATION

The role of clinical and neuroimaging features in the diagnosis of CADASIL

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Received: 1 August 2018 / Revised: 18 September 2018 / Accepted: 20 September 2018 / Published online: 11 October 2018

Abstract

Background Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common familial cerebral small vessel disease, caused by *NOTCH3* gene mutations. The aim of our study was to identify clinical and neuroradiological features which would be useful in identifying which patients presenting with lacunar stroke and TIA are likely to have CADASIL.

Methods Patients with lacunar stroke or TIA were included in the present study. For each patient, demographic and clinical data were collected. MRI images were centrally analysed for the presence of lacunar infarcts, microbleeds, temporal lobe involvement, global atrophy and white matter hyperintensities.

Results 128 patients (mean age 56.3 ± 12.4 years) were included. A *NOTCH3* mutation was found in 12.5% of them. A family history of stroke, the presence of dementia and external capsule lesions on MRI were the only features significantly associated with the diagnosis of CADASIL. Although thalamic, temporal pole gliosis and severe white matter hyperintensities were less specific for CADASIL diagnosis, the combination of a number of these factors together with familial history for stroke result in a higher positive predictive value and specificity.

Conclusions A careful familial history collection and neuroradiological assessment can identify patients in whom *NOTCH3* genetic testing has a higher yield.

Keywords CADASIL · NOTCH3 gene · Stroke genetics · Diagnosis · Neuroimaging · Monogenic disorders

Introduction

CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy; OMIM #125310) is the most common cause of familial cerebral small vessel disease [1]. It usually manifests at middle adulthood with a highly variable clinical phenotype including recurrent TIA or ischemic stroke, migraine with aura, cognitive deficits and mood disorders [2, 3]. T2-White matter hyperintensities (WMHs), with involvement of the external capsules and anterior pole of the temporal lobes and multiple

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00415-018-9072-8) contains supplementary material, which is available to authorized users.

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Funding The Lombardia GENS project has received funding from the Regione Lombardia Government as a Research Independent Project (DGR n°VIII/006128-12/12/2007). Lombardia GENS is an investigator-driven, academic, non-profit consortium and is publicly funded. Hugh Markus is supported by an NIHR Senior Investigator award and his work is supported by the Cambridge University Hospitals NIHR Biomedical Research Centre.

Compliance with ethical standards

Conflicts of interest The authors report no disclosures relevant to the manuscript.

Ethical standards The author hereby declares that the research documented in the submitted manuscript has been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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frontiers in Neurology

CASE REPORT
published: 15 October 2018
doi: 10.3389/fneur.2018.00859

Stormorken Syndrome Caused by a p.R304W *STIM1* Mutation: The First Italian Patient and a Review of the Literature

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OPEN ACCESS

Edited by:
Angelo Schenone, Università di Genova, Italy

Reviewed by:
Rosanna Cardani, Policlinico San Donato (IRCCS), Italy
Nicolas Demaurex, Université de Genève, Switzerland

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Specialty section:
This article was submitted to Neuromuscular Diseases, a section of the journal Frontiers in Neurology

Received: *29 April 2018*
Accepted: *24 September 2018*
Published: *15 October 2018*

Citation:
Borsani O, Piga D, Costa S, Govoni A, Magri F, Artoni A, Cinnante CM, Fagiolari G, Ciscato P, Moggio M, Bresolin N, Comi GP and Corti S (2018) Stormorken Syndrome Caused by a p.R304W STIM1 Mutation: The First Italian Patient and a Review of the Literature. Front. Neurol. 9:859. doi: 10.3389/fneur.2018.00859

Stormorken syndrome is a rare autosomal dominant disease that is characterized by a complex phenotype that includes tubular aggregate myopathy (TAM), bleeding diathesis, hyposplenism, mild hypocalcemia and additional features, such as miosis and a mild intellectual disability (dyslexia). Stormorken syndrome is caused by autosomal dominant mutations in the *STIM1* gene, which encodes an endoplasmic reticulum Ca^{2+} sensor. Here, we describe the clinical and molecular aspects of a 21-year-old Italian female with Stormorken syndrome. The *STIM1* gene sequence identified a c.910C > T transition in a STIM1 allele (p.R304W). The p.R304W mutation is a common mutation that is responsible for Stormorken syndrome and is hypothesized to cause a gain of function action associated with a rise in Ca^{2+} levels. A review of published *STIM1* mutations ($n = 50$) and reported Stormorken patients ($n = 11$) indicated a genotype-phenotype correlation with mutations in a coiled coil cytoplasmic domain associated with complete Stormorken syndrome, and other pathological variants outside this region were more often linked to an incomplete phenotype. Our study describes the first Italian patient with Stormorken syndrome, contributes to the genotype/phenotype correlation and highlights the possibility of directly investigating the p.R304W mutation in the presence of a typical phenotype.

Highlights

- Stormorken syndrome is a rare autosomal dominant disease.
- Stormoken syndrome is caused by autosomal dominant mutations in the *STIM1* gene.
- We present the features of a 21-year-old Italian female with Stormorken syndrome.
- Our review of published *STIM1* mutations suggests a genotype-phenotype correlation.
- The p.R304W mutation should be investigated in the presence of a typical phenotype.

Keywords: stormorken syndrome, STIM1, tubular aggregate myopathy, muscle, myopathy

Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 28 (2018) 532–537

www.elsevier.com/locate/nmd

A new case of limb girdle muscular dystrophy 2G in a Greek patient, founder effect and review of the literature

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Received 20 October 2017; received in revised form 16 January 2018; accepted 10 April 2018

Abstract

Limb girdle muscular dystrophy (LGMD) type 2G is a rare form of muscle disease, described only in a few patients worldwide, caused by mutations in *TCAP* gene, encoding the protein telethonin. It is characterised by proximal limb muscle weakness associated with distal involvement of lower limbs, starting in the first or second decade of life.

We describe the case of a 37-year-old woman of Greek origin, affected by disto-proximal lower limb weakness. No cardiac or respiratory involvement was detected. Muscle biopsy showed myopathic changes with type I fibre hypotrophy, cytoplasmic vacuoles, lipid overload, multiple central nuclei and fibre splittings; ultrastructural examination showed metabolic abnormalities. Next generation sequencing analysis detected a homozygous frameshift mutation in the *TCAP* gene (c.90_91del), previously described in one Turkish family. Immunostaining and Western blot analysis showed complete absence of telethonin. Interestingly, Single Nucleotide Polymorphism analysis of the 10 Mb genomic region containing the *TCAP* gene showed a shared homozygous haplotype of both the Greek and the Turkish patients, thus suggesting a possible founder effect of *TCAP* gene c.90_91del mutation in this part of the Mediterranean area.

Keywords: Limb girdle muscular dystrophy 2G; Telethonin; *TCAP* gene; Founder effect

1. Introduction

Telethonin (or titin-cap) is a 19 kDa protein, consisting of 167 amino acids, encoded by the *TCAP* gene, which includes two exons and maps on chromosome 17q12 [1]. It is one of the most abundant transcripts in skeletal muscle and localizes to the Z-line of sarcomere in adult skeletal and cardiac muscles [2]. The protein is involved in normal sarcomere assembly and development, as well as in sarcomere-membrane interaction and signalling [1]. It interacts with the titin N-terminus, joining two antiparallel titin (Z1-Z2) domains together, and with other Z-disc proteins, such as LIM protein, Ankyrin Repeat Domain 2 protein, myostatin, potassium channel B subunit minK, protein kinase D and murine double minute 2. In the *TCAP* null mouse and in patients without telethonin expression [3,4], the sarcomere architecture is generally normal. Pathogenesis seems to be related to disruption of sarcomere-T-tubular interaction, as shown in zebrafish models [5].

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https://doi.org/10.1016/j.nmd.2018.04.006

0960-8966/

frontiers in Neurology

ORIGINAL RESEARCH
published: 29 March 2018
doi: 10.3389/fneur.2018.00213

The Length of *SNCA* Rep1 Microsatellite May Influence Cognitive Evolution in Parkinson's Disease

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OPEN ACCESS

Edited by:
Giuseppe De Michele,
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Reviewed by:
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Specialty section:
This article was submitted to Movement Disorders, a section of the journal Frontiers in Neurology

Received: 17 December 2017
Accepted: 19 March 2018
Published: 29 March 2018

Citation:
Corrado L, De Marchi F, Tunesi S, Oggioni GD, Carecchio M, Magistrelli L, Tesei S, Riboldazzi G, Di Fonzo A, Locci C, Trezzi I, Zangaglia R, Cereda C, D'Alfonso S, Magnani C, Comi GP, Bono G, Pacchetti C, Cantello R, Goldwurm S and Comi C (2018) The Length of SNCA Rep1 Microsatellite May Influence Cognitive Evolution in Parkinson's Disease. Front. Neurol. 9:213. doi: 10.3389/fneur.2018.00213

Background: Alpha-synuclein is a constituent of Lewy bodies and mutations of its gene cause familial Parkinson's disease (PD). A previous study showed that a variant of the alpha-synuclein gene (*SNCA*), namely the 263 bp allele of Rep1 was associated with faster motor progression in PD. On the contrary, a recent report failed to detect a detrimental effect of Rep1 263 on both motor and cognitive outcomes in PD. Aim of this study was to evaluate the influence of the Rep1 variants on disease progression in PD patients.

Methods: We recruited and genotyped for *SNCA* Rep1 426 PD patients with age at onset ≥40 years and disease duration ≥4 years. We then analyzed frequency and time of occurrence of wearing-off, dyskinesia, freezing of gait, visual hallucinations, and dementia using a multivariate Cox's proportional hazards regression model.

Results: *SNCA* Rep1 263 carriers showed significantly increased risk of both dementia (HR = 3.03) and visual hallucinations (HR = 2.69) compared to 263 non-carriers. Risk of motor complications did not differ in the two groups.

Conclusion: *SNCA* Rep1 263 allele is associated with a worse cognitive outcome in PD.

Keywords: dementia, hallucinations, genetic markers, disease progression, Parkinson's disease

INTRODUCTION

Parkinson's disease (PD) is the second most frequent neurodegenerative disease (1), and is clinically characterized by the presence of asymmetric motor signs, including resting tremor, rigidity, and bradykinesia (2). Nonetheless, non-motor symptoms, such as mood deflection, anosmia, and sleep disturbances may also be present and can at times pre-date motor impairment (3). As disease

Mol Neurobiol (2018) 55:2789–2813
DOI 10.1007/s12035-017-0532-4

Therapeutic Strategies Under Development Targeting Inflammatory Mechanisms in Amyotrophic Lateral Sclerosis

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Received: 2 February 2017 / Accepted: 6 April 2017 / Published online: 28 April 2017

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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Rhabdomyolysis-Associated Acute Kidney Injury

Pasquale Esposito, Luca Estienne, Nicoletta Serpieri, Dario Ronchi, Giacomo P. Comi, Maurizio Moggio, Lorenzo Peverelli, Stefania Bianzina, and Teresa Rampino

Clinical Presentation

A 66-year-old man presented to the emergency department with tremors, dyspnea, nausea, polyarthralgia, and dysuria. He had a history of hypertension and ischemic heart disease. His medications included amlodipine, ramipril, and low-dose aspirin.

At admission, the patient was sweaty and showed generalized tremors. Blood pressure was 210/110 mm Hg, heart rate was 80 beats/min, and axillary temperature was 36.5°C. Physical examination showed diffuse muscle tenderness, mild peripheral edema, and basal pulmonary rales, with normal heart sounds. Laboratory studies showed elevated serum creatinine (201.5 μmol/L), urea nitrogen (10.3 mmol/L), transaminase (aspartate aminotransferase, 3,488 U/L; and alanine aminotransferase, 746 U/L), and lactate dehydrogenase levels (3,416 U/L), along with marked elevation of serum creatinine kinase (CK) level (167,000 U/L). Serum potassium level was 3.6 mmol/L; calcium, 2 mmol/L; and phosphate, 1.6 mmol/L. Urine dipstick was positive for blood and leukocyte esterase; microscopic examination of urine showed only about 2 red blood cells per high-power field.

A diagnosis of severe rhabdomyolysis was made. Shortly after admission, the patient's condition worsened and he was transferred to the intensive care unit. An echocardiogram documented severely depressed right and left ventricular function (ejection fraction, 20%) with generalized hypokinesis. Electromyography showed the presence of severe muscular injury without evidence of acute denervation. The patient developed oliguric acute kidney injury (AKI), and continuous renal replacement therapy (CRRT) was initiated.

Further history revealed that the patient had recently been engaged in what was for him unusually intense physical activity. It was also learned that he had presented with a similar episode of muscle damage 6 years before, when after coronary artery bypass surgery, he developed a marked increase in CK levels (27,000 U/L) that was not associated with AKI.

In the following days, respiratory and cardiac conditions improved, while kidney injury persisted and the patient was switched from CRRT to hemodialysis therapy. Two weeks after admission, kidney function improved and hemodialysis treatments were discontinued. Extensive testing was performed to elucidate the cause of the muscle injury, including a muscle biopsy (Fig 1).

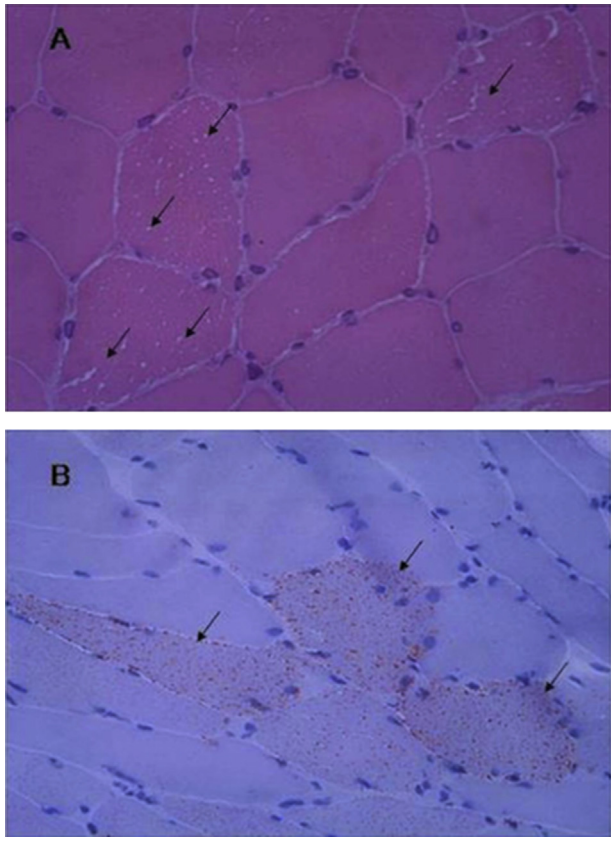

Figure 1. Skeletal muscle biopsy specimen shows small intracytoplasmic vacuoles (arrows) with (A) hematoxylin and eosin stain and (B) confirming the presence of lipid droplets (arrows) with Oil Red O stain (original magnification, ×400).

- **What is the differential diagnosis of the underlying cause of rhabdomyolysis in patients such as this?**
- **What are the effects of rhabdomyolysis on kidney function?**
- **What is the optimal management of patients with AKI secondary to rhabdomyolysis?**

to administer. The rationale for using sodium bicarbonate infusions rather than isotonic saline solutions is that urinary alkalization could avoid myoglobin precipitation and inhibit reduction–oxidation cycling of myoglobin and lipid peroxidation. However, although widely used, this approach is not evidence based and may increase the risk for metastatic tissue calcification and ionized hypocalcemia. The use of diuretics (ie, mannitol or furosemide) to increase diuresis has not been tested in clinical studies and is not generally recommended.

Various forms of dialytic therapy have been used if AKI develops, but there is no evidence supporting the use of a specific dialysis modality. Dialysis has also been hypothesized to enhance recovery through removal of myoglobin released into the circulation from injured muscles. Although some hemodialysis and CRRT filter membranes can remove myoglobin, there is little evidence that this is beneficial after AKI has been established. Intermittent hemodialysis is generally ineffective in sustaining a reduction in plasma myoglobin levels because of rapid rebound in levels following a single hemodialysis treatment. CRRT or hemofiltration is more effective, with much greater clearance of myoglobin than conventional filters,[1,3] but again, without evidence that this prevents AKI or improves its subsequent course once developed. In the current case, because of oliguria and severe AKI, continuous venovenous hemodiafiltration therapy with a high-flux membrane (AN69; Hospal Medical) was initiated. After 5 days, CK levels decreased to 22,000 U/L. Two weeks after admission, the patient was switched from CRRT to intermittent hemodialysis therapy. In the following days, diuresis ensued and kidney function progressively improved, so that it was possible to withdraw hemodialysis therapy.

CPT II deficiency is a rare disorder of the fatty acid beta-oxidation cycle, caused by homozygous or compound heterozygous mutations in the *CPT2* gene.[4] CPT II works as a shuttle for long-chain fatty acids to enter mitochondria, where they are used as the main energy source of muscles during prolonged exercise. CPT II deficiency leads to ATP depletion and dysfunction of the adenosine triphosphatase sodium/potassium pump (Na^+/K^+-ATPase) and the calcium-transporting adenosine triphosphatase (Ca^{2+}-ATPase) and activates phospholipases and proteases, which lyse cellular membranes and disrupt mitochondrial function. Diagnosis of CPT II deficiency, when clinically suspected, is confirmed by a combination of enzyme assay and molecular genetic testing for pathogenic mutations in the *CPT2* gene.

This patient represents a typical case of CPT II deficiency, with a history of recurrent rhabdomyolysis exacerbated by physical activity and postsurgery stress. After resolution of the AKI, prophylactic measures consist of preventing muscle energy depletion by avoiding intense physical activities. Following this recommendation, the patient did not develop other rhabdomyolysis episodes during 2 years of follow-up, at which time serum creatinine level was 111.8 μmol/L and CK (51 U/L) and myoglobin (65 ng/mL) levels were within the reference ranges.

Final Diagnosis

Acute kidney injury caused by rhabdomyolysis due to a mutation in the gene for the carnitine palmitoyltransferase II enzyme.

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Support: None.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Peer Review: Received November 23, 2017. Direct editorial input from the Pathology Editor, the Education Editor, and a Deputy Editor. Accepted in revised form March 9, 2018.

 doi: 10.1053/j.ajkd.2018.03.009

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Mol Neurobiol (2018) 55:4428–4436
DOI 10.1007/s12035-017-0671-7

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

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Received: 8 May 2017 / Accepted: 20 June 2017 / Published online: 29 June 2017

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

The American Journal of Pathology, Vol. 189, No. 2, February 2019

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The American Journal of
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MUSCULOSKELETAL PATHOLOGY

Fibrosis Rescue Improves Cardiac Function in Dystrophin-Deficient Mice and Duchenne Patient—Specific Cardiomyocytes by Immunoproteasome Modulation

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Accepted for publication October 10, 2018.

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Patients affected by Duchenne muscular dystrophy (DMD) develop a progressive dilated cardiomyopathy characterized by inflammatory cell infiltration, necrosis, and cardiac fibrosis. Standard treatments consider the use of β-blockers and angiotensin-converting enzyme inhibitors that are symptomatic and unspecific toward DMD disease. Medications that target DMD cardiac fibrosis are in the early stages of development. We found immunoproteasome dysregulation in affected hearts of *mdx* mice (murine animal model of DMD) and cardiomyocytes derived from induced pluripotent stem cells of patients with DMD. Interestingly, immunoproteasome inhibition ameliorated cardiomyopathy in *mdx* mice and reduced the development of cardiac fibrosis. Establishing the immunoproteasome inhibition—dependent cardioprotective role suggests the possibility of modulating the immunoproteasome as new and clinically relevant treatment to rescue dilated cardiomyopathy in patients with DMD. *(Am J Pathol 2019, 189: 339—353; https://doi.org/10.1016/j.ajpath.2018.10.010)*

Skeletal myopathy and muscular dystrophy progression are commonly associated with cardiac dysfunctions and a consequent high mortality attributable to heart failure.[1—3] In particular, patients with Duchenne muscular dystrophy (DMD) present with early diastolic dysfunction and myocardial fibrosis that turn into a dilated cardiomyopathy, complicated by heart failure and arrhythmia.[4] Even though recent improvements in the management of respiratory insufficiency have improved the lifespan and overall prognosis of patients with DMD, sudden deaths attributable to heart failure negatively affect their quality of life. Prompt treatment and early detection of cardiomyopathy represent the requirements for successful cardioprotective therapies that block or at least slow the processes of cardiac remodeling and heart failure.[3] Unfortunately, the current treatments for dilated cardiomyopathy are still inadequate because a deep understanding of the specific mechanisms underlying DMD-attributable heart failure is

A.F. and A.G. contributed equally to this work.

Supported by Italian Ministry of Health Conte Capitale 2015 (G.P.), Fondazione Umberto Veronesi (A.G.), and Fondazione IEO CCM (D.R.).

Disclosures: None declared.

https://doi.org/10.1016/j.ajpath.2018.10.010

Internal and Emergency Medicine (2018) 13:1287–1303
https://doi.org/10.1007/s11739-018-1909-8

CE - SYSTEMATIC REVIEW

Secondary prevention of cryptogenic stroke in patients with patent foramen ovale: a systematic review and meta-analysis

Elisa Maria Fiorelli[1] · Tiziana Carandini[2] · Delia Gagliardi[3] · Viviana Bozzano[1] · Mattia Bonzi[1] · Eleonora Tobaldini[1] · Giacomo Pietro Comi[3] · Elio Angelo Scarpini[2] · Nicola Montano[1] · Monica Solbiati[1]

Received: 14 May 2018 / Accepted: 12 July 2018 / Published online: 21 July 2018

Abstract

The aim of our study is to compare patent foramen ovale (PFO) closure versus medical treatment and antiplatelet versus anticoagulant therapy in patients with cryptogenic stroke (CS) and PFO. We conducted a systematic review and meta-analysis with trial sequential analysis (TSA) of randomized trials. Primary outcomes are stroke or transient ischemic attack (TIA) and all-cause mortality. Secondary outcomes are peripheral embolism, bleeding, serious adverse events, myocardial infarction and atrial dysrhythmias. We performed an intention to treat meta-analysis with a random-effects model. We include six trials (3677 patients, mean age 47.3 years, 55.8% men). PFO closure is associated with a lower recurrence of stroke or TIA at a mean follow-up of 3.88 years compared to medical therapy [risk ratio (RR) 0.55, 95% CI 0.38–0.81; $I^2 = 40\%$]. The TSA confirms this result. No difference is found in mortality (RR 0.74, 95% CI 0.35–1.60; $I^2 = 0\%$), while PFO closure is associated with a higher incidence of atrial dysrhythmias (RR 4.55, 95% CI 2.16–9.60; $I^2 = 25\%$). The rate of the other outcomes is not different among the two groups. The comparison between anticoagulant and antiplatelet therapy shows no difference in terms of stroke recurrence, mortality and bleeding. There is conclusive evidence that PFO closure reduces the recurrence of stroke or TIA in patients younger than 60 years of age with CS. More data are warranted to assess the consequences of the increase in atrial dysrhythmias and the advantage of PFO closure over anticoagulants.

Keywords Ischemic stroke · Patent foramen ovale · Closure device · Secondary prevention · Systematic reviews · Meta-analysis

Introduction

Ischemic stroke is one of the leading causes of disability worldwide [1]. The percentage of cryptogenic stroke (CS) represents 23–36% of all ischemic strokes [2–4]. Patent foramen ovale (PFO) is a common condition in the general population [5]. The probability of finding an incidental PFO in patients with CS has been estimated to reach 48%, but its role in CS is still doubtful [6]. No consensus about the optimal therapy for stroke secondary prevention in patients with PFO exists [7–16]. The American guidelines recommend antiplatelet therapy in patients with CS and PFO, unless oral anticoagulation (OAC) is indicated for other reasons [17]. Conversely, the European Stroke Organization suggests PFO closure in patients with CS and PFO that is likely to be stroke-related [18, 19]. Recently, two RCTs, the follow-up extension of a previous RCT and several systematic reviews were published, showing a benefit of PFO closure over medical therapy in terms of stroke recurrence [20–33]. Another

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frontiers in Neurology

CASE REPORT
published: 02 August 2018
doi: 10.3389/fneur.2018.00619

Bilateral Cavernous Carotid Aneurysms: Atypical Presentation of a Rare Cause of Mass Effect. A Case Report and a Review of the Literature

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Neuro-Ophthalmology, a section of the journal Frontiers in Neurology

Received: 28 February 2018
Accepted: 10 July 2018
Published: 02 August 2018

Citation:
Gagliardi D, Faravelli I, Villa L, Pero G, Cinnante C, Brusa R, Mauri E, Tresoldi L, Magri F, Govoni A, Bresolin N, Comi GP and Corti S (2018) Bilateral Cavernous Carotid Aneurysms: Atypical Presentation of a Rare Cause of Mass Effect. A Case Report and a Review of the Literature. Front. Neurol. 9:619. doi: 10.3389/fneur.2018.00619

Bilateral cavernous carotid aneurysms (CCAs) represent a rare medical condition that can mimic other disorders. We present a rare case of bilateral CCAs simulating an ocular myasthenia. A 76-year-old woman presented with a history of fluctuating bilateral diplopia and unilateral ptosis, which led to the suspicion of ocular myasthenia. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) of the brain showed the presence of large bilateral aneurysms of the carotid cavernous tract. After an unsuccessful attempt with steroid therapy, the patient underwent endovascular treatment, with mild improvement. Bilateral CCAs can cause pupil sparing third nerve palsies and other cranial neuropathies which can mimic signs and symptoms of disorders of the neuromuscular junction. Therefore, the possibility of a vascular lesion simulating ocular myasthenia should be considered especially in older patients.

Keywords: bilateral cavernous carotid aneurysms, internal carotid artery, cranial nerves palsy, pseudomyasthenia, diplopia

INTRODUCTION

Aneurysms of the cavernous tract of the carotid artery are a rare occurrence, with a reported prevalence varying from 0.3 to 1.4% of all intracranial aneurysms (1). These aneurysms are more common in women and can be idiopathic or due to trauma and infections. CCAs are also different from other intracranial aneurysms in terms of natural history and clinical presentation: they are often asymptomatic, have a low risk of life-threatening complications and are usually considered as benign lesions. Though uncommon, complications of CCAs have been reported, which include rupture into either the cavernous sinus, causing the formation of a carotido-cavernous fistula, or into the subarachnoid space, determining a subarachnoid hemorrhage. Other than spontaneous rupture, complications can derive from acute thrombosis or progressive compression of cranial nerves in the cavernous sinus.

genes

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Article

Copy Number Variants Account for a Tiny Fraction of Undiagnosed Myopathic Patients

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Received: 14 September 2018; Accepted: 23 October 2018; Published: 26 October 2018

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Molecular Neurobiology (2018) 55:6307–6318
https://doi.org/10.1007/s12035-017-0831-9

Time Is Motor Neuron: Therapeutic Window and Its Correlation with Pathogenetic Mechanisms in Spinal Muscular Atrophy

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Received: 26 June 2017 / Accepted: 8 December 2017 / Published online: 2 January 2018

Abstract

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by the degeneration of lower motor neurons (MNs) in the spinal cord and brain stem, which results in relentless muscle weakness and wasting, leading to premature death due to respiratory complications. The identification of the specific mutations in the survival motor neuron 1 (*SMN1*) gene that causes SMA has led to the development of experimental therapeutic strategies to increase SMN protein expression, including antisense oligonucleotides, small molecules, and gene therapy, which have so far shown promising results. The timing of therapeutic intervention is crucial since most of the degeneration in MNs occurs in the first months of life in patients with SMA type 1, which is the most severe and common form of SMA. Nevertheless, a precise temporal window for therapeutic intervention has not yet been identified. Evidence from in vivo studies in mice and large animals suggested that early therapeutic intervention for SMA correlated with better motor performance, longer survival, and, occasionally, rescue of the pathological phenotype. Indeed, the need to compensate for the loss of SMN protein function seemed to diminish during adulthood (even though repair ability after nerve injury remained impaired), suggesting the possibility of tapering the therapy administration late in the disease course. Moreover, recent clinical trials on children afflicted with SMA type 1 have shown a more rapid achievement of motor milestones and diminished disease severity when therapy was administered at an early age and earlier in the disease course. Finally, these results highlight the importance of newborn screening for SMA to facilitate early diagnosis and present the patient with available treatments while they are still in the presymptomatic stage.

Keywords Spinal muscular atrophy · SMA · Therapeutic window · Antisense oligonucleotides · Gene therapy

Introduction

Spinal muscular atrophy (SMA) is a hereditary and progressive neuromuscular disease that is characterized by proximal-distal muscular weakness and atrophy, which results from the degeneration of lower motor neurons (MNs) in the ventral horns of the spinal cord and in lower brainstem nuclei [1, 2]. SMA is the most common autosomal recessive disease after cystic fibrosis and is the leading genetic cause of death in infancy [3–5]. The disease incidence is about 1 in 10,000 live births, whereas the carrier status incidence is 1 in 40 to 60 depending on the ethnicity [6–8].

In 98% of cases, the disease is caused by a homozygous mutation, deletion, or rearrangement in the survival motor neuron 1 (*SMN1*) gene on chromosome 5 (SMA5q, OMIM #253300), which encodes the SMN protein. Only the human genome, and no other species, encodes a highly homologous copy of *SMN1* called *SMN2*; *SMN2* differs from *SMN1* by a few nucleotides, of which the most critical is a C to T transition in exon 7 that causes the skipping of this exon in > 90% of *SMN2* transcripts [6]. For this reason, *SMN2* mainly produces a truncated protein (Δ7), without exon 7, much of which is unstable and rapidly degraded [9]; moreover, *SMN2* expression constitutes only 10% of the full-length fully functional SMN protein [10] and thus partially compensates for the loss of *SMN1*.

In SMA patients, *SMN2* is the major modulator of the disease phenotype, and the number of *SMN2* copies inversely correlates with the severity of the phenotype. SMA types are classified by the higher motor milestones achieved such that untreated SMA type 1 patients never acquire the ability to sit and stand, and SMA type 2 patients are able to sit but cannot

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J. Cell. Mol. Med. Vol 22, No 2, 2018 pp. 755-767

miRNA in spinal muscular atrophy pathogenesis and therapy

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Received: April 19, 2017; Accepted: October 3, 2017

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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doi: 10.1111/jcmm.13450

Neurological Sciences (2018) 39:1491–1492
https://doi.org/10.1007/s10072-018-3311-8

LETTER TO THE EDITOR

Novel Lys215Asn mutation in an Italian family with Thomsen myotonia

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Received: 9 January 2018 / Accepted: 8 March 2018 / Published online: 15 March 2018

Dear Sir,

Myotonia congenita is a non-dystrophic muscular disease characterized by impaired muscle relaxation after voluntary or evoked contraction and muscle stiffness. Myotonia typically occurs after a period of rest and decreases after repetitive movement, the so-called warm-up phenomenon [1]. Muscular hypertrophy is another important clinical sign.

Myotonia congenita, both in dominant (Thomsen disease) and in recessive form (Becker disease), is caused by mutations in the *CLCN1* gene that encodes the major skeletal muscle chloride channel [2]. Dysfunction of this channel causes hyperexcitability of the skeletal muscle membrane and repetitive firing of muscle action potentials [3]. More than 150 *CLCN1* pathogenic variants have been identified.

We present an Italian family with Thomsen myotonia (Fig. 1) in which a novel mutation in the *CLCN1* gene was detected.

A 60-year-old man (patient II:1) complained from childhood of transient stiffness and weakness that improved with activity and got worse with cold temperatures. Neurological examination revealed generalized muscle hypertrophy, most prominent in quadriceps and gastrocnemius muscles, with normal strength. Grip and lip myotonia were evident. Percussion of the thenar eminence elicited brief opposition. CPK were 360 U/L (nv 60-190). EMG revealed profuse myotonic discharges in all examined muscles with normal motor unit potentials.

The 32-year-old firstborn son (patient III:1) presented the same symptoms of the father from infancy. Neurological examination showed Hercules-like appearance. Myotonia was present in all four limbs and in facial muscles including lids. Percussion of the thenar eminence elicited brief opposition. CPK were 420 U/L. EMG showed abundant myotonic discharges in all tested muscles and mild myopathic changes in iliopsoas and deltoids muscles.

In both patients Mexiletine 200 mg bid was started with improvement.

After genetic counseling, probands and their familiars underwent to the screening for *CLCN1* gene. Sequence analysis evidenced the previously reported p.Phe167Leu on exon 4, and the nucleotide change c.645G>T on exon 5 leading to the missense p.Lys215Asn (Fig. 2). This variant was unreported; therefore, 160 control alleles were checked for this novel variant, and none resulted positive. The possible pathological meaning of the variant was evaluated by in silico tools (PROVEAN provean.jcvi.org; MutationTaster www.mutationtaster.org; MutPred mutpred.mutdb.org) and was unanimously predicted as pathogenic.

Subjects II:1 and III:2 were heterozygous for p.Lys215Asn; II:2 carried p.Phe167Leu, III:1 was a compound heterozygote of both mutations.

Thereupon, we clinically evaluated subject III:2, who did not complain any symptoms. Neurological examination revealed only generalized muscle hypertrophy. CPK were normal. EMG showed myotonic discharges of proximal and distal muscles but not at masseter.

Mutations in *CLCN1* gene are causative of congenital myotonia. We report a novel mutation in a family with two males suffering from myotonia congenita and one

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Mauri et al. BMC Neurology (2018) 18:220
https://doi.org/10.1186/s12883-018-1227-9

BMC Neurology

CASE REPORT Open Access

Subclinical Leber's hereditary optic neuropathy with pediatric acute spinal cord onset: more than meets the eye

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Abstract

Background: Leber's hereditary optic neuropathy (LHON) is a mitochondrial disease characterized by visual loss consequent to optic nerve atrophy. In some cases, LHON is associated with heterogeneous neurological extraocular manifestations and is referred to as "Leber plus disease"; rarely it is associated with a multiple sclerosis (MS)-like syndrome known as Harding disease, but no pediatric extraocular acute spinal onset is reported.

Case presentation: We describe the case of a 5-year-old girl carrying the G3460A mtDNA mutation who was referred to clinical examination for bilateral upper and lower limb weakness with no sign of optic neuropathy. Spinal cord MRI showed hyperintense signal alterations in T2-weighted and restricted diffusion in DWI sequences in the anterior portion of the cervical and dorsal spinal cord resembling a spinal cord vascular injury. No association between this mutation and pediatric spinal cord lesions has previously been reported. Alternative diagnostic hypotheses, including infective, ischemic and inflammatory disorders, were not substantiated by clinical and instrumental investigations.

Conclusions: Our case reports a novel pediatric clinical manifestation associated with the m.3460G > A mtDNA mutation, broadening the clinical spectrum of this disease. Early identification of new cases and monitoring of carriers beginning in childhood is important to prevent neurological deterioration and preserve long-term function.

Keywords: Leber's hereditary optic neuropathy, Spinal cord, Pediatric, Mitochondrial pathology

Background

Leber's hereditary optic neuropathy (LHON) is a maternally inherited genetic disease that occurs due to a mitochondrial DNA (mtDNA) mutation that causes central, bilateral, painless, progressive visual loss due to optic nerve atrophy, particularly in young adult men [1]. Three disease-causing mutations that affect subunits of complex I of the mitochondrial respiratory chain (MTND1: m.3460G > A, MTND4: m.11778G > A, and MTND6: m.14484 T > C) are responsible for 90% of the cases. The extraocular manifestations of the disease, known as "Leber plus disease", include movement disorders, mental retardation, seizures, cerebellar ataxia, and peripheral neuropathy [1]. Other associations, such as multiple sclerosis (MS)-like syndrome, referred to as "Harding disease" or "LHON-MS" [2], Leigh-like encephalopathy and MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes)/LHON overlap syndromes, have also been reported [3]. We describe a 5-year-old girl affected by an acute spinal cord lesion mimicking a vascular lesion. The patient had a family history of LHON due to the G3460A mtDNA mutation.

Case presentation

A 5-year-old girl was admitted to our Emergency Department after an episode of acute interscapular back pain occurring without trauma and followed by bilateral upper and lower limb weakness.

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BBA - Molecular Basis of Disease 1864 (2018) 3022–3037

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

The MELAS mutation m.3243A > G promotes reactivation of fetal cardiac genes and an epithelial-mesenchymal transition-like program via dysregulation of miRNAs

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ARTICLE INFO

Keywords:
OXPHOS diseases
Heteroplasmy
Hypertrophic cardiomyopathy
miR-218-5p
miR-4775

ABSTRACT

The pathomechanisms underlying oxidative phosphorylation (OXPHOS) diseases are not well-understood, but they involve maladaptive changes in mitochondria-nucleus communication. Many studies on the mitochondria-nucleus cross-talk triggered by mitochondrial dysfunction have focused on the role played by regulatory proteins, while the participation of miRNAs remains poorly explored. MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) is mostly caused by mutation m.3243A > G in mitochondrial $tRNA^{Leu(UUR)}$ gene. Adverse cardiac and neurological events are the commonest causes of early death in m.3243A > G patients. Notably, the incidence of major clinical features associated with this mutation has been correlated to the level of m.3243A > G mutant mitochondrial DNA (heteroplasmy) in skeletal muscle. In this work, we used a transmitochondrial cybrid model of MELAS (100% m.3243A > G mutant mitochondrial DNA) to investigate the participation of miRNAs in the mitochondria-nucleus cross-talk associated with OXPHOS dysfunction. High-throughput analysis of small-RNA-Seq data indicated that expression of 246 miRNAs was significantly altered in MELAS cybrids. Validation of selected miRNAs, including miR-4775 and miR-218-5p, in patient muscle samples revealed miRNAs whose expression declined with high levels of mutant heteroplasmy. We show that miR-218-5p and miR-4775 are direct regulators of fetal cardiac genes such as *NODAL*, *RHOA*, *ISL1* and *RXRB*, which are up-regulated in MELAS cybrids and in patient muscle samples with heteroplasmy above 60%. Our data clearly indicate that TGF-β superfamily signaling and an epithelial-mesenchymal transition-like program are activated in MELAS cybrids, and suggest that down-regulation of miRNAs regulating fetal cardiac genes is a risk marker of heart failure in patients with OXPHOS diseases.

1. Introduction

Mitochondria are pivotal organelles to eukaryotic cells. They play a crucial role in ATP production, via the oxidative phosphorylation (OXPHOS) system, and in several metabolic pathways, cell signaling and apoptosis [1–4].

Mitochondrial DNA (mtDNA) encodes 13 of the about 100 proteins that make up the OXPHOS system, and the 2 rRNAs and 22 tRNAs necessary for mitochondrial translation. The rest of the OXPHOS subunits and mitochondrial translation factors are encoded by the nucleus (nDNA). Therefore, defects in the OXPHOS system (OXPHOS diseases), which have extremely variable clinical manifestations, ranging from

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https://doi.org/10.1016/j.bbadis.2018.06.014
Received 21 February 2018; Received in revised form 25 May 2018; Accepted 13 June 2018
Available online 19 June 2018

Parkinsonism and Related Disorders 48 (2018) 109–111

Contents lists available at ScienceDirect

Parkinsonism and Related Disorders

journal homepage: www.elsevier.com/locate/parkreldis

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

Keywords:
NBIA
MPAN
C19orf12
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

https://doi.org/10.1016/j.parkreldis.2017.12.025

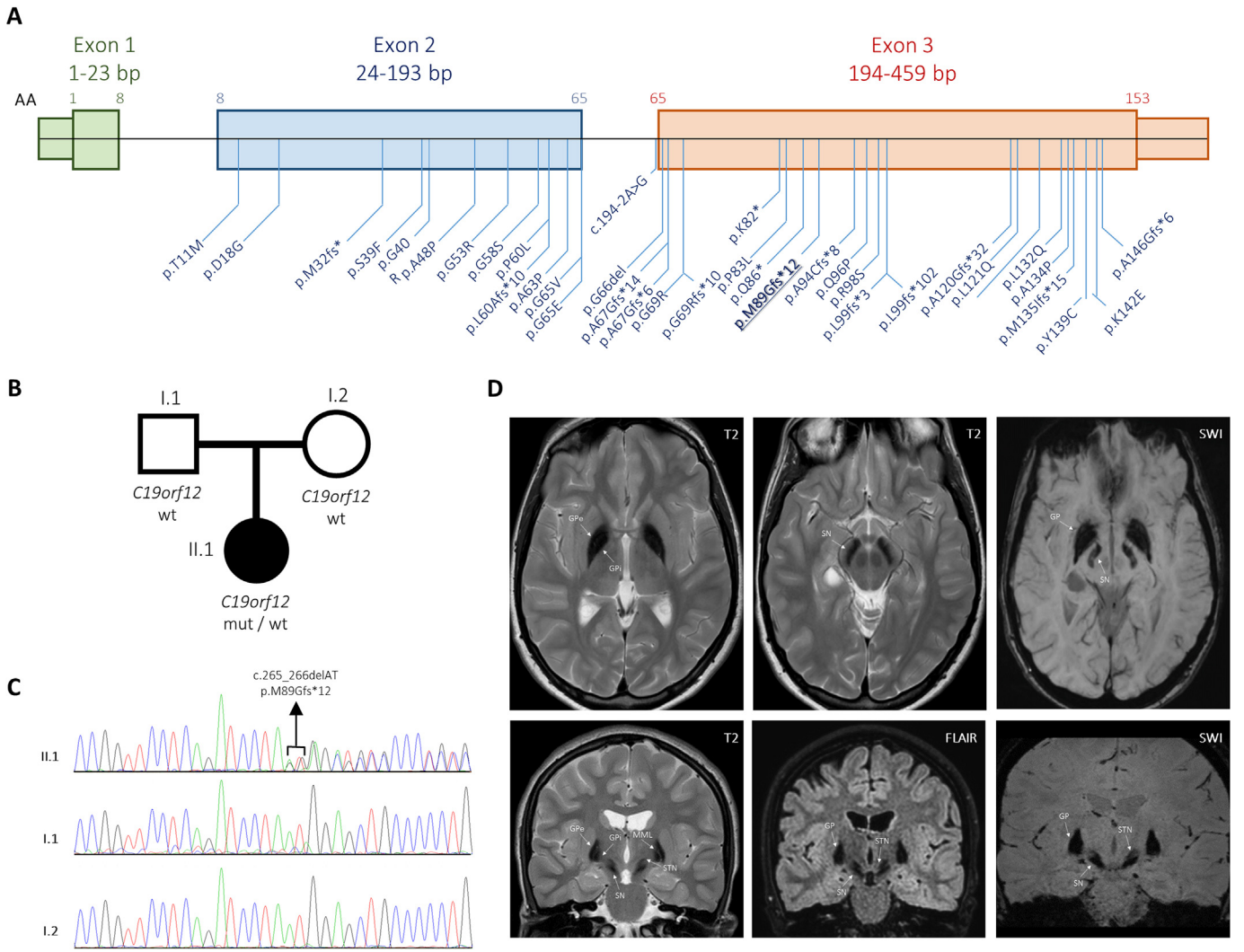

Fig. 1. A) Representation of the *C19orf12* gene mutations reported in MPAN phenotypes so far. B) Pedigree of the family under study. Black symbol denotes affected individual. C) Electropherograms of the mutation c.265_266delAT of *C19orf12* gene in the proband (II.1) and wild type sequence in her parents (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.

Funding sources

This work was supported by ADF's funds, from Intesa San Paolo and Fresco Institute.

Ethics

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

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BBA - Molecular Basis of Disease 1864 (2018) 3588–3597

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Mitochondrial dysfunction in fibroblasts of Multiple System Atrophy

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ARTICLE INFO

Keywords:
Multiple System Atrophy
Cellular models
Fibroblasts
Mitochondria

ABSTRACT

Multiple System Atrophy is a severe neurodegenerative disorder which is characterized by a variable clinical presentation and a broad neuropathological spectrum. The pathogenic mechanisms are almost completely unknown. In the present study, we established a cellular model of MSA by using fibroblasts' primary cultures and performed several experiments to investigate the causative mechanisms of the disease, with a particular focus on mitochondrial functioning.

Fibroblasts' analyses (7 MSA-P, 7 MSA-C and 6 healthy controls) displayed several anomalies in patients: an impairment of respiratory chain activity, in particular for succinate Coenzyme Q reductase ($p < 0.05$), and a reduction of complex II steady-state level ($p < 0.01$); a reduction of Coenzyme Q10 level ($p < 0.001$) and an up-regulation of some CoQ10 biosynthesis enzymes, namely COQ5 and COQ7; an impairment of mitophagy, demonstrated by a decreased reduction of mitochondrial markers after mitochondrial inner membrane depolarization ($p < 0.05$); a reduced basal autophagic activity, shown by a decreased level of LC3 II ($p < 0.05$); an increased mitochondrial mass in MSA-C, demonstrated by higher TOMM20 levels ($p < 0.05$) and suggested by a wide analysis of mitochondrial DNA content in blood of large cohorts of patients.

The present study contributes to understand the causative mechanisms of Multiple System Atrophy. In particular, the observed impairment of respiratory chain activity, mitophagy and Coenzyme Q10 biosynthesis suggests that mitochondrial dysfunction plays a crucial role in the pathogenesis of the disease. Furthermore, these findings will hopefully contribute to identify novel therapeutic targets for this still incurable disorder.

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https://doi.org/10.1016/j.bbadis.2018.09.018
Received 6 June 2018; Received in revised form 7 September 2018; Accepted 17 September 2018
Available online 19 September 2018

Stem Cell Reports
Article
OPEN ACCESS

Mitochondrial Dysregulation and Impaired Autophagy in iPSC-Derived Dopaminergic Neurons of Multiple System Atrophy

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https://doi.org/10.1016/j.stemcr.2018.09.007

SUMMARY

Multiple system atrophy (MSA) is a progressive neurodegenerative disease that affects several areas of the CNS, whose pathogenesis is still widely unclear and for which an effective treatment is lacking. We have generated induced pluripotent stem cell-derived dopaminergic neurons from four MSA patients and four healthy controls and from two monozygotic twins discordant for the disease. In this model, we have demonstrated an aberrant autophagic flow and a mitochondrial dysregulation involving respiratory chain activity, mitochondrial content, and CoQ10 biosynthesis. These defective mechanisms may contribute to the onset of the disease, representing potential therapeutic targets.

INTRODUCTION

Multiple system atrophy (MSA) is a severe and progressive neurodegenerative disease. Parkinsonism, cerebellar ataxia, dysautonomia, and pyramidal features are the main clinical hallmarks. According to the predominant symptomatology at onset, either parkinsonian or cerebellar, two different subtypes of the disease can be distinguished: MSA-P and MSA-C, respectively (Fanciulli and Wenning, 2015).

Although many preclinical and clinical trials are in progress (Valera et al., 2016), an effective treatment is still lacking.

Neuropathologically, MSA is characterized by atrophy mainly in the putamen in MSA-P and in the cerebellum, middle cerebellar peduncles, and pontine basis in MSA-C. α-Synuclein accumulation is the neuropathological hallmark of the disease. Differently from other α-synucleinopathies, it occurs mainly in oligodendrocytes in the form of glial cytoplasmic inclusions. However, α-synuclein aggregates can be detected also in glial nuclei, neuronal cytoplasm, neuronal nuclei, and astroglial cytoplasm. Moreover, astrogliosis and microglial activation are common findings in MSA. Despite the peculiarity of oligodendroglial involvement, neuronal systems are strongly affected. A prominent degeneration of striatonigral pathway (both striatal medium spiny neurons and substantia nigra dopaminergic neurons) is observed in MSA-P. MSA-C displays a remarkable degeneration of Purkinje cells and cerebellopontine fibers; however, substantia nigra is also affected (Jellinger, 2014).

The role of mitochondrial dysfunction in the onset and progression of MSA has been debated. The most direct evidence supporting this scenario is the report of mutations in *COQ2*, a gene involved in the synthesis of CoenzymeQ10 (CoQ10), in familial and sporadic MSA cases (Multiple-System Atrophy Research Collaboration, 2013), but this finding has not been replicated in independent MSA cohorts (Sharma et al., 2014; Schottlaender et al., 2014; Ronchi et al., 2016). The assessment of the activity of respiratory chain complexes in autoptic substantia nigra and platelets of patients has not provided significant results (Gu et al., 1997). Two independent groups have recently described a reduction of CoQ10 levels selectively in

Genome-wide Analyses Identify KIF5A as a Novel ALS Gene

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SUMMARY

To identify novel genes associated with ALS, we undertook two lines of investigation. We carried out a genome-wide association study comparing 20,806 ALS cases and 59,804 controls. Independently, we performed a rare variant burden analysis comparing 1,138 index familial ALS cases and 19,494 controls. Through both approaches, we identified *kinesin family member 5A* (*KIF5A*) as a novel gene associated with ALS. Interestingly, mutations predominantly in the N-terminal motor domain of KIF5A are causative for two neurodegenerative diseases: hereditary spastic paraplegia (SPG10) and Charcot-Marie-Tooth type 2 (CMT2). In contrast, ALS-associated mutations are primarily located at the C-termin cargo-binding tail domain and patients harboring loss-of-function mutations displayed an extended survival relative to typical ALS cases. Taken together, these results broaden the phenotype spectrum resulting from mutations in *KIF5A* and strengthen the role of cytoskeletal defects in the pathogenesis of ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS; OMIM: 05400) is a neurodegenerative disorder clinically characterized by rapidly progressive muscle weakness and death due to respiratory failure, typically within 2 to 4 years of symptom onset (van Es et al., 2017). Although ALS is perceived as being rare, approximately 6,000 Americans die annually from the condition

BBA - Molecular Basis of Disease 1864 (2018) 3407–3417

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Glucose-free/high-protein diet improves hepatomegaly and exercise intolerance in glycogen storage disease type III mice

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ARTICLE INFO

Keywords:
Glycogen storage disease type III
Glucose-free diet
High-protein diet
Glycogen debranching enzyme

ABSTRACT

Glycogen disease type III (GSDIII), a rare incurable autosomal recessive disorder due to glycogen debranching enzyme deficiency, presents with liver, heart and skeletal muscle impairment, hepatomegaly and ketotic hypoglycemia. Muscle weakness usually worsens to fixed myopathy and cardiac involvement may present in about half of the patients during disease. Management relies on careful follow-up of symptoms and diet. No common agreement was reached on sugar restriction and treatment in adulthood.

We administered two dietary regimens differing in their protein and carbohydrate content, high-protein (HPD) and high-protein/glucose-free (GFD), to our mouse model of GSDIII, starting at one month of age. Mice were monitored, either by histological, biochemical and molecular analysis and motor functional tests, until 10 months of age.

GFD ameliorated muscle performance up to 10 months of age, while HPD showed little improvement only in young mice. In GFD mice, a decreased muscle glycogen content and fiber vacuolization was observed, even in aged animals indicating a protective role of proteins against skeletal muscle degeneration, at least in some districts. Hepatomegaly was reduced by about 20%. Moreover, the long-term administration of GFD did not worsen serum parameters even after eight months of high-protein diet. A decreased phosphofructokinase and pyruvate kinase activities and an increased expression of Krebs cycle and gluconeogenesis genes were seen in the liver of GFD fed mice.

Our data show that the concurrent use of proteins and a strictly controlled glucose supply could reduce muscle wasting, and indicate a better metabolic control in mice with a glucose-free/high-protein diet.

1. Introduction

Glycogen storage disease type III (GSDIII; OMIM #232400) is a rare autosomal recessive disease [1,2] caused by deficiency of glycogen debranching enzyme, one of the two enzymes responsible for glycogenolysis. Hepatomegaly, ketotic hypoglycemia, hyperlipidemia, elevated transaminases and failure to thrive are the usual presenting symptoms in the first year of life in GSDIIIa (the most common subtype) [1–3]. Hepatic symptoms usually improve and tend to resolve in adolescence although liver fibrosis and cirrhosis may develop [4,5]. Cardiac involvement, such as left ventricular wall thickness and mass increase, occurs in about half of the patients, but generally is stationary [3,6]. In young adults, myopathy initially presents mostly as exercise intolerance, with involvement of both proximal and distal muscle and elevated CK levels. A fixed myopathy with proximo-distal involvement of variable severity occurs in the following decades, eventually leading to loss of independent walking. Muscle weakness is often described also in young patients revealing that myopathy may occur earlier than usually reported [2].

To date, there is no cure for GSDIII and the current

Abbreviations: GSDIII, glycogen disease type III; GDE, glycogen debranching enzyme; HPD, high-protein diet; GFD, glucose-free diet; SD, standard diet; CKs, creatine kinases; BUN, blood urea nitrogen; M, male mice; F, female mice

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https://doi.org/10.1016/j.bbadis.2018.07.031
Received 20 February 2018; Received in revised form 6 July 2018; Accepted 30 July 2018
Available online 01 August 2018

Ther Adv Neurol Disord
2018, Vol. 11: 1–13
DOI: 10.1177/1756285618754501

Advances in spinal muscular atrophy therapeutics

Valeria Parente and Stefania Corti

Abstract: Spinal muscular atrophy (SMA) is a progressive, recessively inherited neuromuscular disease, characterized by the degeneration of lower motor neurons in the spinal cord and brainstem, which leads to weakness and muscle atrophy. SMA currently represents the most common genetic cause of infant death. SMA is caused by the lack of survival motor neuron (SMN) protein due to mutations, which are often deletions, in the *SMN1* gene. In the absence of treatments able to modify the disease course, a considerable burden falls on patients and their families. Greater knowledge of the molecular basis of SMA pathogenesis has fuelled the development of potential therapeutic approaches, which are illustrated here. Nusinersen, a modified antisense oligonucleotide that modulates the splicing of the *SMN2* mRNA transcript, is the first approved drug for all types of SMA. Moreover, the first gene therapy clinical trial using adeno-associated virus (AAV) vectors encoding SMN reported positive results in survival and motor milestones achievement. In addition, other strategies are in the pipeline, including modulation of *SMN2* transcripts, neuroprotection, and targeting an increasing number of other peripheral targets, including the skeletal muscle. Based on this premise, it is reasonable to expect that therapeutic approaches aimed at treating SMA will soon be changed, and improved, in a meaningful way. We discuss the challenges with regard to the development of novel treatments for patients with SMA, and depict the current and future scenarios as the field enters into a new era of promising effective treatments.

Keywords: antisense oligonucleotides, gene therapy, neuromuscular disease, spinal muscular atrophy

Received: 21 May 2017; accepted in revised form: 24 October 2017.

Introduction

Spinal muscular atrophy (SMA) is a devastating autosomal recessive neuromuscular disease characterized by motor neuron degeneration in the brain stem and spinal cord, resulting in progressive muscle weakness and atrophy.[1] SMA occurs in approximately 1 in 10,000 newborns, and represents the most common hereditary disease-causing childhood death to date.[2] This disease arises from mutations in the survival motor neuron 1 (*SMN1*) gene. These mutations, that are often deletions, lead to the deficiency of the ubiquitous SMN protein.[3] The human genome contains a *SMN1* paralogous gene, *SMN2*, which produces a truncated unstable protein (SMNΔ7) due to alternative splicing which excludes exon 7 from the final transcript. Therefore, the low level (approximately 10%) of full-length functional SMN protein produced only partially compensates for the lack of *SMN1*.[4] All patients with SMA have at least one copy of the *SMN2* gene. They are classified as having SMA type 1–4 (SMA1–SMA4) on the basis of their age of onset and their highest motor milestones, and the number of *SMN2* copies inversely correlates with the clinical severity of the disease phenotype.[5]

The SMA field has been revolutionized during recent months following therapeutic advances that have led to the approval of the first therapy regimen for SMA. In addition, progress has been made on other specific approaches, such as gene

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Molecular Neurobiology
https://doi.org/10.1007/s12035-018-1246-y

R-Loops in Motor Neuron Diseases

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Received: 29 April 2018 / Accepted: 15 July 2018

Abstract
R loops are transient three-stranded nucleic acid structures that form physiologically during transcription when a nascent RNA transcript hybridizes with the DNA template strand, leaving a single strand of displaced nontemplate DNA. However, aberrant persistence of R-loops can cause DNA damage by inducing genomic instability. Indeed, evidence has emerged that R-loops might represent a key element in the pathogenesis of human diseases, including cancer, neurodegeneration, and motor neuron disorders. Mutations in genes directly involved in R-loop biology, such as SETX (senataxin), or unstable DNA expansion eliciting R-loop generation, such as *C9ORF72* HRE, can cause DNA damage and ultimately result in motor neuron cell death. In this review, we discuss current advancements in this field with a specific focus on motor neuron diseases associated with deregulation of R-loop structures. These mechanisms can represent novel therapeutic targets for these devastating, incurable diseases.

Keywords R-loops · DNA damage · Motor neuron disease · Amyotrophic lateral sclerosis · Spinal muscular atrophy

Introduction

The etiopathogenesis of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), remains largely unknown, which hampers the rational development of effective treatments. These disorders have in common the selective loss of a specific neuronal subpopulation. In several neurodegenerative diseases, this progressive cell death has been related to a common neuropathological feature, namely the accumulation of proteins that aggregate in cells due to their misfolded or altered structure [1, 2]. Although a common underlying pathological feature of many neurodegenerative diseases is the accumulation of protein aggregates, it is not clear whether such structures are universally toxic to neurons. Indeed, recent studies have highlighted the possible role of RNA alterations in the pathogenesis of these disorders [3]. In particular, the defect in the resolution of transcriptional R-loops, a consequence of abnormal mRNA metabolism, is thought to be responsible for DNA damage and genome instability [4–6]. This notion represents a new perspective in the comprehension of the pathogenesis of these diseases and may possibly open paths to new therapeutic targets.

R-loops are transient nucleic acid structures composed of three strands (mRNA, template DNA strand, and not-template DNA strand) that form during the transcriptional process. In the course of transcription, dsDNA is unwound, and one strand of the DNA double helix is copied into a complementary RNA transcript. Within the transcription bubble, the two strands of DNA are physically separated, and a transient 8-bp RNA/DNA hybrid forms [7]. In most cases, the two DNA strands reanneal as the RNA is synthesized along the DNA. However, R-loops can develop in particular over DNA regions characterized by high G density, negative supercoiling, and DNA nicks [8] during transcription. The exact mechanism of R-loops generation remains unclear. In specific circumstances, the RNA:DNA hybrid could be maintained and extended as the polymerase moves forward, leaving the other DNA strand unbound. Another mechanism proposed for R-loops generation is the thread-back model. In this model, the nascent RNA transcript exiting the active site of RNA polymerase can anneal to the template DNA strand before the reannealing of the two strands of the DNA duplex, generating a RNA:DNA hybrid that is more stable than dsDNAs [9] and discarding the non-template, single-stranded DNA [10–12].

A number of cellular processes are controlled by the programmed formation of R-loops, e.g., Ig class switch recombination and termination of transcription [12]. Normally, R-loop

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Published online: 25 July 2018

International Journal of
Molecular Sciences

MDPI

Article

Investigation of New Morpholino Oligomers to Increase Survival Motor Neuron Protein Levels in Spinal Muscular Atrophy

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Received: 29 November 2017; Accepted: 2 January 2018; Published: 6 January 2018

Abstract: Spinal muscular atrophy (SMA) is an autosomal-recessive childhood motor neuron disease and the main genetic cause of infant mortality. SMA is caused by deletions or mutations in the survival motor neuron 1 (*SMN1*) gene, which results in SMN protein deficiency. Only one approved drug has recently become available and allows for the correction of aberrant splicing of the paralogous *SMN2* gene by antisense oligonucleotides (ASOs), leading to production of full-length SMN protein. We have already demonstrated that a sequence of an ASO variant, Morpholino (MO), is particularly suitable because of its safety and efficacy profile and is both able to increase SMN levels and rescue the murine SMA phenotype. Here, we optimized this strategy by testing the efficacy of four new MO sequences targeting *SMN2*. Two out of the four new MO sequences showed better efficacy in terms of SMN protein production both in SMA induced pluripotent stem cells (iPSCs) and SMAΔ7 mice. Further, the effect was enhanced when different MO sequences were administered in combination. Our data provide an important insight for MO-based treatment for SMA. Optimization of the target sequence and validation of a treatment based on a combination of different MO sequences could support further pre-clinical studies and the progression toward future clinical trials.

Keywords: spinal muscular atrophy; morpholino; therapy

1. Introduction

Spinal muscular atrophy (SMA) is an autosomal-recessive neurodegenerative disease with an infantile or early onset and represents the main genetic cause of infant mortality and the second most common autosomal-recessive disease in the Caucasian population. SMA is caused by a homozygous mutation of the survival motor neuron 1 (*SMN1*) gene, which results in reduced levels of the functional SMN protein [1]. SMA is characterized by progressive loss of motor neurons (MNs) in the ventral horns of the spinal cord, which causes progressive muscle weakness, paralysis and premature death [2].

In humans, two nearly identical copies of the *SMN* gene are located on chromosome 5q: telomeric *SMN1* and centromeric *SMN2*. The latter differs from *SMN1* by a single C > T nucleotide substitution within the coding region, precisely within exon 7 (c.840 C > T) [3], which causes alternative splicing of *SMN2* and the removal of exon 7 from 90% of *SMN2* mature mRNAs [4]. The SMN protein that lacks exon 7 (SMNΔ7) is not functional, has a reduced ability to oligomerize and is susceptible to rapid degradation [5]. Only 10% of *SMN2* mRNAs retain exon 7 and produce functional SMN

Mol Neurobiol (2018) 55:2617–2630
DOI 10.1007/s12035-017-0537-z

MicroRNA Metabolism and Dysregulation in Amyotrophic Lateral Sclerosis

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Received: 9 February 2017 / Accepted: 7 April 2017 / Published online: 18 April 2017

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

Paola Rinchetti and Mafalda Rizzuti are co-first authors.

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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],

Received: 16 March 2017 | Revised: 9 May 2018 | Accepted: 9 May 2018

DOI: 10.1002/jnr.24263

RESEARCH ARTICLE

WILEY

Purkinje cell COX deficiency and mtDNA depletion in an animal model of spinocerebellar ataxia type 1

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Funding information
Fondazione Cariplo Grant 2014-1010 (to Dario Ronchi and Associazione Amici del Centro Dino Ferrari—University of Milan)

Abstract

Spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of cerebellar degenerative disorders, characterized by progressive gait unsteadiness, hand incoordination, and dysarthria. Ataxia type 1 (SCA1) is caused by the expansion of a CAG trinucleotide repeat in the SCA1 gene resulting in the atypical extension of a polyglutamine (polyQ) tract within the ataxin-1 protein. Our main objective was to investigate the mitochondrial oxidative metabolism in the cerebellum of transgenic SCA1 mice. SCA1 transgenic mice develop clinical features in the early life stages (around 5 weeks of age) presenting pathological cerebellar signs with concomitant progressive Purkinje neuron atrophy and relatively little cell loss; this evidence suggests that the SCA1 phenotype is not the result of cell death per se, but a possible effect of cellular dysfunction that occurs before neuronal demise. We studied the mitochondrial oxidative metabolism in cerebellar cells from both homozygous and heterozygous transgenic SCA1 mice, aged 2 and 6 months. Histochemical examination showed a cytochrome-c-oxidase (COX) deficiency in the Purkinje cells (PCs) of both heterozygous and homozygous mice, the oxidative defect being more prominent in older mice, in which the percentage of COX-deficient PC was up to 30%. Using a laser-microdissector, we evaluated the mitochondrial DNA (mtDNA) content on selectively isolated COX-competent and COX-deficient PC by quantitative Polymerase Chain Reaction and we found mtDNA depletion in those with oxidative dysfunction. In conclusion, the selective oxidative metabolism defect observed in neuronal PC expressing mutant ataxin occurs as early as 8 weeks of age thus representing an early step in the PC degeneration process in SCA1 disease.

KEYWORDS

laser microdissector, mitochondria, mitochondrial DNA depletion, oxidative damage, Purkinje cell, spinocerebellar ataxia type 1, transgenic mice

1 | INTRODUCTION

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant inherited neurodegenerative disease characterized by neurodegeneration in multiple central nervous system (CNS) regions, including spinal cord, brain stem, and cerebellum (Zoghbi & Orr, 1995).

The disease is associated with an unstable trinucleotide CAG repeat expansion in the open reading frame of the ataxin-1 gene. This specific CAG repeat expansion leads to the expression of an expanded polyglutamine (polyQ) tract in the mutant ataxin-1 protein (ATXN1), thereby acquiring a toxic gain-of-function property (Orr, 2012; Zoghbi, 1995; Zoghbi & Orr, 2009).

SCIENTIFIC REPORTS

OPEN

MicroRNA expression analysis identifies a subset of downregulated miRNAs in ALS motor neuron progenitors

Received: 28 February 2018
Accepted: 18 June 2018
Published online: 04 July 2018

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Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder that is characterized by a progressive degeneration of motor neurons (MNs). The pathomechanism underlying the disease is largely unknown, even though increasing evidence suggests that RNA metabolism, including microRNAs (miRNAs) may play an important role. In this study, human ALS induced pluripotent stem cells were differentiated into MN progenitors and their miRNA expression profiles were compared to those of healthy control cells. We identified 15 downregulated miRNAs in patients' cells. Gene ontology and molecular pathway enrichment analysis indicated that the predicted target genes of the differentially expressed miRNAs were involved in neurodegeneration-related pathways. Among the 15 examined miRNAs, miR-34a and miR504 appeared particularly relevant due to their involvement in the p53 pathway, synaptic vesicle regulation and general involvement in neurodegenerative diseases. Taken together our results demonstrate that the neurodegenerative phenotype in ALS can be associated with a dysregulation of miRNAs involved in the control of disease-relevant genetic pathways, suggesting that targeting entire gene networks can be a potential strategy to treat complex diseases such as ALS.

Amyotrophic lateral sclerosis (ALS) is the most common and severe form of motor neuron disease (MND) in adults[1]. It is a fatal neurodegenerative disorder that affects motor neurons (MNs) leading to progressive muscle weakness and atrophy. Death usually occurs within 3–5 years after diagnosis due to respiratory failure[1,2]. Currently, due to the complexity of its etiopathogenesis and poor knowledge, there is no effective treatment and patients can rely only on supportive care and on Riluzole and Edaravone, the only two drugs approved for ALS treatment, which modestly prolong patient survival[3].

The pathomechanisms underlying the disease are multifactorial and due to a complex interplay between genetics and environmental components, such as toxic exposure, diet and circulating inflammatory cytokines[4]. Patients without a familial history are generally recognized as sporadic (sALS) and account for the majority of cases, while familial forms of the disease (fALS) represent only 10% of clinical records[5]. To date, the most relevant genes associated with the disease are *C9ORF72, SOD1, TARDBP* and *FUS*, though several mutations in other genes have been reported to be involved in ALS pathogenesis[6,7].

Currently, RNA pathway dysregulation appears to be a major contributor to ALS etiopathogenesis. Indeed, mutations in *C9ORF72*, which is the most common gene associated with ALS, lead to a toxic mRNA gain of function through RNA foci formation, and the subsequent sequestration and altered activity of RNA-binding proteins (RBPs)[8]. TDP-43 and FUS are also deeply involved in RNA metabolism. In a pathological context, such as cellular stress, the association between TDP-43, FUS and the mRNA can lead to aberrant phosphorylation, ubiquitination and the aggregation of proteins, as well as formation of stress granules (SGs)[9]. Aggregated RBPs are sequestered

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doi:10.1093/brain/awx302 BRAIN 2018: 141; 1–3 | e4

BRAIN
A JOURNAL OF NEUROLOGY

LETTER TO THE EDITOR

Reply: *DGUOK* recessive mutations in patients with CPEO, mitochondrial myopathy, parkinsonism and mtDNA deletions

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Sir,

We read with interest the letter from Caporali *et al.* (2017) reporting three novel patients from two independent families displaying adult onset mitochondrial disorders due to recessive *DGUOK* mutations, confirming the findings of our original paper (Ronchi *et al.*, 2012). Since then, the molecular screening of *DGUOK* in our cohort of patients featuring the accumulation of multiple mitochondrial DNA (mtDNA) deletions in skeletal muscle has not detected additional cases. However, the use of traditional and next generation sequencing (NGS) has expanded the number of variants in canonical and novel genes (Kornblum *et al.*, 2013; Ronchi *et al.*, 2013, 2015; Reyes *et al.*, 2015) associated with impaired muscle mtDNA maintenance (Table 1). *DGUOK* mutations now represent the 4.7% cause of disease in our cohort of patients and, considering the novel cases described by Caporali *et al.* (2017), they account for 6.9% of adult patients with mtDNA maintenance disorders in which a molecular diagnosis has been established (the third most frequent molecular defect after mutations in *POLG* and *PEO1/TWNK* encoding, respectively, for the mtDNA polymerase POLG and the helicase TWINKLE).

Clinical features of the novel probands include progressive bilateral ptosis followed by muscle weakness in upper and lower limbs. Serum creatine kinase levels were found to be increased in two patients and reached large amounts episodically in Patient 3, similar to one of our cases. Interestingly the same patient displayed visual, auditory and CNS involvement. Neuroimaging demonstrated a diffuse hypometabolism in basal ganglia and extending bilaterally to the occipital cortex, as observed in other mitochondrial encephalomyopathies (Martikainen *et al.*, 2016). DATscan showed a bilateral reduction of basal ganglia uptake. Consistently, clinical features of this patient included rigidity and bradykinesia but parkinsonism seems an isolated finding since it was not observed in other *DGUOK* patients. Nevertheless, mutations in *DGUOK* add to the group of defects impairing mtDNA maintenance found to segregate with parkinsonism, which include *POLG* (Miguel *et al.*, 2014), *POLG2* (Van Maldergem *et al.*, 2016), *PEO1/TWNK* (Kiferle *et al.*, 2013), *OPA1* (Carelli *et al.*, 2015), *MPV17* (Garone *et al.*, 2012). Nigrostriatal dysfunction has been observed *in vivo* (Tzoulis *et al.*, 2016) and *ex vivo* (Tzoulis *et al.*, 2013) in patients harbouring *POLG* and *PEO1* mutations as well as in transgenic mice selectively expressing mutant forms of these enzymes in striatal midbrain neurons (Song *et al.*, 2012; Perier *et al.*, 2013). In this regard, the massive accumulation of mtDNA deletions in the proband presenting parkinsonism supports the hypothesis that mtDNA homeostasis is essential for dopaminergic survival.

The authors evaluated mtDNA content and integrity by using standard techniques as well as digital PCR, an

Advance Access publication December 8, 2017

JAMA Neurology | Original Investigation

Interpreting Genetic Variants in Titin in Patients With Muscle Disorders

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IMPORTANCE Mutations in the titin gene (*TTN*) cause a wide spectrum of genetic diseases. The interpretation of the numerous rare variants identified in *TTN* is a difficult challenge given its large size.

OBJECTIVE To identify genetic variants in titin in a cohort of patients with muscle disorders.

DESIGN, SETTING, AND PARTICIPANTS In this case series, 9 patients with titinopathy and 4 other patients with possibly disease-causing variants in *TTN* were identified. Titin mutations were detected through targeted resequencing performed on DNA from 504 patients with muscular dystrophy, congenital myopathy, or other skeletal muscle disorders. Patients were enrolled from 10 clinical centers in April 2012 to December 2013. All of them had not received a diagnosis after undergoing an extensive investigation, including Sanger sequencing of candidate genes. The data analysis was performed between September 2013 and January 2017. Sequencing data were analyzed using an internal custom bioinformatics pipeline.

MAIN OUTCOMES AND MEASURES The identification of novel mutations in the *TTN* gene and novel patients with titinopathy. We performed an evaluation of putative causative variants in the *TTN* gene, combining genetic, clinical, and imaging data with messenger RNA and/or protein studies.

RESULTS Of the 9 novel patients with titinopathy, 5 (55.5%) were men and the mean (SD) age at onset was 25 (15.8) years (range, 0-46 years). Of the 4 other patients (3 men and 1 woman) with possibly disease-causing *TTN* variants, 2 (50%) had a congenital myopathy and 2 (50%) had a slowly progressive distal myopathy with onset in the second decade. Most of the identified mutations were previously unreported. However, all the variants, even the already described mutations, require careful clinical and molecular evaluation of probands and relatives. Heterozygous truncating variants or unique missense changes are not sufficient to make a diagnosis of titinopathy.

CONCLUSIONS AND RELEVANCE The interpretation of *TTN* variants often requires further analyses, including a comprehensive evaluation of the clinical phenotype (deep phenotyping) as well as messenger RNA and protein studies. We propose a specific workflow for the clinical interpretation of genetic findings in titin.

Editorial page 539

Supplemental content

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JAMA Neurol. 2018;75(5):557-565. doi:10.1001/jamaneurol.2017.4899
Published online February 12, 2018. Corrected on August 6, 2018.

should be used to assess the presence of large deletions or duplications[39] in unsolved cases. Western blotting is an effective strategy, although with well-recognized limitations. Inframe deletions, the skipping of inframe exons or truncating variants in exons not expressed in the adult muscles, and small size variations would still not be recognizable by a titin Western blot.

Several recent studies suggest that heterozygous titin truncating variants cause dominant dilated cardiomyopathy.[40,41] However, a positional effect and an incomplete and age-dependent penetrance (probably related to other genetic or environmental factors) may explain the lack of any cardiac symptoms in some individuals with mono or biallelic PTVs (eg, patient V and VIII).[41] A systematic follow-up to evaluate the cardiac status of such individuals, as well as their asymptomatic relatives who carry truncating variants, is highly recommended.

Missense Variants

The clinical significance of missense variants in *TTN* represents a major issue related to NGS investigation in the field of neuromuscular disorders.[5] A WB analysis is not effective in the presence of missense variants, as demonstrated in cases IX and X. The evaluation of *TTN* missense variants should reflect the current genetic guidelines.[42] A segregation analysis and/or in silico predictions can only suggest a pathogenic or a noncausative effect of a missense variant.[42]

In the presence of monoallelic truncating variants, as well as of missense variants, the possible causative effect of mutations in genes other than titin has to be ruled out and the presence of the aforementioned key clinical points has to be assessed by deep phenotyping.

However, the definitive proof of pathogenicity for missense variants can only be established by functional tests, segregation studies in very large families, and/or identifying unrelated patients or families with the same mutations. The interpretation of *TTN* missense variants may also benefit from the establishment of clinical and research consortia able to combine cohorts of patients into larger groups.[43]

Limitations

Our study has limitations. First, we enrolled, in a multicenter study, patients with clinically and genetically heterogenous conditions and specific clinical studies (magnetic resonance imaging or cardiac tests) were unavailable or not performed for some patients.

Second, we report missense variants with an unconfirmed causative role (cases IX and X). Although further studies are needed to attribute causality to missense changes, reporting possible causative variants is an effective strategy to improve consistency in the interpretation of molecular findings in titin. Ultimately, the proposed workflow is meant for interpreting titin variants in a mendelian disorder. The possible role of titin variants as modifiers or within a digenic or multigenic disease is not discussed here.

Conclusions

An increasing number of rare, ultrarare, and private variants in the titin gene is detected in any sequencing approach, and NGS has dramatically expanded the spectrum of skeletal muscle disorders associated with causative mutations in *TTN*.[5] Our workflow results in a greater understanding and more consistent interpretation of titin variants by neurologists, pediatricians, and geneticists less familiar with the titin gene and titinopathies.

ARTICLE INFORMATION

Accepted for Publication: August 6, 2017.

Published Online: February 12, 2018. doi:10.1001/jamaneurol.2017.4899

Correction: This article was corrected online August 8, 2018, to correct Ms Ruggieri's degree.

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Author Contributions: Dr Savarese had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Udd and Nigro contributed equally to this work.
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Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Savarese, Maggi, Vihola, Jonson, Tasca, Bello, Giugliano, Di Fruscio, Vanakker, Rubegni, Santorelli, Udd, Nigro.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Savarese, Di Fruscio.
Obtained funding: Savarese, Angelini, Udd, Nigro.
Administrative, technical, or material support: Savarese, Vanakker, Vercelli, Janssens, Pasanisi, Raimondi, Politano, Moggio, Mongini, Comi, Mora, Udd.
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Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported by Telethon Foundation, Telethon-Unione Italiana Lotta alla Distrofia Muscolare, Association Française contre les

Available online at www.sciencedirect.com

ScienceDirect

Neuromuscular Disorders 28 (2018) 586–591

www.elsevier.com/locate/nmd

Targeted gene panel screening is an effective tool to identify undiagnosed late onset Pompe disease

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Received 4 December 2017; received in revised form 12 March 2018; accepted 29 March 2018

Abstract

Mutations in the *GAA* gene may cause a late onset Pompe disease presenting with proximal weakness without the characteristic muscle pathology, and therefore a test for *GAA* activity is the first tier analysis in all undiagnosed patients with hyperCKemia and/or limb-girdle muscular weakness. By using MotorPlex, a targeted gene panel for next generation sequencing, we analyzed *GAA* and other muscle disease-genes in a large cohort of undiagnosed patients with suspected inherited skeletal muscle disorders (n = 504). In this cohort, 275 patients presented with limb-girdle phenotype and/or an isolated hyperCKemia. Mutational analysis identified *GAA* mutations in ten patients. Further seven affected relatives were identified by segregation studies. All the patients carried the common *GAA* mutation c.-32-13T >G and a second, previously reported mutation. In the subcohort of 275 patients with proximal muscle weakness and/or hyperCKemia, we identified late-onset Pompe disease in 10 patients. The clinical overlap between Pompe disease and LGMDs or other skeletal muscle disorders suggests that *GAA* and the genes causing a metabolic myopathy should be analyzed in all the gene panels used for testing neuromuscular patients. However, enzymatic tests are essential for the interpretation and validation of genetic results.

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https://doi.org/10.1016/j.nmd.2018.03.011
0960-8966/

Molecular Neurobiology (2018) 55:6850–6862
https://doi.org/10.1007/s12035-018-0888-0

In Vivo Transient and Partial Cell Reprogramming to Pluripotency as a Therapeutic Tool for Neurodegenerative Diseases

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Received: 6 October 2017 / Accepted: 8 January 2018 / Published online: 20 January 2018

Abstract

In theory, human diseases in which a specific cell type degenerates, such as neurodegenerative diseases, can be therapeutically addressed by replacement of the lost cells. The classical strategy for cell replacement is exogenous cell transplantation, but now, cell replacement can also be achieved with in situ reprogramming. Indeed, many of these disorders are age-dependent, and "rejuvenating" strategies based on cell epigenetic modifications are a possible approach to counteract disease progression. In this context, transient and/or partial reprogramming of adult somatic cells towards pluripotency can be a promising tool for neuroregeneration. Temporary and controlled in vivo overexpression of Yamanaka reprogramming factors (Oct3/4, Sox2, Klf4, and c-Myc (OSKM)) has been proven feasible in different experimental settings and could be employed to facilitate in situ tissue regeneration; this regeneration can be accomplished either by producing novel stem/precursor cells, without the challenges posed by exogenous cell transplantation, or by changing the epigenetic adult cell signature to the signature of a younger cell. The risk of this procedure resides in the possible lack of perfect control of the process, carrying a potential oncogenic or unexpected cell phenotype hazard. Recent studies have suggested that these limits can be overcome by a tightly controlled cyclic regimen of short-term OSKM expression in vivo that prevents full reprogramming to the pluripotent state and avoids both tumorigenesis and the presence of unwanted undifferentiated cells. On the other hand, this strategy can enhance tissue regeneration for therapeutic purposes in aging-related neurological diseases as well. These data could open the path to further research on the therapeutic potential of in vivo reprogramming in regenerative medicine.

Keywords In vivo reprogramming · Rejuvenation · Tissue repair · Senescence · Aging · Yamanaka Factors · Progeria · Regenerative medicine

Introduction

In Vivo Reprogramming for Tissue Repair: General Ideas and Hypotheses

Neurodegenerative diseases are characterized by a progressive loss of specific neuronal cells that are not replaced due to the apparent inability of the central nervous system (CNS) to effectively regenerate, resulting in a major obstacle to self-repair. Cell replacement is a possible, even if still partially theoretical, approach to tackle cell degeneration such as that taking place in neurodegenerative diseases. So far, the standard strategy has been exogenous cell transplantation and consists of exogenous neural stem cell (NSC) transplantation. NSCs can be obtained from primary tissue such as the fetal CNS or can be differentiated from pluripotent stem cells, a new source for cell replacement therapy [1]. This process in not faultless because transplanting externally reprogrammed cells into the CNS is associated with many obstacles such as the invasiveness of the injection, risk of immunorejection, and poor functional integration into the receiving tissue. To overcome these challenges, several groups have recently proposed in vivo direct reprogramming as an alternative successful strategy in which endogenous glial cells can be reprogrammed into functional neurons within the brain and spinal cord for reparative purposes [2].

Thus, a proposed therapeutic strategy consists of reprogramming resident tissue-adult cells into the cell types that are lost due to disease by a process called in vivo lineage reprogramming [2–4].

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Molecular Therapy
Original Article

Rescue of GSDIII Phenotype with Gene Transfer Requires Liver- and Muscle-Targeted GDE Expression

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Glycogen storage disease type III (GSDIII) is an autosomal recessive disorder caused by a deficiency of glycogen-debranching enzyme (GDE), which results in profound liver metabolism impairment and muscle weakness. To date, no cure is available for GSDIII and current treatments are mostly based on diet. Here we describe the development of a mouse model of GSDIII, which faithfully recapitulates the main features of the human condition. We used this model to develop and test novel therapies based on adeno-associated virus (AAV) vector-mediated gene transfer. First, we showed that overexpression of the lysosomal enzyme alpha-acid glucosidase (GAA) with an AAV vector led to a decrease in liver glycogen content but failed to reverse the disease phenotype. Using dual overlapping AAV vectors expressing the GDE transgene in muscle, we showed functional rescue with no impact on glucose metabolism. Liver expression of GDE, conversely, had a direct impact on blood glucose levels. These results provide proof of concept of correction of GSDIII with AAV vectors, and they indicate that restoration of the enzyme deficiency in muscle and liver is necessary to address both the metabolic and neuromuscular manifestations of the disease.

INTRODUCTION

Glycogen storage disease type III (GSDIII) is a rare (incidence of 1 in 100,000 at birth)[1] autosomal recessive disorder caused by mutations in the *Agl* gene encoding for the glycogen-debranching enzyme (GDE or amylo-alpha-1,6-glucosidase, ExPASy: EC 3.2.1.33, UniProt: P35573). GDE is an enzyme with two catalytic sites involved in the conversion of cytosolic glycogen to glucose.[2]

The clinical manifestations of GSDIII are characterized by two phases: during childhood, the disease has mainly the features of a metabolic disorder with hepatomegaly and severe fasting hypoglycemia, hyperlipidemia, and hyperketonemia; and during adolescence and adulthood, a progressive debilitating myopathy, with a heterogeneous involvement of different muscle groups and exercise intolerance, appears, rendering the metabolic impairment less prominent.[2] GSDIII disease burden is important especially in patients who experience severe skeletal muscle weakness and exercise intolerance.[1,2] Histological analysis of muscle biopsies from GSDIII patients confirms the muscle involvement, and it shows the accumulation of glycogen in large vacuoles that disrupt the myofibrils architecture.[3] Additionally, most GSDIII patients have a cardiac involvement, although only a small percentage (15%) of them develops cardiomyopathy.[2] Additionally, liver complications, such as cirrhosis, development of hepatocellular adenomas (HCAs), and hepatocellular carcinomas (HCCs), have been described in a significant proportion of adult GSDIII patients.[2]

To date, the only therapeutic approach available for GSDIII is symptomatic.[2,4] During childhood, to avoid recurrent hypoglycemia, patients follow a strict diet regimen with frequent meals

Received 28 June 2017; accepted 20 December 2017;
https://doi.org/10.1016/j.ymthe.2017.12.019.

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LRP10 genetic variants in familial Parkinson's disease and dementia with Lewy bodies: a genome-wide linkage and sequencing study

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Summary

Background Most patients with Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies do not carry mutations in known disease-causing genes. The aim of this study was to identify a novel gene implicated in the development of these disorders.

Methods Our study was done in three stages. First, we did genome-wide linkage analysis of an Italian family with dominantly inherited Parkinson's disease to identify the disease locus. Second, we sequenced the candidate gene in an international multicentre series of unrelated probands who were diagnosed either clinically or pathologically with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies. As a control, we used gene sequencing data from individuals with abdominal aortic aneurysms (who were not examined neurologically). Third, we enrolled an independent series of patients diagnosed clinically with Parkinson's disease and controls with no signs or family history of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies from centres in Portugal, Sardinia, and Taiwan, and screened them for specific variants. We also did mRNA and brain pathology studies in three patients from the international multicentre series carrying disease-associated variants, and we did functional protein studies in in-vitro models, including neurons from induced pluripotent stem-like cells.

Findings Molecular studies were done between Jan 1, 2008, and Dec 31, 2017. In the initial kindred of ten affected Italian individuals (mean age of disease onset 59·8 years [SD 8·7]), we detected significant linkage of Parkinson's disease to chromosome 14 and nominated *LRP10* as the disease-causing gene. Among the international series of 660 probands, we identified eight individuals (four with Parkinson's disease, two with Parkinson's disease dementia, and two with dementia with Lewy bodies) who carried different, rare, potentially pathogenic *LRP10* variants; one carrier was found among 645 controls with abdominal aortic aneurysms. In the independent series, two of these eight variants were detected in three additional Parkinson's disease probands (two from Sardinia and one from Taiwan) but in none of the controls. Of the 11 probands from the international and independent cohorts with *LRP10* variants, ten had a positive family history of disease and DNA was available from ten affected relatives (in seven of these families). The *LRP10* variants were present in nine of these ten relatives, providing independent—albeit limited—evidence of co-segregation with disease. Post-mortem studies in three patients carrying distinct *LRP10* variants showed severe Lewy body pathology. Of nine variants identified in total (one in the initial family and eight in stage 2), three severely affected *LRP10* expression and mRNA stability (1424+5delG, 1424+5G→A, and Ala212Serfs*17, shown by cDNA analysis), four affected protein stability (Tyr307Asn, Gly603Arg, Arg235Cys, and Pro699Ser, shown by cycloheximide-chase experiments), and two affected protein localisation (Asn517del and Arg533Leu; shown by immunocytochemistry), pointing to loss of LRP10 function as a common pathogenic mechanism.

Interpretation Our findings implicate *LRP10* gene defects in the development of inherited forms of α-synucleinopathies. Future elucidation of the function of the LRP10 protein and pathways could offer novel insights into mechanisms, biomarkers, and therapeutic targets.

Funding Stichting ParkinsonFonds, Dorpmans-Wigmans Stichting, Erasmus Medical Center, ZonMw—Memorabel programme, EU Joint Programme Neurodegenerative Disease Research (JPND), Parkinson's UK, Avtal om Läkarutbildning och Forskning (ALF) and Parkinsonfonden (Sweden), Lijf and Leven foundation, and cross-border grant of Alzheimer Netherlands–Ligue Européene Contre la Maladie d'Alzheimer (LECMA).

Lancet Neurol 2018; 17: 597–608

Published Online June 7, 2018 http://dx.doi.org/10.1016/S1474-4422(18)30179-0

See Comment page 571

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For the second stage, we analysed samples and clinical data that had been obtained from an international series of unrelated probands with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies between Jan 1, 2000, and Dec 31, 2017. These participants were enrolled from the International Parkinsonism Genetics Network, the Netherlands Brain Bank at the Netherlands Institute of Neuroscience in Amsterdam (selected based on presence of α-synuclein-positive pathology), and the Laboratory of Neuropathology at the University of Bologna in Italy (appendix). We also included as a control whole exome sequencing data from a Dutch study of patients with abdominal aortic aneurysms (unpublished data). Data for neurological diseases were not available in that study.

For the third study stage, we enrolled an independent series of unrelated patients with clinically diagnosed Parkinson's disease and unaffected controls from centres in Portugal, Sardinia, and Taiwan. As controls, we included population-matched series of spouses of individuals with Parkinson's disease or unrelated individuals examined at the same centres with no signs or family history of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies.

We obtained written informed consent for use of clinical data and biological samples for this study from patients with a clinical diagnosis of Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies (or their next of kin, for patients with dementia) and unaffected relatives. For patients diagnosed pathologically from the Netherlands Brain Bank and the Laboratory of Neuropathology, written informed consent for brain autopsy and use of clinical information and material for research purposes had been obtained previously from the donor or from the next of kin. Participants in the abdominal aortic aneurysms study had provided written informed consent for use of whole exome sequencing data for genetic research. Relevant ethics authorities approved study protocols (appendix).

Procedures

We made clinical diagnoses of Parkinson's disease according to the UK Parkinson's Disease Society Brain Bank criteria.[17] We diagnosed Parkinson's disease dementia in patients developing dementia after 1 year from the onset of Parkinson's disease symptoms.[3] We based our clinical diagnosis of dementia with Lewy bodies on the third report of the Dementia with Lewy Body Consortium.[3]

In the first stage of the study, after confirming the absence of pathogenic mutations in genes causing autosomal-dominant Parkinson's disease (ie, *SNCA*, *LRRK2*, *VPS35*, and *CHCHD2*, as well as *GBA* variants; sequencing and multiplex ligation-dependent probe amplification [MLPA] protocols are reported in the appendix), we did genome-wide single nucleotide polymorphism (SNP) array genotyping in ten affected relatives from the Italian family. We also ran a parametric multipoint linkage analysis, assuming an autosomal-dominant mode of inheritance, and we did copy number analysis with Nexus Copy Number (appendix). We did whole exome sequencing in the index patient. We annotated variants with Annovar (version 2016Feb01)[18] and the Mendelian Clinically Applicable Pathogenicity (M-CAP) score.[19] We then filtered variants located within the linkage interval using the following criteria: (1) the variant being present in the heterozygous state; (2) rarity, defined as a minor allele frequency (MAF) less than 0·1% by the Exome Aggregation Consortium (ExAC), dbSNP, the National Heart, Lung, and Blood Institute's Exome Sequencing Project exome variant server, Genome of the Netherlands (GoNL), and the genome aggregation database (gnomAD); (3) exonic and non-synonymous, or predicted to affect splicing in silico; and (4) pathogenicity, defined as being predicted as pathogenic by at least five of 11 in-silico tools (appendix). This work led to nomination of *LRP10* (low-density lipoprotein receptor-related protein 10) as the candidate disease-causing gene in the Italian family. We used Sanger sequencing for validation and co-segregation analysis in all members of this family for whom DNA was available.

In the second stage of the study, we sequenced the entire *LRP10* open reading frame and exon–intron boundaries in 660 unrelated probands with Parkinson's disease, Parkinson's disease dementia, or dementia with Lewy bodies. We did Sanger sequencing in 659 participants and whole exome sequencing in one. Protocols and primers are detailed in the appendix. We judged of interest variants fulfilling the same criteria mentioned in the first study stage. We used Sanger sequencing for co-segregation analysis when DNA from additional relatives was available. We also searched for *LRP10* variants (entire coding region and exon–intron boundaries) in the whole exome sequencing database of individuals from the abdominal aortic aneurysm study (average *LRP10* depth coverage 100·7 times). We included variants fulfilling the same, above-specified, criteria and compared their frequency with that in our series of 660 patients with Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies.

In stage three of the study, we used high-resolution melting analysis to study three of the identified *LRP10* variants in the independent population-matched series of patients and controls from Portugal, Sardinia, and Taiwan. We also used Sanger sequencing and MLPA to analyse genes causing autosomal-dominant Parkinson's disease (ie, *SNCA*, *LRRK2*, *VPS35*, and *CHCHD2*, and the risk gene *GBA*) in all probands from the second and third study stages in whom *LRP10* variants were identified (appendix).

For pathological analyses, we obtained autopsy tissue blocks from 23 different brain regions in three patients from the international multicentre series of unrelated

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See **Online** for appendix

For the **Netherlands Brain Bank** see http://www.brainbank.nl

Clinical Reasoning: A 75-year-old man with parkinsonism, mood depression, and weight loss

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Neurology® 2018;90:572-575. doi:10.1212/WNL.0000000000005177

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Section 1

A 75-year-old man presented to the emergency department with a 1-year history of 66-pound weight loss and alternating bowel habits. He was admitted to the hospital, where he underwent several examinations to investigate the presence of a malignancy. A colonoscopy, a gastroscopy, an ultrasound of the abdomen, and a contrast-enhanced CT scan of thorax and abdomen did not detect any neoplasia. The only findings consisted of a prostatic hypertrophy and a basal pleural–parenchymal hyperdensity in the left lung, which was described as the result of an infective process. Neoplastic markers CA19.9, carcinoembryonic antigen, neuron-specific enolase, and α-fetoprotein were also negative. Wide-spectrum blood tests were unremarkable, except for hypogammaglobulinemia and elevated β2 microglobulin.

Upon a more thorough collection of the patient's history and examination, progressive stiffness and slowness of movements were reported to have appeared a few months before, along with a longer history of mood depression, apathy, hyporexia, hyposmia, constipation, micrographia, dysphagia, hypophonia, and sleep disturbances including sleeplessness and REM sleep behavior disorder.

A neurologic examination of the motor system revealed a symmetric and severe plastic hypertonia in the trunk and all 4 extremities with cogwheel rigidity at the wrists and marked bradykinesia. In the cranial district, hypomimia, hypophonia, and upward gaze limitation were noticed. Deep tendon reflexes were normal on all 4 limbs. No tremor was recorded. Inducible small-amplitude polyminimyoclonus could be elicited in the upper extremities. The patient could stand aided and walked a few steps with a slow and normal-base gait. Findings on the remainder of the neurologic examination were unremarkable. Family history was negative for neurodegenerative diseases, although a sister had multiple sclerosis and a daughter had depression.

Questions for consideration:

1. What are the main neurologic systems involved?
2. What medical conditions could be considered in the differential diagnosis?

GO TO SECTION 2

From the Dino Ferrari Center, Neuroscience Section, Department of Pathophysiology and Transplantation (E.F., E.M., G.B., N.B., M.C.S., A.D.F.), Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre (B.F.), and Internal Medicine, Department of Pathophysiology and Transplantation (S.A.), IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy.

Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Internal and Emergency Medicine
https://doi.org/10.1007/s11739-018-1871-5

IM - CASE RECORD

Syncope and autonomic failure in a middle-aged man

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Received: 13 January 2018 / Accepted: 6 May 2018

Colombo, Solbiati (internal medicine)

An Albanian 54-year-old man presented to the Emergency Department (ED) after a transient loss of consciousness that occurred while walking in the street. The episode was preceded by dizziness, vertigo and palpitations, and was followed by fatigue and paraesthesia in the right arm and leg. A long-standing history (1–2 years) of lower limb fatigue with progressive walking problems, slow speech rate, confusion and severe weight loss (about 70 kg) was reported. The patient also described previous syncopal episodes similar to the present one. A patient's friend, who had not seen him in years, noticed that the patient was more confused and slow in moving and speaking than what he recalled.

His past medical history was positive for type-2 diabetes, visual impairment in the left eye and a previous vitreous haemorrhage. The family medical history was unremarkable.

On admission to the Internal Medicine Unit, the patient was alert, attentive and partially oriented, with no sign of dehydration, hypo-perfusion or congestive heart failure. Chest and abdomen physical examinations were normal. Blood pressure was 95/50 mmHg in the supine position, and the systolic blood pressure dropped to 65 mmHg while standing. Heart rate was 78 beats/min and regular, peripheral oxygen saturation was 96% in room air and body temperature was 36 °C. Neurological examination showed slurred speech, severe loss of muscle mass with diffuse limb fasciculations, diffuse absence of tendon reflexes, no response to plantar cutaneous stimulation, distal anaesthesia, postural tremor along with lower limb weakness, ataxic gait, bilateral foot drop and postural instability, distal anaesthesia for epicritic and proprioceptive sensibility without any impairment in the cranial nerves.

Routine blood tests and chest X-ray study were normal. The ECG showed low voltages in both precordial and limb leads. The brain computed tomography was normal.

Preliminary investigations

Colombo, Solbiati, Ceriani (internal medicine)

The transient loss of consciousness was interpreted as syncope due to orthostatic hypotension. Considering the patient history of recurrent syncopal episodes and the abnormal ECG, 72-h ECG telemetry and a trans-thoracic echocardiography were performed. The monitoring was negative for significant dysrhythmic events, and the echocardiography showed left ventricular diastolic dysfunction and hypertrophy, especially at the interventricular septum (14 mm), which had a sparkling appearance.

Orthostatic hypotension along with muscular hypotrophy, widespread absence of tendon reflexes and loss of sensitivity suggested a problem of the peripheral nervous system. In agreement with our consultant neurologists, we obtained an electromyography: it showed a severe chronic and active axonal polyneuropathy. To deepen the differential diagnosis of the polyneuropathy that was the leading condition at that moment, we asked our neurologist for a consultation.

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Published online: 15 May 2018

Current Neurology and Neuroscience Reports (2018) 18: 37
https://doi.org/10.1007/s11910-018-0847-1

MOVEMENT DISORDERS (S FOX, SECTION EDITOR)

Genetics of Movement Disorders and the Practicing Clinician; Who and What to Test for?

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Published online: 23 May 2018

Abstract

Purpose of Review This review aims to provide the basic knowledge on the genetics of hypokinetic and hyperkinetic movement disorders to guide clinicians in the decision of "who and what to test for?"

Recent Findings In recent years, the identification of various genetic causes of hypokinetic and hyperkinetic movement disorders has had a great impact on a better definition of different clinical syndromes. Indeed, the advent of next-generation sequencing (NGS) techniques has provided an impressive step forward in the easy identification of genetic forms. However, this increased availability of genetic testing has challenges, including the ethical issue of genetic testing in unaffected family members, "commercially" available home testing kits and the increasing number and relevance of "variants of unknown significance."

Summary The emergent role of genetic factors has important implications on clinical practice and counseling. As a consequence, it is fundamental that practicing neurologists have a proper knowledge of the genetic background of the diseases and perform an accurate selection of who has to be tested and for which gene mutations.

Keywords Genetics · Movement disorders · Next-generation sequencing · Parkinson's disease · Dystonia · Chorea

Introduction

In the last 20 years, the identification of genetic causes of movement disorders has had a significant impact on the comprehension of pathological mechanisms and on a better definition of different clinical syndromes. Moreover, the advent of next-generation sequencing (NGS) techniques has provided an impressive step forward in the ease of identification of several genetic forms of hypokinetic and hyperkinetic movement disorders [1].

The emergent role of genetic factors also has important implications for clinical practice and counseling.

This article is part of the Topical Collection on *Movement Disorders*

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Genetic testing is a very complex subject, which involves clinicians, geneticists, patients, and their families. Clinical genetic testing in patients or relatives can serve a variety of purposes. If causal or symptomatic treatments depend on the molecular diagnosis, the purpose of genetic analysis is evident (e.g. chelating therapy in Wilson's disease). However, even if there are no clear therapeutic consequences, a patient can still benefit from genetic diagnosis. For example, defining the molecular etiology of the disease can be a reassuring final explanation of the signs and symptoms for the patient and can put an end to a long and painful series of medical visits and expensive diagnostic tests. Life or family planning could be another reasonable cause to perform a genetic test, in particular if a highly penetrant dominant or X-linked pattern of inheritance is present. In the clinical setting, genetic testing should be performed only if there is a clear and informed wish of the patient, always following genetic counseling. Therefore, patients or people- at-risk should be comprehensively informed and counseled about the possibility of transmission, penetrance, expressivity, and available therapeutic solutions [2].

It is important to highlight that genetic analysis in the research setting—with or without disclosure of the test results—can be performed in every patient who gives appropriate

Neurological Sciences (2018) 39:733–739
https://doi.org/10.1007/s10072-018-3272-y

ORIGINAL ARTICLE

Real life evaluation of safinamide effectiveness in Parkinson's disease

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Received: 15 November 2017 / Accepted: 1 February 2018 / Published online: 13 February 2018

Abstract

In this retrospective study, we evaluated both efficacy and effectiveness of safinamide 50 and 100 mg in the treatment of motor fluctuations and disabling dyskinesias in a cohort of patients with idiopathic Parkinson's disease (PD). Ninety-one PD patients were evaluated during the first year of commercialization of the drug, both prior to starting safinamide and at the last available follow-up. Evaluations were based on the Unified Parkinson's Disease Scale part III (UPDRS III), Hoehn & Yahr (HY), Unified Dyskinesia Rating Scale (UDysRS) walking and balance item 9 score, daily time spent in OFF and in ON with disabling dyskinesias (1 week diary), mean daily dose of levodopa (LD), dopamine-agonists (DA), catechol-O-methyl transferase inhibitor (COMT-I), monoamine oxidase B inhibitor (MAOB-I), and their LD equivalent dose (LEDD). Eight patients withdrew safinamide within the first month for minor side effects. At the follow-up evaluation, after a mean time with safinamide of 7.5 months ± 3.4, all patients showed a significant improvement of all the scale scores, except for HY, and of the daily dosages of the drugs and the LEDD. The same results were shown by PD patients treated with safinamide 50 mg and patients who started safinamide without switching from a previous MAOBI. PD patients with safinamide 100 mg and patients who started safinamide switching from a previous MAOBI significantly improved in time spent in OFF and LEDD. In conclusion, safinamide is safe and effective in improving motor complications in patients with idiopathic PD and can be considered a useful levodopa sparing strategy.

Keywords Parkinson's disease · Motor fluctuations · Dyskinesias · Safinamide

Introduction

Parkinson's disease (PD) is the world's second most spread chronic neurodegenerative disorder of the elderly. It rarely occurs before the age of 50 and a sharp increase in incidence is reported after the age of 60 [1, 2]. PD is traditionally defined as a progressive disorder characterized by the triad of rigidity, bradykinesia, and tremor accompanied by several non-motor symptoms that show a nonlinear progression during the course of the disease [3]. The pathogenetic changes include the loss of dopaminergic neurons in the substantia nigra pars compacta and the appearance of Lewy bodies within the pigmented neurons of the SN. It is presumed that motor symptoms occur at a loss of about 60–80% of the dopaminergic neurons in the SN. Non-dopaminergic neurotransmitter systems, such as the serotonergic, cholinergic, adrenergic, and glutamatergic are also involved in the pathophysiological of the disease [4].

Levodopa (LD) is still the gold standard of symptomatic efficacy on PD symptoms. Since the amount of its daily dose is associated with the development of motor complications

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RESEARCH PAPER

CSF β-amyloid and white matter damage: a new perspective on Alzheimer's disease

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Received 6 June 2017
Revised 13 September 2017
Accepted 28 September 2017
Published Online First 20 October 2017

ABSTRACT

Objective To assess the connection between amyloid pathology and white matter (WM) macrostructural and microstructural damage in demented patients compared with controls.

Methods Eighty-five participants were recruited: 65 with newly diagnosed Alzheimer's disease (AD), non-AD dementia or mild cognitive impairment and 20 age-matched and sex-matched healthy controls. β-amyloid$_{1-42}$ (Aβ) levels were determined in cerebrospinal fluid (CSF) samples from all patients and five controls. Among patients, 42 had pathological CSF Aβ levels (Aβ(+)), while 23 had normal CSF Aβ levels (Aβ(−)). All participants underwent neurological examination, neuropsychological testing and brain MRI. We used T2-weighted scans to quantify WM lesion loads (LLs) and diffusion-weighted images to assess their microstructural substrate. Non-parametric statistical tests were used for between-group comparisons and multiple regression analyses.

Results We found an increased WM-LL in Aβ(+) compared with both, healthy controls (p=0.003) and Aβ(−) patients (p=0.02). Interestingly, CSF Aβ concentration was the best predictor of patients' WM-LL (r=−0.30, p<0.05) when using age as a covariate. Lesion apparent diffusion coefficient value was higher in all patients than in controls (p=0.0001) and correlated with WM-LL (r=0.41, p=0.001). In Aβ(+), WM-LL correlated with WM microstructural damage in the left peritrigonal WM (p<0.0001).

Conclusions WM damage is crucial in AD pathogenesis. The correlation between CSF Aβ levels and WM-LL suggests a direct link between amyloid pathology and WM macrostructural and microstructural damage.

INTRODUCTION

In patients with Alzheimer's disease (AD), MRI often shows focal hyperintensities in the deep and subcortical white matter (WM).[1–5] Their nature remains unclear: the main hypothesis considers them as chronic ischaemic lesions caused by cerebral microangiopathy,[6 7] while neuropathological studies show evidence of demyelination and axonal loss.[5 8] Thus, other mechanisms could be implicated, including blood–brain barrier leakage, inflammation, neurodegeneration and amyloid angiopathy.[5] A direct link between WM hyperintensities (WMHs) and the severity of cognitive decline has already been demonstrated in literature.[9 10] The incidence of WMHs is higher in patients with AD,[11–13] vascular dementia (VaD),[14] dementia with Lewy body[14] and frontotemporal dementia (FTD)[15] (including some inherited forms of FTD).[16–18] Moreover, the presence of WMHs seems to increase the risk for conversion from mild cognitive impairment (MCI) to AD and to predict the progression of cognitive symptoms.[10 11 19 20] Diffusion-weighted imaging (DWI) studies have demonstrated the presence of WM microstructural changes in AD brains at preclinical stages.[3] In our study, we chose to use apparent diffusion coefficient (ADC) maps, obtained from DWI scans, as metrics to evaluate the integrity of WM at microscopic level. The Dominantly Inherited Alzheimer Network analysed the severity and distribution of WMHs in presymptomatic presenilin 1, presenilin 2 and amyloid precursor protein mutation carriers, investigating the extent to which WMHs manifest genetically predisposed individuals.[21] This study found that WMHs are elevated well before symptom onset, suggesting that WMHs are a core feature of AD pathogenesis.[21]

Against this background, the contribution of WMHs to AD pathogenesis is still debated, and WMHs are mostly considered as a comorbidity rather than part of AD pathophysiology.[4 10 21]

To the best of our knowledge, only few data are available in literature on the relationship between measures of macrostructural and microstructural WM damage and cerebrospinal fluid (CSF) biomarkers of neurodegeneration. Kalheim and colleagues reported a remarkable extent of WM microstructrual damage in patients with MCI who showed pathological CSF levels of β-amyloid$_{1-42}$ (Aβ).[22] Additionally, an elegant paper by Dean III *et al* has contributed in clarifying the relationship between amyloid pathology and myelin alteration in preclinical AD.[23] Measuring whole-brain longitudinal and transverse relaxation times and the myelin water fraction (MWF), a significantly negative relationship between MWF and CSF Aβ levels was observed. Concerning inherited forms of AD, Lee and colleagues[21] reported a correlation between WMHs and CSF Aβ levels. Finally, Noh and colleagues,[24] using 11C-Pittsburgh compound B positron emission tomography (PET) imaging, demonstrated an association between WMH extension and amyloid burden.

► http://dx.doi.org/10.1136/jnnp-2017-317053

To cite: Pietroboni AM, Scarioni M, Carandini T, *et al*. *J Neurol Neurosurg Psychiatry* 2018;**89**:352–357.

Journal of Alzheimer's Disease 61 (2018) 47–52
DOI 10.3233/JAD-170712
IOS Press

Short Communication

Word and Picture Version of the Free and Cued Selective Reminding Test (FCSRT): Is There Any Difference?

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Handling Associate Editor: Marco Bozzali

Accepted 31 August 2017

Abstract. The Free and Cued Selective Reminding Test (FCSRT) is the most commonly used neuropsychological test to evaluate episodic memory. Two variants of FCSRT exist, using the recall of words (FCSRT-w) or pictures (FCSRT-p). Fourteen patients with mild cognitive impairment underwent neuropsychological evaluation and brain magnetic resonance. We found differences in FCSRT-w and FCSRT-p variants scores. FCSRT-p was correlated with atrophy in areas involved in visual stimuli processing while FCSRT-w was correlated to hippocampal atrophy. Our study suggests that FCSRT-w and FCSRT-p scores are not equivalent, but a larger cohort of patients is needed to validate these results.

Keywords: Atrophy, dementia, magnetic resonance imaging, memory, neuropsychology

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia worldwide. Although AD may have protean manifestations, an early and significant impairment in episodic memory is the main clinical feature and represents a core criterion for the diagnosis of AD [1]. Evaluation of episodic memory impairment is therefore essential in the diagnostic work-up and several neuropsychological tests have been developed in order to optimize diagnostic accuracy.

In clinical practice, the Free and Cued Selective Reminding Test (FCSRT) is the most commonly used neuropsychological test to evaluate episodic memory and to predict AD development in patients with mild cognitive impairment (MCI) [2]. Patients are asked to

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Neurobiology of Aging 62 (2018) 191–196

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Patterns of gray matter atrophy in genetic frontotemporal dementia: results from the GENFI study

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ARTICLE INFO

Article history:
Received 24 April 2017
Received in revised form 8 October 2017
Accepted 10 October 2017
Available online 19 October 2017

Keywords:
Frontotemporal dementia
Magnetic resonance imaging
Atrophy
Voxel-based morphometry
Preclinical dementia

ABSTRACT

Frontotemporal dementia (FTD) is a highly heritable condition with multiple genetic causes. In this study, similarities and differences of gray matter (GM) atrophy patterns were assessed among 3 common forms of genetic FTD (mutations in *C9orf72*, *GRN*, and *MAPT*). Participants from the Genetic FTD Initiative (GENFI) cohort with a suitable volumetric T1 magnetic resonance imaging scan were included (319): 144 nonmutation carriers, 128 presymptomatic mutation carriers, and 47 clinically affected mutation carriers. Cross-sectional differences in GM volume between noncarriers and carriers were analyzed using voxel-based morphometry. In the affected carriers, each genetic mutation group exhibited unique areas of atrophy but also a shared network involving the insula, orbitofrontal lobe, and anterior cingulate. Presymptomatic GM atrophy was observed particularly in the thalamus and cerebellum in the *C9orf72* group, the anterior and medial temporal lobes in *MAPT*, and the posterior frontal and parietal lobes as well as striatum in *GRN*. Across all presymptomatic carriers, there were significant decreases in the anterior insula. These results suggest that although there are important differences in atrophy patterns for each group (which can be seen presymptomatically), there are also similarities (a fronto-insula-anterior cingulate network) that help explain the clinical commonalities of the disease.

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https://doi.org/10.1016/j.neurobiolaging.2017.10.008

Journal of Alzheimer's Disease 61 (2018) 1289–1294
DOI 10.3233/JAD-170861
IOS Press

Short Communication

Profiling of Specific Gene Expression Pathways in Peripheral Cells from Prodromal Alzheimer's Disease Patients

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Accepted 15 November 2017

Abstract. Herein, we performed a gene expression profiling in a cohort of 10 mild cognitive impairment (MCI), subdivided, according to the analysis of cerebrospinal fluid biomarkers, in prodromal Alzheimer's disease (AD) and non-AD MCI, as compared with 27 AD patients and 24 controls, in order to detect early gene expression alterations. We observed a significant upregulation of *insulin (INS*) and *INS Receptor (INSR)* expression levels in AD both prodromal and fully symptomatic, as compared with controls, but not in MCI subjects. Our results suggest an early dysregulation of *INS* and *INSR* in AD pathogenesis and pave the way to a possible utility of these transcripts as peripheral biomarkers.

Keywords: Gene expression, insulin, insulin receptor, peripheral biomarkers, prodromal Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that slowly destroys memory and thinking skills. Currently, there is an ever-growing need to find biomarkers for the early diagnosis of AD as well as to predict disease progression, especially as the number of persons affected by this disease is nearing approximately 46 million worldwide [1]. At the histopathological level, AD is defined by the presence of senile plaques and neurofibrillary tangles. Current scientific evidence suggests that in preclinical AD, brain changes may begin years before symptoms, and the transition between normal cognition and full-blown dementia is represented by mild cognitive impairment (MCI) [3]. Although a few pathogenic mechanisms have been suggested, such as inflammation and oxidative damage, an extensive study of transcripts deregulated in AD pathogenesis is currently lacking. Moreover, the identification of early changes occurring in peripheral cells may be also useful to identify potential

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OPEN ACCESS

RESEARCH PAPER

Downregulation of exosomal miR-204-5p and miR-632 as a biomarker for FTD: a GENFI study

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► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2017-317492).

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Received 20 October 2017
Revised 20 December 2017
Accepted 14 January 2018
Published Online First 6 February 2018

ABSTRACT

Objective To determine whether exosomal microRNAs (miRNAs) in cerebrospinal fluid (CSF) of patients with frontotemporal dementia (FTD) can serve as diagnostic biomarkers, we assessed miRNA expression in the Genetic Frontotemporal Dementia Initiative (GENFI) cohort and in sporadic FTD.

Methods GENFI participants were either carriers of a pathogenic mutation in progranulin, chromosome 9 open reading frame 72 or microtubule-associated protein tau or were at risk of carrying a mutation because a first-degree relative was a known symptomatic mutation carrier. Exosomes were isolated from CSF of 23 presymptomatic and 15 symptomatic mutation carriers and 11 healthy non-mutation carriers. Expression of 752 miRNAs was measured using quantitative PCR (qPCR) arrays and validated by qPCR using individual primers. MiRNAs found differentially expressed in symptomatic compared with presymptomatic mutation carriers were further evaluated in a cohort of 17 patients with sporadic FTD, 13 patients with sporadic Alzheimer's disease (AD) and 10 healthy controls (HCs) of similar age.

Results In the GENFI cohort, miR-204-5p and miR-632 were significantly decreased in symptomatic compared with presymptomatic mutation carriers. Decrease of miR-204-5p and miR-632 revealed receiver operator characteristics with an area of 0.89 (90% CI 0.79 to 0.98) and 0.81 (90% CI 0.68 to 0.93), respectively, and when combined an area of 0.93 (90% CI 0.87 to 0.99). In sporadic FTD, only miR-632 was significantly decreased compared with AD and HCs. Decrease of miR-632 revealed an area of 0.90 (90% CI 0.81 to 0.98).

Conclusions Exosomal miR-204-5p and miR-632 have potential as diagnostic biomarkers for genetic FTD and miR-632 also for sporadic FTD.

INTRODUCTION

Frontotemporal dementia (FTD) is now recognised as the most common cause of early-onset dementia in people under the age of 60 years.[1] FTD usually presents with either behavioural or language impairment. The pathogenic mechanisms resulting in FTD remain largely unknown, but current knowledge suggests that genetic, epigenetic and environmental factors contribute to disease development.[2] Approximately 40% of patients with FTD have a positive family history of dementia[3] and about 25% of patients with FTD have an identified genetic form of the disease.[1] The vast majority of genetic FTD is inherited in an autosomal dominant pattern caused by mutations in one of three genes: chromosome 9 open reading frame 72 (*C9orf72*), progranulin (*GRN*) or microtubule-associated protein tau (*MAPT*). These genes provide an opportunity to study the disease in its presymptomatic phase and offer great hope for elucidating the pathogenic mechanisms that cause FTD. There is some mounting evidence that alterations in microRNA (miRNAs) may occur in FTD.[4–6] MiRNAs are small, non-coding RNAs that regulate gene expression through post-transcriptional silencing of target mRNAs.[7] The same miRNA may regulate hundreds of target mRNAs affecting complex disease pathways.[8] MiRNAs are stable in body fluids and can be enriched in extracellular vesicles termed exosomes. These vesicles were thought to be a means for cells to discard unnecessary molecules into the extracellular space,[9] but more recent studies have shown that cells can transfer proteins, lipids, DNA, RNA and miRNA to other cells via exosomes.[10] Exosomes display different miRNA profiles compared with serum and cells, suggesting that a specific selection of exosomal miRNAs provides signals to regulate pathways in recipient cells.[11] This intercellular transfer can influence a multitude of biological processes relevant to the nervous system such as neuronal survival, neurite outgrowth and synaptic plasticity.[12–15] Disease-relevant miRNAs may be enriched within exosomes,[16] and since miRNA expression can vary in different disease states, exosomal miRNAs are attractive targets for biomarker profiling.[17 18]

Genetic FTD is a rare condition, and single groups have only been able to study small numbers of patients. Through the Genetic Frontotemporal Dementia Initiative (GENFI), we obtained CSF from individuals who were either symptomatic or presymptomatic carriers of a known pathogenic mutation in *GRN*, *MAPT* or *C9orf72* or who were non-affected first-degree relatives of a known symptomatic carrier (healthy non-mutation carriers). We characterised miRNA expression profiles and

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To cite: Schneider R, McKeever P, Kim TH, *et al*. *J Neurol Neurosurg Psychiatry* 2018;**89**:851–858.

found miR-204-5p and miR-632 significantly decreased in symptomatic compared with presymptomatic mutation carriers, suggesting low miR-204-5p and miR-632 as potential diagnostic biomarkers. In a separate cohort, we found miR-632 significantly decreased in sporadic FTD compared with sporadic Alzheimer's disease (AD) and healthy controls (HCs), highlighting its potential as a diagnostic biomarker for sporadic FTD.

METHODS

Ethics statements, sample collection and clinical data

Written informed consent and local research ethics boards' approval was obtained. Six GENFI centres contributed CSF (Karolinska Institute, Department of Neurobiology, Stockholm, Sweden; Erasmus Medical Center, Department of Neurology, Rotterdam, The Netherlands; University College London, Dementia Research Centre, London, England; Université Laval, Département des Sciences Neurologiques, Quebec City, Canada; University of Milan, Centro Dino Ferrari, Fondazione Ca' Granda IRCCS Ospedale Policlinico, Milan, Italy; and University of Toronto, Sunnybrook Health Sciences Centre, Toronto, Canada). The GENFI cohort consisted of 49 subjects: 38 mutation carriers (22 *GRN*, 11 *C9orf72* and 5 *MAPT*) and 11 first-degree relatives who tested negative for a mutation in the gene that had been found mutated in their affected first-degree relative (healthy non-mutation carriers). Twenty-three mutation carriers were presymptomatic, and 15 mutation carriers were symptomatic. The clinical presentation was behavioural variant FTD (bvFTD) (n=12), non-fluent variant primary progressive aphasia (nfvPPA) (n=1), semantic variant primary progressive aphasia (svPPA) (n=1) or dementia not otherwise specified (D-NOS) (n=1) (online supplementary table 1). Mini-Mental State Examination (MMSE[19]) was carried out in all individuals. A cohort of sporadic FTD, sporadic AD and HCs was recruited at the University Health Network Memory Clinic, Toronto, and the University of California San Francisco Memory and Aging Center. This sporadic disease cohort consisted of bvFTD (n=7), bvFTD/amyotrophic lateral sclerosis (ALS) (n=4), svPPA (n=3), nfvPPA/ALS (n=1), svPPA/ALS (n=1), nfvPPA (n=1), sporadic AD (n=13) and HCs (n=10) (online supplementary table 2). BvFTD met the Rascovsky diagnostic criteria,[20] PPA met the Gorno-Tempini diagnostic criteria,[21] ALS met the El Escorial diagnostic criteria[22] and AD met the McKhann diagnostic criteria.[23]

Samples for miRNA detection

Lumbar puncture was performed with a 20-gauge or 24-gauge spinal needle, and fluid was collected in polypropylene tubes according to local standards. Most sites follow ADNI procedures manual (http://www.adni-info.org/). CSF was stored in aliquots at −80°C until use.

Real-time PCR

For the genetic cohort (n=49), 500 µL of each CSF sample was thawed and centrifuged at 10 000 × g for 5 min to pellet any debris. To isolate exosomes, the supernatant was transferred to a new reaction vial, and 200 µL precipitation buffer (miRCURY Exosome Isolation Kit, Exiqon, Copenhagen, Denmark) was mixed with the supernatant. The mix was incubated at 4°C for 60 min and spun for 30 min at 10 000 × g at 20°C. The supernatant was discarded, and lysis buffer containing synthetic spike-ins (UniSp2, UniSp4 and UniSp5) was added to the pellet. RNA was extracted using spin column chromatography (miRCURY RNA Isolation Kit, Exiqon). To obtain cDNA, each RNA sample was incubated for 60 min at 42°C in the presence of reaction buffer, nuclease-free water, enzyme mix and synthesis RNA spike-in mix (cel-miR-39-3p and UniSp6) (miRCURY RNA Isolation Kit, Exiqon). Reverse transcriptase (RT) was heat-inactivated for 5 min at 95°C, and the cDNA samples were immediately stored at −80°C. Immediately prior to real-time PCR, each cDNA sample was thawed and added to a Master Mix working-solution containing SYBR Green (Exiqon). Ten microlitres of this mix was added to each of the 768 wells of the ready-to-use Human microRNA panel I+II, V4.M (Exiqon). Panel I+II contained a total of 752 individual miRNA primer sets plus control assays. Plates were spun at 1500 × g for 1 min. Plates were run on the Applied Biosystems 7900HT Real-Time PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Only miRNAs detected with Ct <40 were included in the analysis. After normalisation to cel-mir-39-3p, as previously described by Freischmidt *et al*,[24] cycle threshold (Ct) values were converted to linear scale relative to the control group (healthy non-mutation carriers), and Log2 conversion was applied (Exiqon Data Analysis Guide for miRCURY GenEx software v 3). For the sporadic disease cohort (n=40), CSF was thawed, and cDNA was obtained from the exosomal miRNA content as described above. For technical validation of the results obtained in the GENFI cohort and for the sporadic disease cohort, a Master Mix working-solution containing either hsa-204-5p or hsa-miR-632 PCR primer set (both Exiqon) and SYBR Green (Exiqon) was prepared. Master Mix and samples were added to 96-well plates and run on the Applied Biosystems Step One Plus Real-Time PCR System (Thermo Fisher Scientific). MiRNA expression changes were calculated relative to HCs using the $2^{-\Delta\Delta Ct}$ method[25] with $^{\Delta}Ct = Ct_{miRNA} - Ct_{reference}$ and $^{\Delta\Delta}Ct = {}^{\Delta}Ct_{patient\ or\ mutation\ carrier} - {}^{\Delta}Ct_{control\ pool}$. UniSp6 spike-in was used as a reference for normalisation. RNA and DNA spike-ins showed steady levels across samples indicating accurate RT reaction and PCR. Applied Biosystems SDS V.2.2.2. software (Thermo Fisher Scientific) and GenEx 6 (MultiD Analyses, Göteborg, Sweden) were used for miRNA expression processing prior to statistical analysis.

Statistical analysis

Welch's t-test was performed and corrected for multiple comparisons using the Holm-Sidak method when relative miRNAs expression changes passed D'Agostino & Pearson normality test. When relative miRNA expression changes calculated as $2^{-\Delta\Delta Ct}$ were not normally distributed, Mann-Whitney U test was performed. Fisher's exact test was used to detect differences in miRNA detection frequency. Correlations between clinical data and miRNA expression were calculated using Spearmans's rank order correlation. Receiver operating characteristics (ROC) curves and the area under the curve (AUC) were established to evaluate the diagnostic value of miRNA expression changes. For cross-validation, we used 50% of the dataset to train linear models and 50% to validate the results. We then calculated Pearson's bivariate correlation. Statistical analysis was performed using GraphPad Prism V.7.01 (La Jolla, California, USA). IBM SPSS V.24.0 was used for logistic regression, ROC calculations and cross-validation. P values <0.05 were considered significant. When the 90% CI included 1, P values were reported as P trend.

Target prediction and gene ontology analysis

Targets of each significantly different miRNA were predicted using miRWalk 2.0, which combines information from 12 existing miRNA-target prediction programs (DIANA-microTv4.0, DIANA-microT-CDS, miRanda-rel2010, mirBridge,

Received: 3 August 2017 | Accepted: 11 December 2017

DOI: 10.1111/jcmm.13545

ORIGINAL ARTICLE

WILEY

Microtubule defects in mesenchymal stromal cells distinguish patients with Progressive Supranuclear Palsy

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Funding information
Regione Lombardia - Independent Research call 2012; Fondazione Grigioni per il Morbo di Parkinson

Abstract

Progressive Supranuclear Palsy (PSP) is a rare neurodegenerative disease whose etiopathogenesis remains elusive. The intraneuronal accumulation of hyperphosphorylated Tau, a pivotal protein in regulating microtubules (MT), leads to include PSP into tauopathies. Pathological hallmarks are well known in neural cells but no word yet if PSP-linked dysfunctions occur also in other cell types. We focused on bone marrow mesenchymal stromal cells (MSCs) that have recently gained attention for therapeutic interventions due to their anti-inflammatory, antiapoptotic and trophic properties. Here, we aimed to investigate MSCs biology and to disclose if any disease-linked defect occurs in this non-neuronal compartment. First, we found that cells obtained from patients showed altered morphology and growth. Next, Western blotting analysis unravelled the imbalance in α-tubulin post-translational modifications and in MT stability. Interestingly, MT mass is significantly decreased in patient cells at baseline and differently changes overtime compared to controls, suggesting their inability to efficiently remodel MT cytoskeleton during ageing in culture. Thus, our results provide the first evidence that defects in MT regulation and stability occur and are detectable in a non-neuronal compartment in patients with PSP. We suggest that MSCs could be a novel model system for unravelling cellular processes implicated in this neurodegenerative disorder.

KEYWORDS

bone marrow mesenchymal stromal cells, microtubules, neurodegeneration, Progressive Supranuclear Palsy

1 | INTRODUCTION

PSP, also known as Richardson-Steele-Olszewski syndrome, is a sporadic neurodegenerative disease described for the first time in 1963[1] and for whom there are not available treatments to date. PSP results in severe disability, as it is characterized by frequent falls, supranuclear vertical gaze palsy, pseudobulbar palsy and rigidity of the neck.[2] Thanks to its wide spectrum of clinical phenotypes, now this pathology has been recognized as a range of motor and behavioural syndromes[3] and related to multiple pathological mechanisms.[4] The disease is characterized by a neurodegenerative process that

Alessandra Maria Calogero and Mariele Viganò contributed equally to the work.
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BIOMARKERS
2018, VOL. 23, NO. 6, 603–608
https://doi.org/10.1080/1354750X.2018.1468825

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Comparison of β2-microglobulin serum level between Alzheimer's patients, cognitive healthy and mild cognitive impaired individuals

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ABSTRACT

Background: Several studies performed in the last years on the brain, showed that beta2-microglobulin (β2m) and MHC can act independently of their canonical immune function to regulate normal brain development, synaptic plasticity and behaviour. Increased systemic levels of soluble β2m have been implicated in cognitive impairments like that associated with chronic haemodialysis, or aortic valve replacement. Increased soluble β2m has also been detected in the cerebral spinal fluid (CSF) of patients with HIV-associated dementia and Alzheimer's disease (AD).

Objective: To compare plasma β2m levels in healthy subjects and subjects with dementia or cognitive impairment.

Methods: We measured the concentration of β2m in a cohort of 245 individuals and compared sex matched, cognitive healthy individuals.

Results: We found higher levels of β2m in AD patients compared to non-AD MCI and healthy controls (2063 ng/mL ±852 versus 1613 ± 503 and 1832 ± 382 ng/mL, $p < 0.001$ and < 0.033, respectively), while there was no difference between mild cognitive impairment (MCI) and healthy controls ($p > 0.05$).

Conclusions: Our data confirm that β2m could play a role in AD. However, a replication study in an independent cohort would be necessary to confirm our preliminary results.

ARTICLE HISTORY
Received 31 January 2018
Revised 9 April 2018
Accepted 20 April 2018

KEYWORDS
β2-microglobulin; Alzheimer disease; mild cognitive impairment; serum biomarkers

Introduction

Imaging, cerebrospinal fluid (CSF) and blood-based biomarkers have the potential to improve the accuracy by which specific causes of dementia can be diagnosed *in vivo*, provide insights into the underlying pathophysiology, and may be used as inclusion criteria and outcome measures for clinical trials. While a number of imaging and CSF biomarkers are currently used for each of these purposes, this is an evolving field, with numerous potential biomarkers in varying stages of research and development. β2-microglobulin (β2m) is a low molecular weight (11.8 KDa) non-glycosylated polypeptide. The gene is located on chromosome 15q21.1 and the encoded, functional protein forms the invariant or light β-chain of HLA class I molecules on the surface of all nucleated cells, in non-covalent association with the 43 KDa heavy α-chain of MHC class I antigens (Cunningham and Berggard 1974, Mátrai *et al.* 2009). The small size of the molecule allows β2m to pass through the glomerular membrane, but normally less of 1% of protein is excreted in the urine; the remainder is reabsorbed and catabolized in the proximal tubules of the kidney. Nonetheless, it was firstly identified in the urine of patients with renal tubular disease. Since it is processed by glomerular filtration and subsequent tubular reabsorption, increases in serum concentrations are a sensitive marker of impaired renal function. Increased plasma concentrations have also been found in solid tumours, haematological malignancies, autoimmune diseases and infections including AIDS. In particular, β2m is regarded as a robust marker of disease activity and prognosis in lymphoproliferative conditions like myeloma, chronic lymphocytic leukaemia, other lymphomas and infections. Interestingly, some variants of human β2m were associated to increased β2m-amyloid deposition and to dialysis-related amyloidosis (Corazza *et al.* 2004, Corlin *et al.* 2005, Murray 2008, Foster *et al.* 2013, Kim *et al.* 2017). Increased systemic levels of soluble β2m have been implicated in cognitive impairments associated with chronic haemodialysis (Murray 2008, Kim *et al.* 2017). Despite its important role in prognosis assessment and disease monitoring, relatively few studies are available on its expression in healthy individuals. These reports show remarkable variations in the methods used, age and number of reference individuals and statistical analyses of the data. Further complexity is introduced by the dependence of β2m expression, with respect to race and ethnicity (Cunningham and Berggard 1974, Mátrai *et al.* 2009). Recently a study investigated the

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Potential genetic modifiers of disease risk and age at onset in patients with frontotemporal lobar degeneration and *GRN* mutations: a genome-wide association study

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Lancet Neurol 2018; 17: 548–58

Published Online April 30, 2018 http://dx.doi.org/10.1016/S1474-4422(18)30126-1

See Comment page 488

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Summary

Background Loss-of-function mutations in *GRN* cause frontotemporal lobar degeneration (FTLD). Patients with *GRN* mutations present with a uniform subtype of TAR DNA-binding protein 43 (TDP-43) pathology at autopsy (FTLD-TDP type A); however, age at onset and clinical presentation are variable, even within families. We aimed to identify potential genetic modifiers of disease onset and disease risk in *GRN* mutation carriers.

Methods The study was done in three stages: a discovery stage, a replication stage, and a meta-analysis of the discovery and replication data. In the discovery stage, genome-wide logistic and linear regression analyses were done to test the association of genetic variants with disease risk (case or control status) and age at onset in patients with a *GRN* mutation and controls free of neurodegenerative disorders. Suggestive loci ($p<1\times10^{-5}$) were genotyped in a replication cohort of patients and controls, followed by a meta-analysis. The effect of genome-wide significant variants at the *GFRA2* locus on expression of *GFRA2* was assessed using mRNA expression studies in cerebellar tissue samples from the Mayo Clinic brain bank. The effect of the *GFRA2* locus on progranulin concentrations was studied using previously generated ELISA-based expression data. Co-immunoprecipitation experiments in HEK293T cells were done to test for a direct interaction between GFRA2 and progranulin.

Findings Individuals were enrolled in the current study between Sept 16, 2014, and Oct 5, 2017. After quality control measures, statistical analyses in the discovery stage included 382 unrelated symptomatic *GRN* mutation carriers and 1146 controls free of neurodegenerative disorders collected from 34 research centres located in the USA, Canada, Australia, and Europe. In the replication stage, 210 patients (67 symptomatic *GRN* mutation carriers and 143 patients with FTLD without *GRN* mutations pathologically confirmed as FTLD-TDP type A) and 1798 controls free of neurodegenerative diseases were recruited from 26 sites, 20 of which overlapped with the discovery stage. No genome-wide significant association with age at onset was identified in the discovery or replication stages, or in the meta-analysis. However, in the case-control analysis, we replicated the previously reported *TMEM106B* association (rs1990622 meta-analysis odds ratio [OR] 0·54, 95% CI 0·46–0·63; $p=3\cdot54\times10^{-16}$), and identified a novel genome-wide significant locus at *GFRA2* on chromosome 8p21.3 associated with disease risk (rs36196656 meta-analysis OR 1·49, 95% CI 1·30–1·71; $p=1\cdot58\times10^{-8}$). Expression analyses showed that the risk-associated allele at rs36196656 decreased *GFRA2* mRNA concentrations in cerebellar tissue ($p=0\cdot04$). No effect of rs36196656 on plasma and CSF progranulin concentrations was detected by ELISA; however, co-immunoprecipitation experiments in HEK293T cells did suggest a direct binding of progranulin and GFRA2.

Interpretation TMEM106B-related and GFRA2-related pathways might be future targets for treatments for FTLD, but the biological interaction between progranulin and these potential disease modifiers requires further study.

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genetic modifiers in *GRN* mutation carriers through genome-wide association analyses in unrelated symptomatic patients with *GRN* mutations.

Methods

Study design and participants

The study was done in three stages: a discovery stage, a replication stage, and finally a meta-analysis of the discovery and replication data. Participants were recruited at 40 international clinical or pathological research centres in Italy, the USA, France, Spain, the UK, Canada, the Netherlands, Sweden, Australia, Denmark, Poland, and Germany between Sept 16, 2014, and Oct 5, 2017 (appendix). No restriction in terms of age, sex, or race was applied to the initial selection; however, the statistical analyses only included white individuals to limit genetic heterogeneity (appendix). Identification of *GRN* mutations and assessment of TDP-43 pathological subtype was done at each individual site. In the discovery stage, we obtained DNA from 33 centres from symptomatic *GRN* carriers from the USA, Canada, Europe, and Australia, and healthy controls from Italy and Spain. We also obtained genetic data from 1986 controls free from neurodegenerative diseases from the Genome-Wide Association Study of Parkinson Disease: Genes and Environment from the Center for Inherited Disease Research (CIDR) consortium (NCBI dbGaP phs000196.v3.p1;[12] hereafter referred to as the CIDR dataset and considered one site; appendix). Additional and non-overlapping patients and controls free from neurodegenerative diseases were recruited for the replication stage from 26 centres, 20 of which overlapped with the discovery stage and six of which were newly identified (appendix).

Age at onset was defined as the age at which the first disease symptoms appeared, including initial cognitive dysfunction in judgment, language, or memory, or changes in behaviour or personality. Written informed consent for genetic studies was given by patients and controls who were alive, or by next of kin at the time of death for autopsy material, with approval from each institution's institutional review board.

Procedures and statistical analysis

Genotyping and quality control procedures for the discovery stage are described in detail in the appendix. Genome-wide association analyses, using logistic and linear regressions, were done to test the association of genetic variants with patient or control status (disease risk) and age at onset, respectively, under an additive model for allele effects and adjusting for age, sex, and the first two principal components of genetic variation when appropriate (appendix). Minor alleles were treated as effect alleles. As exploratory analyses, association of variants with absence or presence of specific first clinical symptoms (memory, behaviour, or language impairment) or presence of parkinsonism at any time during the course of the disease was tested among patients by logistic regression adjusting for age, sex, and the first two principal components (appendix). Association of previously reported putative genetic modifier variants in known neurodegenerative disease genes with disease presentation and age at onset were also established.

Lead variants or a proxy associated at a p value of less than 1×10^{-5} with disease risk or age at onset in the discovery stage were selected for the replication stage. Genotyping and quality control measures for this stage are described in the appendix. Association analyses were done using logistic or linear regressions to replicate association of genetic variants associated suggestively with disease risk or age at onset, adjusting for age and sex when appropriate under an additive model. 36 variants at 34 loci were analysed in the replication stage, and thus a Bonferroni-corrected significance threshold of p less than $1{\cdot}5\times10^{-3}$ was used in this stage. A meta-analysis of the discovery and replication results was done under a fixed-effects model. We also calculated I^2 heterogeneity statistics to assess the degree of heterogeneity of the effects in the discovery and replication stages; for single nucleotide polymorphisms with an I^2 value suggestive of moderate or high heterogeneity ($I^2>0{\cdot}3$) we also did a random effects meta-analysis to verify that conclusions regarding associations would not change under this model. Using the discovery data, a test of interaction was done for the

	Discovery stage		Replication stage		
	GRN mutation carriers (n=382)	Controls (n=1146)	*GRN* mutation carriers (n=67)	Controls (n=1798)	*GRN*-negative FTLD-TDP type A (n=143)
Age (years)					
At onset	60·0 (55·0–66·0)	NA	59·0 (55·0–65·0)	NA	70·0 (62·0–76·8)
At death	66·0 (61·0–73·0)	NA	65·0 (60·8–71·0)	77·0 (64·0–81·0)	79·0 (68·0–85·0)
At last healthy visit	NA	62·0 (56·0–67·0)	NA	62·0 (53·0–71·0)	NA
Sex					
Women	211 (55%)	630 (55%)	35 (52%)	853 (47%)	61 (43%)
Men	171 (45%)	516 (45%)	32 (48%)	945 (53%)	82 (57%)

Data are median (IQR) or number (%). NA=not applicable. FTLD-TDP=frontotemporal lobar degeneration with TAR DNA-binding protein 43.

Table 1: **Demographics**

Journal of Alzheimer's Disease 62 (2018) 913–932
DOI 10.3233/JAD-170702
IOS Press

Review

Role of Genetics and Epigenetics in the Pathogenesis of Alzheimer's Disease and Frontotemporal Dementia

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Accepted 15 September 2017

Abstract. Alzheimer's disease (AD) and frontotemporal dementia (FTD) represent the first cause of dementia in senile and pre-senile population, respectively. A percentage of cases have a genetic cause, inherited with an autosomal dominant pattern of transmission. The majority of cases, however, derive from complex interactions between a number of genetic and environmental factors. Gene variants may act as risk or protective factors. Their combination with a variety of environmental exposures may result in increased susceptibility to these diseases or may influence their course. The scenario is even more complicated considering the effect of epigenetics, which encompasses mechanisms able to alter the expression of genes without altering the DNA sequence. In this review, an overview of the current genetic and epigenetic progresses in AD and FTD will be provided, with particular focus on 1) causative genes, 2) genetic risk factors and disease modifiers, and 3) epigenetics, including methylation, non-coding RNAs and chromatin remodeling.

Keywords: Alzheimer's disease, epigenetics, frontotemporal dementia, genetics

INTRODUCTION

Most neurological disorders, including Alzheimer's disease (AD) and frontotemporal dementia (FTD), are multifactorial diseases. Despite a small percentage of these diseases occurring in families with an autosomal dominant pattern of transmission, the majority of cases are sporadic, and derive from complex interactions between a number of genetic and environmental factors. Therefore, these diseases are defined as "multifactorial" or "complex" [1]. The familial clustering can be explained by recognizing that family members share a greater proportion of their genetic information and environmental exposures than do individuals chosen randomly in the population. Thus, the relatives of an affected individual are more likely to experience the same gene-gene and gene-environment interactions that led to disease in the first place than are individuals who are unrelated to the patient. The multifactorial inheritance pattern represents an interaction between the collective effect of the genotype at one or, more commonly, multiple loci (polygenic or multigenic effects) either to increase or to decrease the susceptibility to the disease, combined with a variety of environmental exposures that may trigger, accelerate, or protect against the ase altered mechanisms.

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EXPERT OPINION ON THERAPEUTIC TARGETS
2018, VOL. 22, NO. 7, 579–585
https://doi.org/10.1080/14728222.2018.1487951

Taylor & Francis
Taylor & Francis Group

REVIEW

Progranulin as a therapeutic target for dementia

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ABSTRACT

Introduction: Progranulin (PGRN) is an acrosomal glycoprotein that is synthesized during spermatogenesis. It is overexpressed in tumors and has anti-inflammatory properties. The protein may be cleaved into granulins which display pro-inflammatory properties. In 2006, mutations in progranulin gene (*GRN*) that cause haploinsufficiency were found in familial cases of frontotemporal dementia (FTD). Patients with null mutations in *GRN* display very low-plasma PGRN levels; this analysis is useful for identifying mutation carriers, independent of the clinical presentation, and in those before the appearance of symptoms.

Areas covered: Here, we review the current knowledge of PGRN physiological functions and *GRN* mutations associated with FTD; we also summarize state of the art clinical trials and those compounds able to replace PGRN loss in preclinical models.

Expert opinion: PGRN represents a promising therapeutic target for FTD. Cohorts suitable for treatment, ideally at the preclinical stage, where pathogenic mechanisms ongoing in the brain are targeted, are available. However, PGRN may have side effects, such as the risk of tumorigenesis, and the risk/benefit ratio of any intervention cannot be predicted. Furthermore, at present, the situation is complicated by the absence of adequate outcome measures.

ARTICLE HISTORY
Received 14 February 2018
Accepted 8 June 2018

KEYWORDS
Frontotemporal dementia (FTD); progranulin (PGRN); progranulin gene (*GRN*); plasma levels; biomarker; therapy

1. Introduction: progranulin structure and functions

Progranulin (PGRN) was identified as an acrosomal glycoprotein (named acrogranin) synthesized during spermatogenesis in 1990 [1]. The gene encoding for PGRN (*GRN*), is localized on chromosome 17q21.32, and contains 12 exons, which results in 3 isoforms [2]. PGRN consists of 593 amino acids and has a molecular weight of 68.5 kDa [3]. The protein has emerged as the prototypic member of a family of structurally unique proteins, evolutionarily conserved, related to growth factors [4]. Full-length PGRN consists of a secretory N-terminal signal peptide of 17 amino acids, and 7.5 granulin domains. Granulins are named, from the N to the C terminus: granulins p (paragranulin domain), G, F, B, A, C, D, and E. Granulin domains are composed of tandem repeats of a 12-cysteine motif (see [5] for details on the molecular structure of the protein). PGRN can be cleaved into granulins by serine and threonine proteases, such as metalloproteinases and elastase. On the other hand, Secretory Leukocytes Protease Inhibitor can bind directly to PGRN, blocking the proteolysis by elastase. Both PGRN and granulins are biologically active, although often with opposing actions. Whereas PGRN has anti-inflammatory properties, granulins display proinflammatory properties. Regarding cell growth, PGRN is a mitogenic and is overexpressed in tumors, whereas the different granulin peptides may have growth-promoting or growth-inhibiting activities, although they seem to act with lower potency than PGRN. Therefore, PGRN-granulins balance is critical in a number of biological processes.

PGRN promotes neurite extension, neuronal survival and differentiation. In animal models of different neurodegenerative diseases, it exerts a neuroprotective role (see [6] for details).

PGRN binds to tumor necrosis factor (TNF)α receptors (TNFR) thanks to the granulins F, A, and C, which mediate the interaction between PGRN and TNFα receptors. PGRN binds to TNFR1 with affinity comparable to that of TNFα, whereas binds to TNFR2 with much higher affinity than TNFα [7]. As TNFR1n signaling induces the apoptotic and proinflammatory pathway, whereas TNFR2 signaling triggers cell survival signal, PGRN can block the TNFα-induced inflammatory pathways by competitively binding to TNFR1 and can also promote cell proliferation through the high affinity binding to TNFR2. Although other Authors did not confirm the binding of PGRN to TNFR [8], more recent studies confirmed that PGRN binds to TNF3 [9,10].

PGRN mutations were found to be the cause of some cases of autosomal dominant frontotemporal lobar degeneration (FTLD) in 2006 [11,12]. Later on, it was shown that PGRN is implicated in a number of autoimmune diseases, including rheumatoid arthritis (RA), osetoarthritis (OA), inflammatory bowel disease (IBD), psoriasis, diabete mellitus (DM), systemic lupus erythematosus (SLE), systemic sclerosis (SS), multiple sclerosis (MS), and Sjogren's syndrome [13].

In 2012, PGRN was shown to mediate high-fat diet-induced insulin resistance by inducing the upregulation of IL-6 expression in adipose tissue, thus being a key adipokine [14]. In conclusion, PGRN may act as growth factor, anti-inflammatory agent or adipokine, depending on the target tissue.

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EXPERT REVIEW OF NEUROTHERAPEUTICS
2018, VOL. 18, NO. 6, 469–475
https://doi.org/10.1080/14737175.2018.1481389

Taylor & Francis
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REVIEW

Epigenetic regulatory modifications in genetic and sporadic frontotemporal dementia

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ABSTRACT

Introduction: Epigenetic modifications have recently been linked to neurodegenerative diseases, such as frontotemporal dementia (FTD), which represents the second most common form of dementia in adulthood after Alzheimer's disease (AD). Epigenetic regulation occurs at different cellular levels and serve as a way to alter genetic information not only in aging but also following environmental signals. Thus, epigenetics mechanisms could exert their function at early stage of the disease, especially in sporadic cases.

Areas covered: Herein, the available evidence supporting the concept that epigenetic-driven changes might shed the light into the pathogenic mechanisms of FTD will be summarized, with particular regard to their influence in underlying sporadic/familiar FTD onset and/or severity, and to the possibility to open a new scenario to facilitate early diagnosis and the identification of novel therapeutic targets. Bibliographic search through PubMed was used to find the studies included in this review.

Expert commentary: Although epigenetic investigation in neurodegenerative disorders is in its infancy, recent advances in the technology of epigenetic change determination has led to novel, challenging findings. In particular, the knowledge and the characterization of epigenetic events could result in novel therapeutic strategies.

ARTICLE HISTORY
Received 13 February 2018
Accepted 23 May 2018

KEYWORDS
Microrna; non-coding RNA; risk factor; dementia; epigenetics

1. Introduction

Frontotemporal Dementia (FTD) is the second most common cause of dementia after Alzheimer's disease (AD), affecting people from 45 to 65 years. The prevalence has been estimated as 3–26% worldwide in people more than 65 years old [1]. The term FTD encompasses three clinically distinct syndromes: behavioral variant (bv) FTD, Progressive Non-Fluent Aphasia (PNFA), and Semantic Dementia (SD) [2]. Clinical features of FTD are different according to the different subtype of disease. In general, typical features of bvFTD are the presence of behavioral disturbances, aggressiveness, lack of empathy, decline in social conduct, often associated with cognitive and executive impairment, whereas in PNFA and SD language impairment is the most prominent feature [2]. At the pathological level, all syndromes described are collectively grouped as Frontotemporal Lobar Degeneration (FTLD). At the histopathological level, based on the type of protein depositing, FTLD is classified into FTLD-Tau, FTLD-TAR DNA Binding protein (TDP) 43, and FTLD fused in Sarcoma (FUS) [3].

Up to 50% of FTD patients show a familial history for dementia [4]. Genetic investigation over the past two decades in FTD with Mendelian inheritance led to the identification of three causal genes: microtubule-associated protein tau (*MAPT*), progranulin (*GRN*), and Chromosome 9 Open Reading Frame 72 (*C9ORF72*), together with an additional small number of rare FTD genes. Altogether the mutations in the above mentioned genes explain about 83% of overall FTD cases with an autosomal dominant mode of inheritance, which was unexplained in the past [5]. Other rare mutations have been found in TDP-43 and FUS, as well as in charged multivesicular body protein 2B (*CHMP2B*). However, altogether these mutations account for less than 5% of genetic cases [3]. Interestingly, *CHMP2B* could exert an epigenetic contribution to the disease since it is also known as chromatin modifying protein 2B.

Many FTD cases with an unclear family history of neurodegenerative disease remain unexplained and the genetic basis have been identified in less than 10% of apparently sporadic cases. These evidences suggest that other genetic risk factors still not known may be present, together with unknown additional non-genetic factors that in combination could be responsible for the disease in the remaining familial and apparently sporadic patients.

With respect to this review, current evidence supporting the concept that epigenetic-driven changes might shed light into the pathogenic mechanisms underlying sporadic/familiar FTD onset and or severity will be summarized, as well as its support to facilitate early diagnosis and the identification of novel therapeutic targets.

2. Epigenetic regulatory mechanisms

The term epigenetics is referred to the investigation of changes in gene expression and chromatin structure caused by mechanisms other than changes in the DNA sequence [6].

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Fumagalli et al. Alzheimer's Research & Therapy (2018) 10:46
https://doi.org/10.1186/s13195-018-0376-9

Alzheimer's Research & Therapy

RESEARCH Open Access

Distinct patterns of brain atrophy in Genetic Frontotemporal Dementia Initiative (GENFI) cohort revealed by visual rating scales

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Abstract

Background: In patients with frontotemporal dementia, it has been shown that brain atrophy occurs earliest in the anterior cingulate, insula and frontal lobes. We used visual rating scales to investigate whether identifying atrophy in these areas may be helpful in distinguishing symptomatic patients carrying different causal mutations in the microtubule-associated protein tau (*MAPT*), progranulin (*GRN*) and chromosome 9 open reading frame *(C9ORF72)* genes. We also analysed asymptomatic carriers to see whether it was possible to visually identify brain atrophy before the appearance of symptoms.

Methods: Magnetic resonance imaging of 343 subjects (63 symptomatic mutation carriers, 132 presymptomatic mutation carriers and 148 control subjects) from the Genetic Frontotemporal Dementia Initiative study were analysed by two trained raters using a protocol of six visual rating scales that identified atrophy in key regions of the brain (orbitofrontal, anterior cingulate, frontoinsula, anterior and medial temporal lobes and posterior cortical areas).

Results: Intra- and interrater agreement were greater than 0.73 for all the scales. Voxel-based morphometric analysis demonstrated a strong correlation between the visual rating scale scores and grey matter atrophy in the same region for each of the scales. Typical patterns of atrophy were identified: symmetric anterior and medial temporal lobe involvement for *MAPT*, asymmetric frontal and parietal loss for *GRN*, and a more widespread pattern for *C9ORF72*. Presymptomatic *MAPT* carriers showed greater atrophy in the medial temporal region than control subjects, but the visual rating scales could not identify presymptomatic atrophy in *GRN* or *C9ORF72* carriers.

Conclusions: These simple-to-use and reproducible scales may be useful tools in the clinical setting for the discrimination of different mutations of frontotemporal dementia, and they may even help to identify atrophy prior to onset in those with *MAPT* mutations.

Keywords: Frontotemporal dementia, Genetics, MRI, Visual rating

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Journal of Alzheimer's Disease 65 (2018) 283–292
DOI 10.3233/JAD-180242
IOS Press

PICALM Gene Methylation in Blood of Alzheimer's Disease Patients Is Associated with Cognitive Decline

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Accepted 22 June 2016

Abstract. Epigenetic mechanisms might be involved in Alzheimer's disease (AD). Genetic polymorphisms in several genes, including *APOE* (Apolipoprotein E), *PSEN1* (Presenilin 1), *CR1* (Complement receptor 1), and *PICALM* (Phosphatidylinositol binding clathrin assembly protein), have been associated to an increased AD risk. However, data regarding methylation of these specific genes are lacking. We evaluated DNA methylation measured by quantitative bisulfite-PCR pyrosequencing in 43 AD patients and 38 healthy subjects (HS). In a multivariate age- and gender-adjusted model, *PICALM* methylation was decreased in AD compared to HS (mean = 3.54 and 4.63, respectively, $p = 0.007$). In AD, *PICALM* methylation level was also positively associated to Mini-Mental Scale Examination (MMSE) score (percent change 3.48%, $p = 0.008$). Moreover, a negative association between *PICALM* methylation and age was observed only in HS (percent change –2.29%, $p = 0.002$). In conclusion, our data suggest a possible role of PICALM methylation in AD, particularly related to cognitive function. Given the small study sample and the associative nature of our study, further prospective investigations are required to assess the dynamics of DNA methylation in the early stages of AD development.

Keywords: Alzheimer's disease, epigenetics, methylation, Mini-Mental State Examination, PICALM

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the elderly, characterized by memory impairment. Several estimates confirm that there are nearly 44 million people with dementia in the world, that are expected to almost double by 2030 and triple by 2050 [1].

AD is characterized by two neuropathological hallmarks: extracellular amyloid-β (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) [2], made of aggregates of hyperphosphorylated tau protein. Decreased levels of Aβ1-42 ($A\beta_{42}$) with increased levels of total tau protein (t-tau) and tau phosphorylated at position 181 (p-tau) have been observed in the cerebrospinal fluid (CSF) of patients affected by AD [3, 4] and are nowadays used in clinical practice for differential diagnosis with an accuracy of 90% for AD [5].

Familial forms of AD, which are generally early onset AD (<65 years), are often associated with known mutations in genes encoding for Amyloid

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Journal of Alzheimer's Disease 65 (2018) 147–163
DOI 10.3233/JAD-180053
IOS Press

Distinct Neuroanatomical Correlates of Neuropsychiatric Symptoms in the Three Main Forms of Genetic Frontotemporal Dementia in the GENFI Cohort

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Accepted 16 June 2018

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Journal of Neuroimmunology 324 (2018) 129–135

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

LncRNAs expression profile in peripheral blood mononuclear cells from multiple sclerosis patients

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ARTICLE INFO

Keywords:
Multiple Sclerosis
Non coding RNA
Gene expression
Biomarker

ABSTRACT

LncRNA PCR arrays containing 90 common LncRNAs were used to screen lncRNA expression levels in PBMC from a discovery population of patients with MS. Data from discovery and replications cohorts showed a generalized dysregulation of lncRNA levels in MS patients compared with controls. *MALAT1, MEG9, NRON, ANRIL, TUG1, XIST, SOX2OT, GOMAFU, HULC, BACE-1AS* were significantly downregulated in MS patients in comparison with controls. Therefore, we performed a validation analysis in an independent cohort of Belgian origin. In this study, *NRON* and *TUG1* downregulations in MS patients compared with controls were confirmed ($p \leq .05$ and $p \leq .0001$ respectively), whereas considering the other lncRNAs, the statistical threshold was not reached. LncRNAs profiling could thus represent a new challenge in the research of easy detectable biomarkers of disease susceptibility and progression.

1. Introduction

Multiple Sclerosis (MS) is a chronic complex autoimmune disease of the Central Nervous System (CNS) characterized by demyelination and axonal degeneration. Indeed, ethnic, environmental and genetic factors are involved in the initiation and progression of MS.

Clinically, different MS subtypes have been described (relapsing-remitting -RR, secondary progressive –SP, and primary progressive -PP) and within each subtype a considerable individual variation in disease course could occur. PPMS likely has a different pathogenesis than RRMS in which inflammatory events seem to have a pivotal role (McFarland and Martin, 2007). In PPMS, neurodegeneration is likely prominent, as shown by the development of motor disability, cognitive deficits and brain atrophy (Trapp and Nave, 2008).

At present, no laboratory measure that reliably correlates with or predicts disease activity and response to therapies exists. In the last few years, several evidence supporting a key role of epigenetic changes in MS pathogenesis has been highlighted (Zhang and Zhang, 2015). Among these, non coding RNAs (ncRNAs) are involved in neural development and in the progression of neurodegenerative (Wan et al., 2017)(Zhou and Xu, 2015)(Lourenco et al., 2015) and demyelinating diseases (Wu et al., 2013). Moreover, they have been largely investigated as potential biomarkers of disease activity (Zhang et al., 2016)(Santoro et al., 2016).

Long non coding RNAs (lncRNAs) are transcripts with a low or no protein-coding potential, with a length of > 200 nucleotides. These non-coding RNA molecules share some common features with microRNAs (miRNA) as they could be spliced, capped and

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https://doi.org/10.1016/j.jneuroim.2018.08.008
Received 22 May 2018; Received in revised form 20 July 2018; Accepted 14 August 2018

Neurobiology of Aging 72 (2018) 171–176

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

CSF pro-orexin and amyloid-β38 expression in Alzheimer's disease and frontotemporal dementia

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ARTICLE INFO

Article history:
Received 22 January 2018
Received in revised form 16 August 2018
Accepted 17 August 2018
Available online 25 August 2018

Keywords:
Alzheimer's disease
Frontotemporal dementia
Orexin
Progranulin
C9orf72
Amyloid β-38

ABSTRACT

There is an unmet need for markers that can stratify different forms and subtypes of dementia. Because of similarities in clinical presentation, it can be difficult to distinguish between Alzheimer's disease (AD) and frontotemporal dementia (FTD). Using a multiplex targeted proteomic LC-MS/MS platform, we aimed to identify cerebrospinal fluid proteins differentially expressed between patients with AD and FTD. Furthermore analysis of 2 confirmed FTD genetic subtypes carrying progranulin (*GRN*) and chromosome 9 open reading frame 72 (*C9orf72*) mutations was performed to give an insight into the differing pathologies of these forms of FTD. Patients with AD (n = 13) demonstrated a significant ($p < 0.007$) 1.24-fold increase in pro-orexin compared to FTD (n = 32). Amyloid beta-38 levels in patients with AD were unaltered but demonstrated a >2-fold reduction ($p < 0.0001$) in the FTD group compared to controls and a similar 1.83-fold reduction compared to the AD group ($p < 0.001$). Soluble TREM2 was elevated in both dementia groups but did not show any difference between AD and FTD. A further analysis comparing FTD subgroups revealed slightly lower levels of proteins apolipoprotein E, CD166, osteopontin, transthyretin, and cystatin C in the *GRN* group (n = 9) compared to the *C9orf72* group (n = 7). These proteins imply GRN FTD elicits an altered inflammatory response to *C9orf72* FTD.

1. Introduction

It is estimated that over 850,000 people live with dementia in the UK alone and this figure is predicted to rise to over 2 million in the next 50 years. Research into new therapies and development of new drugs to stop or slow the progression of neurodegeneration is thus a major priority in health care. Development of treatment has been confounded in the past by difficulties in identifying the correct patients for new drug trials as it can be difficult clinically to distinguish between some forms as dementia such as Alzheimer's disease (AD) and frontotemporal dementia (FTD). Differential diagnosis between AD and FTD may be challenging as AD may manifest with behavioral disturbances (the "frontal variant" (Dubois et al., 2014)) whereas, on the other side, memory disturbances may manifest in FTD, particularly in carriers *of GRN* and chromosome 9 open reading frame (*C9orf72)* mutations (Galimberti et al., 2015; Pietroboni et al., 2011). Cerebrospinal fluid (CSF) biomarkers amyloid beta 1-42 (Aβ), total tau (tau), and Tau phosphorylated at position 181 (Ptau) have a good accuracy in predicting AD (Mulder et al., 2010). In clinical practice, this analysis helps to rule out AD, but apparently normal results cannot exclude FTD, as no specific biomarkers are available for this disease. Moreover, tau may be altered in FTD (as well as in many other neurodegenerative conditions), but surprisingly values are often normal in genetic forms, despite evidence of clinical deterioration (Carecchio et al., 2011). It is also possible that the CSF total- and phospho-tau (t-tau and p-tau, respectively) increase in AD is not a direct effect of tau pathology and neurodegeneration but rather reflects increased tau secretion from AD-affected neurons, as suggested in both

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0197-4580/$ – see front matter © 2018 Published by Elsevier Inc.
https://doi.org/10.1016/j.neurobiolaging.2018.08.019

ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

Presymptomatic white matter integrity loss in familial frontotemporal dementia in the GENFI cohort: A cross-sectional diffusion tensor imaging study

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Funding Information
No funding information is provided.

Received: 4 May 2018; Accepted: 8 June 2018

Abstract

Objective: We aimed to investigate mutation-specific white matter (WM) integrity changes in presymptomatic and symptomatic mutation carriers of the *C9orf72*, *MAPT*, and *GRN* mutations by use of diffusion-weighted imaging within the Genetic Frontotemporal dementia Initiative (GENFI) study. **Methods**: One hundred and forty mutation carriers (54 *C9orf72*, 30 *MAPT*, 56 *GRN*), 104 presymptomatic and 36 symptomatic, and 115 noncarriers underwent 3T diffusion tensor imaging. Linear mixed effects models were used to examine the association between diffusion parameters and years from estimated symptom onset in *C9orf72*, *MAPT*, and *GRN* mutation carriers versus noncarriers. Post hoc analyses were performed on presymptomatic mutation carriers only, as well as left–right asymmetry analyses on *GRN* mutation carriers versus

Astrea et al. Orphanet Journal of Rare Diseases (2018) 13:170
https://doi.org/10.1186/s13023-018-0863-x

Orphanet Journal of Rare Diseases

RESEARCH Open Access

Broad phenotypic spectrum and genotype-phenotype correlations in *GMPPB*-related dystroglycanopathies: an Italian cross-sectional study

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Abstract

Background: Dystroglycanopathy (α-DG) is a relatively common, clinically and genetically heterogeneous category of congenital forms of muscular dystrophy (CMD) and limb-girdle muscular dystrophy (LGMD) associated with hypoglycosylated α-dystroglycan. To date, mutations in at least 19 genes have been associated with α-DG. One of them, *GMPPB*, encoding the guanosine-diphosphate-mannose (GDP-mannose) pyrophosphorylase B protein, has recently been associated with a wide clinical spectrum ranging from severe Walker-Warburg syndrome to pseudo-metabolic myopathy and even congenital myasthenic syndromes.
We re-sequenced the full set of known disease genes in 73 Italian patients with evidence of either reduced or nearly absent α-dystroglycan to assess genotype-phenotype correlations in this cohort. We used innovative bioinformatic tools to calculate the effects of all described *GMPPB* mutations on protein function and attempted to correlate them with phenotypic expressions.

Results: We identified 13 additional cases from 12 families and defined seven novel mutations. Patients displayed variable phenotypes including less typical pictures, ranging from asymptomatic hyperCKemia, to arthrogryposis and congenital clubfoot at birth, and also showed neurodevelopmental comorbidities, such as seizures and ataxic gait, as well as autism-spectrum disorder, which is seldom described in clinical reports of dystroglycanopathies. We also demonstrated that few mutations recur in the Italian *GMPPB*-mutated population and that alterations of protein stability are the main effects of *GMPPB* missense variants.

Conclusion: This work adds to the data on genotype-phenotype correlations in α-DG and offers new bionformatic tools to provide the conceptual framework needed to understand the complexity of these disorders.

Keywords: Congenital muscular dystrophy, Limb-girdle muscular dystrophy, *GMPPB*, Dystroglycanopathies, Genotype-phenotype correlations

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as previously described, and this is in keeping with this mutation's neutral effect on protein stability (Fig. 2a). The reverse is true for p.D287Q whose ΔΔG value predicted a stabilizing effect on the protein. Furthermore, p.R287Q, if combined with the likely destabilizing effect of p. I219T or p.P32L, might predict a severe phenotype (as in P1 or P5, respectively), whereas its association with a neutral variant (e.g. p.G220R in P11 or p.V330I, as in P12) might suggest a less aggressive phenotype. Similar considerations probably apply in the case of association of the most common variant, p.D27H (often seen in LGMD-CMS patients), with the more severe p.P32L mutation.

Consistent with previous findings [26], we observed that patients with *GMPPB* dystroglycanopathy share the unique biochemical feature of a change in electrophoretic mobility of β-dystroglycan. As there was not apparent correlation between the predicted stability of mutated *GMPPB* and residual expression of glycosylated α-dystroglycan or secondary reduction of laminin α2 (Fig. 3 and not shown), the finding that β-dystroglycan is equally affected in all patients regardless of the predicted stability of the mutations suggests that the overall retained function of *GMPPB* may be key to the variability in patients' phenotype.

Conclusions

To summarize, this study describes a sample of 13 Italian patients carrying a total of 15 different mutations in *GMPPB*, representing 18% of our study cohort of α-DG patients. Accordingly, *GMPPB* seems to be one of the more frequent "second generation" α-DG-related genes discovered in the NGS era. Our findings, combined with literature data, show that there are at least three forms of *GMPPB*-related myopathy: i) CMD, ii) early onset LGMD, and iii) adult onset LGMD, often with evidence of neuromuscular junction involvement. Less severe phenotypes are also observed, such as exercise intolerance and myoglobinuria (in P6) or asymptomatic hyperCKemia (P8). In the absence of information on residual enzyme activity in tissues, combining clinical findings with bioinformatic data on variant stability might allow objective assessment of disease severity.

Additional files

Additional file 1: Figure S1. (**A**) Effects of missense mutations on the structure of GMPPB. The images show the close-up of the different mutation sites with the predicted consequences of the amino acid replacement. Wild-type protein is shown in gray and mutated proteins in magenta. The side chain of wild-type and mutated residues are shown as sticks. Mutated residues located in the N-terminal catalytic domain, inter-domains and C-terminal LbH domain are shown on orange, gray and green backgrounds, respectively. (**B**) Distribution of missense mutations between the domains and inter-domains of the GMPPB protein reported as number of mutations per amino acid. Orange bar, N-terminal catalytic domain; gray bar, inter-domains; green bar, C-terminal LbH domain. (PDF 8212 kb)

Additional file 2: Figure S2. Pedigree of the family showing pseudo-dominant inheritance in GMPPB disease. Two patients (uncle and nephew, P9 and P10, respectively) showed onset in early adulthood and similar muscular impairments associated with biallelic mutations (p:Asp27His and p.Val330Ile) in *GMPPB*. Circles are females and squares are males. Slashed symbols indicate deceased individuals. Numbers in symbols indicate number of siblings. (PDF 7 kb)

Abbreviations

CMD: Congenital muscular dystrophy; CMS: Congenital myasthenic syndrome; GMPPB: Guanosine-diphosphate-mannose (GDP-mannose) dyrophosphorylase B gene; LGMD: Limb-girdle muscular dystrophy; MRI: Magnetic resonance imaging; NGS: Next-generation sequencing; WWS: Walker-Warburg syndrome; α-DG: alpha-dystroglycanopathy

Acknowledgements

The authors thank Catherine J. Wrenn, who provided expert editorial assistance.

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Funding

This work was partially funded by Regione Toscana FAS SALUTE 2014 (CUP 4042.16092014.066000060 to FMS) and Telethon Foundation grants GUP13004 to AD, GA, CB, EM and LP). FMu and IZ gratefully acknowledge the European Community's Seventh Framework Programme (FP7/2007–2013) funded grant "Integrated European –omics research project for diagnosis and therapy in rare neuromuscular and neurodegenerative diseases (NEUROMICS)" (grant agreement n° 2012–305121); the Muscular Dystrophy UK Grant on Gene Identification to FMu. FMu is supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London; the views expressed in this article are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.. The support of the Muscular Dystrophy UK to the Dubowitz Neuromuscular Centre and of the MRC Neuromuscular Centre Biobank is also gratefully acknowledged.

Availability of data and materials

The dataset supporting the conclusions of this article are included within the article and its additional files.

Authors' contributions

GA, CF and AD coordinated the collection and elaboration of data. GA and AR drafted the manuscript. CD, RT, FM, FeM, IP, MP, FF, IZ, and WS performed and interpreted the molecular analysis. AR: performed functional in silico studies on selected mutations. RiB and CF reviewed Western blotting in muscle biopsies. CF and AR reviewed the muscle biopsies. CA, CGA, RB, CB, MF, RG, LM, MM, EP, LP, PS and CT, provided patients data. VN and MS reviewed the genetic results. FMS, EB, CB, EM, FMu conceived the study and helped in drafting the manuscript. All authors read and approved the final manuscript.

Received: 16 March 2017 | Revised: 9 May 2018 | Accepted: 9 May 2018

DOI: 10.1002/jnr.24263

RESEARCH ARTICLE

WILEY

Purkinje cell COX deficiency and mtDNA depletion in an animal model of spinocerebellar ataxia type 1

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Funding information
Fondazione Cariplo Grant 2014-1010 (to Dario Ronchi and Associazione Amici del Centro Dino Ferrari—University of Milan)

Abstract

Spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of cerebellar degenerative disorders, characterized by progressive gait unsteadiness, hand incoordination, and dysarthria. Ataxia type 1 (SCA1) is caused by the expansion of a CAG trinucleotide repeat in the SCA1 gene resulting in the atypical extension of a polyglutamine (polyQ) tract within the ataxin-1 protein. Our main objective was to investigate the mitochondrial oxidative metabolism in the cerebellum of transgenic SCA1 mice. SCA1 transgenic mice develop clinical features in the early life stages (around 5 weeks of age) presenting pathological cerebellar signs with concomitant progressive Purkinje neuron atrophy and relatively little cell loss; this evidence suggests that the SCA1 phenotype is not the result of cell death per se, but a possible effect of cellular dysfunction that occurs before neuronal demise. We studied the mitochondrial oxidative metabolism in cerebellar cells from both homozygous and heterozygous transgenic SCA1 mice, aged 2 and 6 months. Histochemical examination showed a cytochrome-c-oxidase (COX) deficiency in the Purkinje cells (PCs) of both heterozygous and homozygous mice, the oxidative defect being more prominent in older mice, in which the percentage of COX-deficient PC was up to 30%. Using a laser-microdissector, we evaluated the mitochondrial DNA (mtDNA) content on selectively isolated COX-competent and COX-deficient PC by quantitative Polymerase Chain Reaction and we found mtDNA depletion in those with oxidative dysfunction. In conclusion, the selective oxidative metabolism defect observed in neuronal PC expressing mutant ataxin occurs as early as 8 weeks of age thus representing an early step in the PC degeneration process in SCA1 disease.

KEYWORDS

laser microdissector, mitochondria, mitochondrial DNA depletion, oxidative damage, Purkinje cell, spinocerebellar ataxia type 1, transgenic mice

1 | INTRODUCTION

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant inherited neurodegenerative disease characterized by neurodegeneration in multiple central nervous system (CNS) regions, including spinal cord, brain stem, and cerebellum (Zoghbi & Orr, 1995).

The disease is associated with an unstable trinucleotide CAG repeat expansion in the open reading frame of the ataxin-1 gene. This specific CAG repeat expansion leads to the expression of an expanded polyglutamine (polyQ) tract in the mutant ataxin-1 protein (ATXN1), thereby acquiring a toxic gain-of-function property (Orr, 2012; Zoghbi, 1995; Zoghbi & Orr, 2009).

Neuropathology and Applied Neurobiology (2018), **44**, 449–462 doi: 10.1111/nan.12414

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.

Nanoscale

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Nanoscale*, 2018, **10**, 7933

Received 25th January 2018,
Accepted 27th March 2018

DOI: 10.1039/c8nr00724a

rsc.li/nanoscale

Demonstration of cellular imaging by using luminescent and anti-cytotoxic europium-doped hafnia nanocrystals†

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Luminescent nanoparticles are researched for their potential impact in medical science, but no materials approved for parenteral use have been available so far. To overcome this issue, we demonstrate that Eu^{3+}-doped hafnium dioxide nanocrystals can be used as non-toxic, highly stable probes for cellular optical imaging and as radiosensitive materials for clinical treatment. Furthermore, viability and biocompatibility tests on artificially stressed cell cultures reveal their ability to buffer reactive oxygen species, proposing an anti-cytotoxic feature interesting for biomedical applications.

A very promising application of luminescent nanomaterials lies in the area of medical science, spanning from imaging to therapy of diseases.[1,2] The poor photostability of genetically encoded probes and organic dyes has indeed strongly motivated researchers to explore different types of luminescent nanomaterials such as quantum dots,[3] nanodiamonds,[4] dye-doped silica particles,[5] and metallic clusters.[6] However, no luminescent nanoparticles approved for parenteral use by the Food and Drug Administration (FDA) have been available so far, although they would revolutionize many areas of medical therapy and diagnostics, such as drug delivery,[7] tissue mapping, real-time detection of intracellular events,[8] biosensing,[9] and tracking of cell migration.[10] For example, fluorescent quantum dots have been extensively tested obtaining high quality optical imaging[11] but, despite the good results achieved, significant efforts are continuously made to improve their biocompatibitly,[12] which remains limited and hinders their approval by the regulatory agencies.[11,13,14] With the aim to overcome this compatibility issue, in the past decade, a lot of research has been devoted to the development of luminescent rare-earth (RE) doped inorganic nanoparticles, whose optical properties make them promising candidates as contrast agents for biological applications.[15] Attractive properties of RE-doped nanoparticles include high photostability, the absence of blinking, extremely narrow emission lines, weak self-absorption and, importantly, facile functionalization strategies.[16] Moreover, the use of specific ions in their compositions is an additional powerful engineering route for fabricating multifunctional systems such as optical nanosensors, *i.e.* highly sensitive oxidant detectors,[17] or for implementing potent contrast agents for magnetic resonance imaging.[18] Therefore, further pushed by their non-toxic nature and by their favorable cellular uptake kinetics, RE-doped *metal fluoride* nanocrystals such as $NaGdF_4$, $NaYF_4$, CaF_2, LaF_3, and SrF_2 were widely investigated.[19]

Conversely, only a few reports are available on luminescent *metal oxide* nanocrystals, and especially on systems including heavy metals such as hafnium. This is surprising, considering that hafnium dioxide (HfO_2, hafnia) is a low energy phonon environment, shows no toxicity effects and possesses a higher density with respect to other host materials such as yttrium oxide, vanadates and phosphates used in fluorescence bioimaging.[20] Indeed, thanks to these advantageous features, *non-luminescent* hafnia and a few other inorganic oxides have been already approved by the FDA for medical injections and radiotheraphy,[21,22] being a safe and effective means for the treatment of radiosensitive and radioresistant tumors thanks to the enhanced interaction of the incorporated heavy elements with high energy radiation.[23,24] The potential of hafnia nanocrystals has been recently further highlighted by Vinogradov and co-workers.[25] They show how the combination of their easily tunable optical properties with an exceptional

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Received: 20 April 2017 | Revised: 21 September 2017 | Accepted: 15 October 2017

DOI: 10.1111/ajt.14547

ORIGINAL ARTICLE

AJT

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

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Funding information
Fondazione Tronchetti Provera; Diabetes Research Institute Foundation; National Institutes of Health (NIH), Grant/Award Number: DK109929; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico; Juvenile Diabetes Research Foundation International, Grant/Award Number: 17-2001-268, 17-2010-5, 17-2012-361

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 lyı ethylene glycol; R, responders; ROS, reactive oxygen species; S, stimulators; SC, subcutaneous; SD, standard deviation; SPL, spleen; T1D, type 1 diabetes.

SCIENTIFIC REPORTS

OPEN

Supplementation with a selective amino acid formula ameliorates muscular dystrophy in *mdx* mice

Received: 26 January 2018
Accepted: 10 September 2018
Published online: 02 October 2018

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Duchenne muscular dystrophy (DMD) is one of the most common and severe forms of muscular dystrophy. Oxidative myofibre content, muscle vasculature architecture and exercise tolerance are impaired in DMD. Several studies have demonstrated that nutrient supplements ameliorate dystrophic features, thereby enhancing muscle performance. Here, we report that dietary supplementation with a specific branched-chain amino acid-enriched mixture (BCAAem) increased the abundance of oxidative muscle fibres associated with increased muscle endurance in dystrophic *mdx* mice. Amelioration of the fatigue index in BCAAem-treated *mdx* mice was caused by a cascade of events in the muscle tissue, which were promoted by endothelial nitric oxide synthase (eNOS) activation and vascular endothelial growth factor (VEGF) expression. VEGF induction led to recruitment of bone marrow (BM)-derived endothelial progenitors (EPs), which increased the capillary density of dystrophic skeletal muscle. Functionally, BCAAem mitigated the dystrophic phenotype of *mdx* mice without inducing dystrophin protein expression or replacing the dystrophin-associated glycoprotein (DAG) complex in the membrane, which is typically lost in DMD. BCAAem supplementation could be an effective adjuvant strategy in DMD treatment.

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy are caused by mutations within the *dystrophin* gene[1] which primarily results in sarcolemmal fragility, muscle damage and respiratory or cardiac muscle fatigue and failure[2,3]. Treatment strategies for DMD have been done using genetic[4–9], pharmacological[10,11], or cellular[12–16] approaches aimed at restoring dystrophin-associated glycoprotein (DAG) complex, reversing sarcolemmal fragility, and abating muscular dystrophy. However many hurdles remain and DMD is still incurable. Dysregulation of pathways associated with muscle fibre plasticity and angiogenesis in DMD are not well understood. Elucidation of such pathways may reveal signalling targets that are amenable to therapeutic manipulation by synthetic drugs. Activation of AMP-activated protein kinase (AMPK) ameliorates DMD mitochondrial activity and promotes oxidative slow-twitch myogenesis in *mdx* mice[17,18]. The transcription factor peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) regulates the neuromuscular junction gene programme, induces a fast-to-slow fibre type transition, and ameliorates DMD pathology[19,20]. The dystrophin protein is expressed not only in skeletal muscle cells but also in vascular smooth muscle and endothelial cells (ECs)[21,22]. Vascular defects, including ultrastructural abnormalities of microvessels, mixed degenerating and regenerating capillaries, replication of the capillary basal lamina, and compression of capillaries and small-calibre veins by

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ADVANCED FUNCTIONAL MATERIALS
www.afm-journal.de

Self-Assembled pH-Sensitive Fluoromagnetic Nanotubes as Archetype System for Multimodal Imaging of Brain Cancer

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Fluoromagnetic systems are recognized as an emerging class of materials with great potential in the biomedical field. Here, it is shown how to fabricate fluoromagnetic nanotubes that can serve as multimodal probes for the imaging and targeting of brain cancer. An ionic self-assembly strategy is used to functionalize the surface of synthetic chrysotile nanotubes with pH-sensitive fluorescent chromophores and ferromagnetic nanoparticles. The acquired magnetic properties permit their use as contrast agent for magnetic resonance imaging, and enable the tracking of tumor cell migration and infiltration responsible for metastatic growth and disease recurrence. Their organic component, changing its fluorescence attitude as a function of local pH, targets the cancer distinctive acidity, and allows localizing and monitoring the tumor occurrence and progression by mapping the acidic spatial distribution within biopsy tissues. The fluoromagnetic properties of nanotubes are preserved from the in vitro to the in vivo condition and they show the ability to migrate across the blood brain barrier, thus spontaneously reaching the brain tumor after injection. The simplicity of the synthesis route of these geomimetic nanomaterials combined with their demonstrated affinity with the in vivo condition strongly highlights their potential for developing effective functional materials for multimodal theranostics of brain cancer.

1. Introduction

A very dynamic research area focuses on the design and synthesis of nanomaterials that simultaneously contain more than one functional component, the so-called *multifunctional*, which are expected to have a significant impact on a wide range of applications.[1–5] Especially for in vivo uses, the availability of multimodal sensing or multifunctional drug delivery/imaging materials can allow the combination of different diagnostic and/or therapeutic techniques in a single platform, thus leading to a potential reduction in side effects, risks, and costs, while increasing the benefits obtained from the synergy of different methods.[6–8] Recently, fluoromagnetic particles have been recognized as emerging class with a huge potential.[9–13] Magnetic nanoparticles can be coupled to drug molecules, fluorescent compounds, and various hydrophobic and hydrophilic coatings, opening up great

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DOI: 10.1002/adfm.201707582

Stem Cell Research 30 (2018) 61–68

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Motor neuron differentiation of iPSCs obtained from peripheral blood of a mutant *TARDBP* ALS patient

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ARTICLE INFO

Keywords:
Amyotrophic Lateral Sclerosis (ALS)
Induced Pluripotent Stem Cells (iPSCs)
Peripheral blood
TDP43
Motor neuron

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease, mainly affecting the motor neurons (MNs) and without effective therapy. Drug screening is hampered by the lack of satisfactory experimental and pre-clinical models. Induced pluripotent stem cells (iPSCs) could help to define disease mechanisms and therapeutic strategies as they could be differentiated into MNs, otherwise inaccessible from living humans. In this study, given the seminal role of TDP-43 in ALS pathophysiology, MNs were obtained from peripheral blood mononuclear cells-derived iPSCs of an ALS patient carrying a p.A382T *TARDBP* mutation and a healthy donor. Venous samples were preferred to fibroblasts for their ease of collection and no requirement for time consuming extended cultures before experimentation. iPSCs were characterized for expression of specific markers, spontaneously differentiated into primary germ layers and, finally, into MNs. No differences were observed between the mutated ALS patient and the control MNs with most of the cells displaying a nuclear localization of the TDP-43 protein. In conclusion, we here demonstrated for the first time that human *TARDBP* mutated MNs can be successfully obtained exploiting the reprogramming and differentiation ability of peripheral blood cells, an easily accessible source from any patient.

1. Introduction

An important limitation in the field of neurodegenerative disorders is the difficulty to translate information provided by preclinical research into effective new treatments for patients. This unmet need is mainly due to the scarcity of adequate experimental models. This is particularly true for amyotrophic lateral sclerosis (ALS), where motor neurons (MNs) and other nervous tissues affected by disease processes are difficult to obtain from alive patients. In addition, when post mortem brain samples are available, differentiated neurons do not anyway undergo cell division, resulting in a limited utility for *in vitro* functional studies.

The majority of ALS patients present a sporadic form of the disease, while around 10% are familial cases with > 20 causative genes identified so far among which *SOD1*, *C9ORF72, TARDBP* and *FUS* represent the main involved (Chen et al., 2013). Several transgenic animal models have been developed in order to investigate the different pathomechanisms of ALS and to test future possible therapies: the first and most commonly used was the human transgenic *SOD1* mouse (Gurney et al., 1994). Later, hemizygous and homozygous mice expressing wild-type and mutated human TDP-43 (Stallings et al., 2010), (Xu et al., 2010) together with mice carrying the *C9ORF72* GGGGCC repeat expansion (Chew et al., 2015) were generated. Albeit animal models have been useful for investigation of some ALS pathological mechanisms, they recapitulate only partial aspects of the disease mostly reproducing familial forms of ALS. Indeed, drug effects in SOD1 transgenic mice were rarely able to predict the same efficacy in humans (Ludolph et al., 2007), (Ludolph et al., 2010). This discrepancy may be explained by the diversity in both structure and development of rodent and human brains together with the absence of naturally occurring ALS in mice. Consequently, animal models may not be the most adequate tool to fully represent the various phenotypes of human ALS. Cellular model systems, such as NSC-34 (Cashman et al., 1992), SH-SY5Y (Biedler et al., 1973) or HEK293T (Graham et al., 1977) have also been widely used and useful to study ALS pathomechanisms *in vitro*. However, they are mostly tumor-derived or engineered cells, not completely

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https://doi.org/10.1016/j.scr.2018.05.009
Received 30 January 2018; Received in revised form 24 April 2018; Accepted 16 May 2018
Available online 17 May 2018

frontiers in Psychology

ORIGINAL RESEARCH
published: 05 April 2018
doi: 10.3389/fpsyg.2018.00450

The Complex Interplay Between Depression/Anxiety and Executive Functioning: Insights From the ECAS in a Large ALS Population

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Antonino Vallesi,
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Reviewed by:
Marco Pitteri,
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Arianna Palmieri,
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Specialty section:
This article was submitted to Cognition, a section of the journal Frontiers in Psychology

Received: *08 January 2018*
Accepted: *19 March 2018*
Published: *05 April 2018*

Citation:
Carelli L, Solca F, Faini A, Madotto F, Lafronza A, Monti A, Zago S, Doretti A, Ciammola A, Ticozzi N, Silani V and Poletti B (2018) The Complex Interplay Between Depression/Anxiety and Executive Functioning: Insights From the ECAS in a Large ALS Population. Front. Psychol. 9:450. doi: 10.3389/fpsyg.2018.00450

Introduction: The observed association between depressive symptoms and cognitive performances has not been previously clarified in patients with amyotrophic lateral sclerosis (pALS). In fact, the use of cognitive measures often not accommodating for motor disability has led to heterogeneous and not conclusive findings about this issue. The aim of the present study was to evaluate the relationship between cognitive and depressive/anxiety symptoms by means of the recently developed Edinburgh Cognitive and Behavioral ALS Screen (ECAS), a brief assessment specifically designed for pALS.

Methods: Sample included 168 pALS (114 males, 54 females); they were administered two standard cognitive screening tools (FAB; MoCA) and the ECAS, assessing different cognitive domains, including ALS-specific (executive functions, verbal fluency, and language tests) and ALS non-specific subtests (memory and visuospatial tests). Two psychological questionnaires for depression and anxiety (BDI; STAI/Y) were also administered to patients. Pearson's correlation coefficient was used to assess the degree of association between cognitive and psychological measures.

Results: Depression assessment negatively correlated with the ECAS, more significantly with regard to the executive functions subdomain. In particular, Sentence Completion and Social Cognition subscores were negatively associated with depression levels measured by BDI total score and Somatic-Performance symptoms subscore. Conversely, no significant correlations were observed between depression level and cognitive functions as measured by traditional screening tools for frontal abilities (FAB) and global cognition (MoCA) assessment. Finally, no significant correlations were observed between state/trait anxiety and the ECAS.

Discussion and conclusion: This represents the first study focusing on the relationship between cognitive and psychological components in pALS by means of the ECAS, the current gold standard for ALS cognitive-behavioral assessment.

frontiers in Molecular Neuroscience

ORIGINAL RESEARCH
published: 28 February 2018
doi: 10.3389/fnmol.2018.00064

The LRRK2 Variant E193K Prevents Mitochondrial Fission Upon MPP+ Treatment by Altering LRRK2 Binding to DRP1

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OPEN ACCESS

Edited by:
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Reviewed by:
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Received: *03 November 2017*
Accepted: *15 February 2018*
Published: *28 February 2018*

Citation:
Perez Carrion M, Pischedda F, Biosa A, Russo I, Straniero L, Civiero L, Guida M, Gloeckner CJ, Ticozzi N, Tiloca C, Mariani C, Pezzoli G, Duga S, Pichler I, Pan L, Landers JE, Greggio E, Hess MW, Goldwurm S and Piccoli G (2018) The LRRK2 Variant E193K Prevents Mitochondrial Fission Upon MPP+ Treatment by Altering LRRK2 Binding to DRP1. Front. Mol. Neurosci. 11:64. doi: 10.3389/fnmol.2018.00064

Mutations in leucine-rich repeat kinase 2 gene (*LRRK2*) are associated with familial and sporadic Parkinson's disease (PD). LRRK2 is a complex protein that consists of multiple domains, including 13 putative armadillo-type repeats at the N-terminus. In this study, we analyzed the functional and molecular consequences of a novel variant, E193K, identified in an Italian family. E193K substitution does not influence LRRK2 kinase activity. Instead it affects LRRK2 biochemical properties, such as phosphorylation at Ser935 and affinity for 14-3-3ε. Primary fibroblasts obtained from an E193K carrier demonstrated increased cellular toxicity and abnormal mitochondrial fission upon 1-methyl-4-phenylpyridinium treatment. We found that E193K alters LRRK2 binding to DRP1, a crucial mediator of mitochondrial fission. Our data support a role for LRRK2 as a scaffolding protein influencing mitochondrial fission.

Keywords: LRRK2, DRP1, mitochondria, protein interaction, Parkinson's disease

INTRODUCTION

Parkinson's disease (PD) is an age-related disorder that affects 2% of the population above 65-years. PD is related to the progressive loss of dopaminergic neurons in the *substantia nigra* (Moore et al., 2005; Poewe et al., 2017) and is clinically characterized by bradykinesia, rigidity and resting tremor. Although the majority of cases do not correlate with clear genetic causes, mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene (PARK8; OMIM 609007) have been unequivocally related to late-onset PD. LRRK2 mutations have been identified in up to 13% of familial PD cases (Paisán-Ruíz et al., 2004; Zimprich et al., 2004) and also account for 1%–2% of not familial cases (Aasly et al., 2005; Goldwurm et al., 2005). LRRK2 protein includes some functional domains such as, from N- to C-terminus armadillo, ankyrin, the namesake leucine-rich repeats, a ROC GTPase

Original Article

Is diabetes a marker of higher risk after carotid revascularization? Experience from a single centre

Diabetes & Vascular Disease Research
2018, Vol. 15(4) 314–321

DOI: 10.1177/1479164118769530
journals.sagepub.com/home/dvr
SAGE

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Abstract

Purpose: This single centre study investigates the influence of diabetes mellitus on outcomes following carotid artery endarterectomy or stenting.

Methods: In total, 752 carotid revascularizations (58.2% carotid artery stenting and 41.8% carotid endarterectomy) were performed in 221 (29.4%) patients with diabetes and 532 (70.6%) patients without diabetes. The study outcomes were death, disabling and non-disabling stroke, transient ischaemic attack and restenosis within 36 months after the procedure.

Results: Patients with diabetes had higher periprocedural risk of any stroke or death (3.6% diabetes vs 0.6% no diabetes; $p < 0.05$), transient ischaemic attack (1.8% diabetes vs 0.2% no diabetes; $p > 0.05$) and restenosis (2.7% diabetes vs 0.6% no diabetes; $p < 0.05$). During long-term follow-up, there were no significant differences in Kaplan–Meier estimates of freedom from death, any stroke and transient ischaemic attack, between people with and without diabetes for each carotid artery stenting and carotid endarterectomy subgroup. Patients with diabetes showed higher rates of restenosis during follow-up than patients without diabetes (36-months estimate risk of restenosis: 21.2% diabetes vs 12.5% no diabetes; $p < 0.05$).

Conclusion: The presence of diabetes was associated with increased periprocedural risk, but no further additional risk emerged during longer term follow-up. Restenosis rates were higher among patients with diabetes.

Keywords

Diabetes mellitus, carotid endarterectomy, stenting, restenosis

Introduction

Diabetes mellitus (DM) is as a major risk factor for both stroke and cardiovascular disease, and its prevalence is high among patients undergoing carotid revascularization.[1,2] In addition, stroke in patients with diabetes is associated with a worse functional outcome and higher mortality compared to their counterparts.[3]

There is conflicting evidence on the effect of DM on the outcomes of patients undergoing carotid endarterectomy (CEA). A number of studies reported increased perioperative risk of stroke and death in patients with diabetes undergoing surgery,[4,5] and in some series, it was a marker of coronary insufficiency and a predictor of cardiac adverse events.[6] On the other hand, other studies have documented similar perioperative outcomes during CEA in both patients with and without diabetes.[7] Moreover, there are few data in the literature concerning the analysis of risk factors influencing early and late results in patient with diabetes undergoing carotid artery stenting (CAS), despite the growing emergence of CAS into clinical practice as an appealing therapeutic alternative.[8–11]

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Received: 6 April 2018 | Revised: 11 September 2018 | Accepted: 7 October 2018

DOI: 10.1002/dmrr.3084

RESEARCH ARTICLE

WILEY

Does metabolic syndrome influence short and long term durability of carotid endarterectomy and stenting?

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Abstract

Aims: The metabolic syndrome (MetS) is composed of a cluster of related cardiovascular risk factors. The aim of the present study was to determine how MetS contributes to short- (30-day) and long-term complications and restenosis after carotid endarterectomy (CEA) or stenting (CAS).

Methods: A consecutive cohort of 752 patients undergoing CEA (n = 314) and CAS (n = 438) in a single institution was examined, of which 296 (39.4%) were identified as having MetS. All patients were followed-up with carotid duplex ultrasound scan of the supraaortic vessels and a neurological assessment of symptoms status at 30-day postprocedure and at 3, 6, and 12 months, with annual follow-up thereafter for 3 years.

Results: Patients with MetS had a significant increased risk in their 30-day death, major adverse events (MAE), and restenosis rates, both after CEA and after CAS (death: 0.7% vs 0.0%; MAE: 5.3% vs 2.7%; and restenosis: 1.7% vs 0.2%; $p < 0.05$). The MAE and restenosis rates remained statistically different at 36 months, with both procedures (29.2% vs 24.2% and 9.5% vs 3.3%, $p < 0.05$, for patients with and without MetS, respectively). Among the components of MetS, high fasting serum glucose, low high-density lipoprotein cholesterol, and elevated body mass index were associated with increased risk of complications at 30 days and within 36 months.

Conclusions: The current study suggested that the presence of MetS is an important risk factor for morbidity and restenosis after CEA and CAS.

KEYWORDS

carotid revascularization, complications, metabolic syndrome, mortality, restenosis

1 | INTRODUCTION

The metabolic syndrome (MetS) is composed of a cluster of related cardiovascular risk factors including hypertension, obesity, and a prediabetic state, which is manifested by high fasting serum blood glucose, high triglycerides, and low levels of high-density lipoprotein (HDL).[1]

The prevalence of MetS is increasing rapidly in the Western world.[2] It is estimated that MetS currently affects between 9% and 35% of the overall population depending on populations studied and MetS definition employed.[3,4] Prior studies have shown a two-fold higher risk of developing cardiovascular disease and a 1.5-fold increased risk of all-cause mortality in patients with MetS compared with patients without MetS.[5]

Consequently, there has been a growing interest in the association between MetS and cardiovascular diseases. People with MetS had both increased mortality from cardiovascular diseases (12.0% vs 2.2%) and increased total mortality (18.0% vs 4.6%) compared with those without MetS.[4] Various studies have shown an increased incidence and more rapid progression of carotid atherosclerosis in

Neurological Sciences
https://doi.org/10.1007/s10072-018-3685-7

ORIGINAL ARTICLE

Heterogeneous brain FDG-PET metabolic patterns in patients with C9orf72 mutation

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Received: 29 September 2018 / Accepted: 10 December 2018

Abstract

Objective The hexanucleotide repeat expansion in C9orf72 is an associated genetic cause in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In the "ALS/FTD" spectrum prevails clinical heterogeneity and an in vivo knowledge of the underling brain dysfunction in patients carrying C9orf72 mutation remain limited and only described at group level. The study aimed to assess the brain metabolic alterations characterizing patients with C9orf72 mutation using FDG-PET in single individuals.

Methods We applied a validated statistical parametric mapping (SPM) voxel-based procedure for FDG-PET data to obtain maps of brain relative hypometabolism and hypermetabolism at single-subject level in six FTD/ALS patients carrying the C9orf72 mutation.

Results Clinical diagnoses classified the patients as right semantic variant of frontotemporal dementia (one case, C9svFTD), behavioral variant of frontotemporal dementia (two cases, C9bvFTD), and bulbar amyotrophic lateral sclerosis (three cases, C9bALS). The FDG-PET SPM revealed a prevalent frontal hypometabolism in C9bvFTD cases, and right temporal polar and lateral involvement in C9svFTD, consistent with the clinical diagnosis. There was a quite comparable occipital and cerebellar hypermetabolism in these cases. The three C9bALS patients showed variable patterns of hypo- and hypermetabolism.

Conclusions The present work is the first in vivo FDG-PET study showing the heterogeneous patterns of brain regional hypo- and hypermetabolism in single patients sharing C9orf72 mutation. Brain hypometabolism was consistent with the clinical phenotypes, supporting the diagnostic importance of neuroimaging functional biomarkers to capture at single-subject level specific brain dysfunction.

Keywords ALS-FTD spectrum · C9orf72 · Heterogeneity · Positron emission tomography · Statistical parametric mapping

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-018-3685-7) contains supplementary material, which is available to authorized users.

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Introduction

In 2011, two international groups have identified in parallel the most common genetic cause of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD): the hexanucleotide repeat expansion C9orf72, a gene strongly implicated in neurodegeneration [1, 2]. The C9orf72 pathogenic expansion is characterized by a clinical heterogeneity, which reflects both the structural and pathological variance of ALS and FTD [3]. At present, according to the clinical, neuropathological, and genetic overlapping features, they are accounted to lay on a continuous spectrum, referred to as ALS/FTD [4–7].

18-Fluorodeoxyglucose positron emission tomography (FDG-PET) studies in patients carrying the C9orf72 mutation are limited and all conducted at a group level, reporting variable cortical and subcortical metabolic alterations [8–11].

Published online: 15 December 2018

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2018; 19: 426–431

RESEARCH ARTICLE

Characterization of the *c9orf72* GC-rich low complexity sequence in two cohorts of Italian and Turkish ALS cases

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Abstract

Large expansions of a noncoding GGGGCC repeat in the *C9orf72* gene are the main cause of amyotrophic lateral sclerosis (ALS). The GGGGCC repeat is contiguous with another GC-rich region. Recent studies reported a significantly higher frequency of insertions/deletions within the GC-rich region in patients carrying the GGGGCC expansion. A GTGGT motif comprised within the GC-rich region, which joins two 100% GC sequences, was frequently deleted, supporting the hypothesis that these deletions could make the region more prone to slippage and pathological expansion. To confirm this hypothesis, we sequenced the GC-rich region adjacent the GGGGCC repeat in ALS patients, 116 *C9orf72* expansion carriers, 219 non-carriers, and 223 healthy controls, from Italian and Turkish cohorts. Deletions were significantly more frequent in *C9orf72* expansion carriers (6%) compared to non-carrier ALS patients (0.46%, OR =14.00, 95% CI =1.71–306.59, $p = 0.003$), to controls (0%, OR =16.29, 95% CI =2.12–725.99, $p = 4.86 \times 10^{-4}$) and to the whole cohort of non-carriers (0.2%, OR =28.51, 95% CI =3.47–618.91, $p = 9.58 \times 10^{-5}$). Among expansion carriers, deletions with or without the GTGGT motif were equally distributed (4 vs. 3). The frequency of insertions was not statistically different between *C9orf72* expansion carriers and any other group including the whole cohort of non-carriers ($p = 0.439$, Fisher's exact test). Our data confirmed the association between deletions within GC-rich region and the GGGGCC expansion in Italian and Turkish cases, although we did not confirm a role of the GTGGT element deletion. Further studies will be therefore necessary to assess the causal relationships between contiguous deletions of the GC-rich region and the GGGGCC expansion.

Keywords: *C9orf72*, *low complexity sequence*, *repeat*, *deletion*, *indel*, *ALS*

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset fatal neurodegenerative disorder affecting mainly the motor system. Degeneration of motor neurons in ALS leads to a progressive and severe muscular weakness with paralysis and death generally occurring within 2–3 years after disease onset because of respiratory failure. Familial forms represent 5–10% of cases, and several causative genes have been identified so far accounting for more than 60% of all inherited forms (1). Large expansions of a noncoding GGGGCC repeat in the *C9orf72* gene have been identified as the main cause of ALS accounting for about 40% of the familial and 7% of the sporadic cases, but also 25% of hereditary forms of frontotemporal degeneration (FTD) (2). The mechanism which induces the GGGGCC repeat to expand from the polymorphic and normal 2–23 unit range to more than 4000 units in ALS/FTD patients are complex and still remains to be elucidated. One

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(Received 1 December 2017; revised 17 January 2018; accepted 3 February 2018)

ISSN 2167-8421 print/ISSN 2167-9223 online © 2018 World F eration of Ne ology on behalf of the Research Group on Motor Neuron Diseases
DOI: 10.1080/21678421.2018.1440407

Neurobiology of Aging xxx (2018) 1.e1–1.e10

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

ALS-associated missense and nonsense *TBK1* mutations can both cause loss of kinase function

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ARTICLE INFO

Article history:
Received 18 April 2018
Received in revised form 12 June 2018
Accepted 12 June 2018

Keywords:
ALS
TBK1
FTD
WES
Familial ALS

ABSTRACT

Mutations in TANK binding kinase 1 (TBK1) have been linked to amyotrophic lateral sclerosis. Some *TBK1* variants are nonsense and are predicted to cause disease through haploinsufficiency; however, many other mutations are missense with unknown functional effects. We exome sequenced 699 familial amyotrophic lateral sclerosis patients and identified 16 *TBK1* novel or extremely rare protein-changing variants. We characterized a subset of these: p.G217R, p.R357X, and p.C471Y. Here, we show that the p.R357X and p.G217R both abolish the ability of TBK1 to phosphorylate 2 of its kinase targets, IRF3 and optineurin, and to undergo phosphorylation. They both inhibit binding to optineurin and the p.G217R, within the TBK1 kinase domain, reduces homodimerization, essential for TBK1 activation and function. Finally, we show that the proportion of TBK1 that is active (phosphorylated) is reduced in 5 lymphoblastoid cell lines derived from patients harboring heterozygous missense or in-frame deletion *TBK1* mutations. We conclude that missense mutations in functional domains of TBK1 impair the binding and phosphorylation of its normal targets, implicating a common loss of function mechanism, analogous to truncation mutations.

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0197-4580/
https://doi.org/10.1016/j.neurobiolaging.2018.06.015

Original Paper

Neuro–degenerative Diseases

Neurodegener Dis 2018;18:38–48
DOI: 10.1159/000486201

Received: July 7, 2017
Accepted after revision: December 8, 2017
Published online: February 9, 2018

Elevated Global DNA Methylation Is Not Exclusive to Amyotrophic Lateral Sclerosis and Is Also Observed in Spinocerebellar Ataxia Types 1 and 2

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Keywords
Amyotrophic lateral sclerosis · Trinucleotide repeat disorder · Spinocerebellar ataxia · ELISA · Global DNA methylation · 5-Methylcytosine

Abstract
Adult-onset neurological disorders are caused and influenced by a multitude of different factors, including epigenetic modifications. Here, using an ELISA kit selected upon careful testing, we investigated global 5-methylcytosine (5-mC) levels in sporadic and familial amyotrophic lateral sclerosis (sALS and fALS), spinocerebellar ataxia types 1 and 2 (SCA1 and SCA2), Huntington's disease, Friedreich's ataxia, and myotonic dystrophy type 1. We report a significant elevation in global 5-mC levels of about 2–7% on average for sALS ($p < 0.01$ [$F(1, 243) = 9.159$, $p = 0.0027$]) and various forms of fALS along with SCA1 ($p < 0.01$ [$F(1, 83) = 11.285$], $p = 0.0012$) and SCA2 ($p < 0.001$ [$F(1, 122) = 29.996$, $p = 0.0001$]) when compared to age- and sex-matched healthy controls. *C9orf72* expansion carrier ALS patients exhibit the highest global 5-mC levels along with *C9orf72* promoter hypermethylation. We failed to measure global 5-hydroxymethylcytosine (5-hmC) levels in blood, probably due to the very low levels of 5-hmC and the limitations of the commercially available ELISA kits. Our results point towards a role for epigenetics modification in ALS, SCA1, and SCA2, and help conclude a dispute on the global 5-mC levels in sALS blood.

Introduction

Epigenetic mechanisms that regulate both coding and noncoding RNA transcription range from DNA methylation to various histone modifications, differential nucleosomal positioning and modifications at the RNA level [1]. One such epigenetic modification is the well-characterized promoter methylation at CpG sites, carried out by DNA methyltransferases (DNMTs) [2]. In addition, another group of enzymes, namely the ten-eleven translocation methylcytosine dioxygenase 1, are responsible for converting 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC), a relatively newly identified DNA

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Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2018; 19: 331–341

RESEARCH ARTICLE

Understanding the use of NIV in ALS: results of an international ALS specialist survey

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Abstract

Objective: To identify common practices of noninvasive ventilation (NIV) use among ALS specialists and how they follow respiratory status in their patients. *Methods:* A 25-item questionnaire on NIV indications/initiation was sent via SurveyMonkey® to ALS specialists identified through membership in NEALS (114 sites in the US) and ENCALS (39 sites in Europe). Descriptive statistics and Cochran–Mantel–Haenszel test for general association were performed. *Results:* In their initial evaluation, US and European specialists ($n = 186$) use upright forced vital capacity (FVC) most (92.8% vs 91.1%; $p = 0.752$). Upright FVC results are most important for US respondents when deciding to prescribe NIV; European respondents consider symptoms of orthopnea and/or dyspnea as most important. European respondents use overnight pulse oximetry (69.8% vs 7.9%; $p < 0.001$) and arterial blood gas analyses (62.8% vs 3.2%; $p < 0.001$) more than US respondents. Insurance regulations/national health care coverage impact NIV initiation more in the US than in Europe (70.0% vs 47.5%; $p = 0.025$). When asked if insurance/other financial constraints affects when they prescribe NIV, more US respondents answered positively (77.2% vs 15.4%; $p < 0.001$). In patients with no respiratory symptoms, most US specialists (68.3%) initiated NIV at VC <50% predicted; European responses showed greater variability. *Conclusions:* Given the impact of NIV on respiratory function and the importance of respiratory function to quality of life and survival, understanding differences that influence NIV prescribing is critical. This information may inform future study design and identify areas warranting additional research to develop best practices for NIV implementation.

Keywords: *Amyotrophic lateral sclerosis*, *noninvasive ventilation*, *respiratory function*, *vital capacity*

Introduction

Amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative disorder, is characterized by the degeneration of motor neurons that causes irreversible weakness of skeletal muscles, including respiratory muscles (1–3). Death often results from respiratory failure (4), typically within 3 to 5 years (5). Declining respiratory muscle function also greatly impacts patients' quality of life (QoL) (6), with weakness leading to dyspnea, orthopnea,

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(Received 9 January 2018; revised 8 March 2018; accepted 18 March 2018)

ISSN 2167-8421 print/ISSN 2167-9223 online © 2018 World Federation of Neurology on behalf of the Research Group on Motor Neuron Diseases
DOI: 10.1080/21678421.2018.1457058

Cardiovascular diseases may play a negative role in the prognosis of amyotrophic lateral sclerosis

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Keywords:
amyotrophic lateral sclerosis, atrial fibrillation, heart diseases, hypertension, platelet disorders, prognostic factors, survival

Received 7 September 2017
Accepted 26 February 2018

European Journal of Neurology 2018, **0:** 1–8

doi:10.1111/ene.13620

Background and purpose: Only a few studies have considered the role of comorbidities in the prognosis of amyotrophic lateral sclerosis (ALS) and have provided conflicting results.

Methods: Our multicentre, retrospective study included patients diagnosed from 1 January 2009 to 31 December 2013 in 13 referral centres for ALS located in 10 Italian regions. Neurologists at these centres collected a detailed phenotypic profile and follow-up data until death in an electronic database. Comorbidities at diagnosis were recorded by main categories and single medical diagnosis, with the aim of investigating their role in ALS prognosis.

Results: A total of 2354 incident cases were collected, with a median survival time from onset to death/tracheostomy of 43 months. According to univariate analysis, together with well-known clinical prognostic factors (age at onset, diagnostic delay, site of onset, phenotype, Revised El Escorial Criteria and body mass index at diagnosis), the presence of dementia, hypertension, heart disease, chronic obstructive pulmonary disease, haematological and psychiatric diseases was associated with worse survival. In multivariate analysis, age at onset, diagnostic delay, phenotypes, body mass index at diagnosis, Revised El Escorial Criteria, dementia, hypertension, heart diseases (atrial fibrillation and heart failure) and haematological diseases (disorders of thrombosis and haemostasis) were independent prognostic factors of survival in ALS.

Conclusions: Our large, multicentre study demonstrated that, together with the known clinical factors that are known to be prognostic for ALS survival, hypertension and heart diseases (i.e. atrial fibrillation and heart failure) as well as haematological diseases are independently associated with a shorter survival. Our findings suggest some mechanisms that are possibly involved in disease progression, giving new interesting clues that may be of value for clinical practice and ALS comorbidity management.

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EUROPEAN JOURNAL OF NEUROLOGY

Original Article

Impact of obstructive sleep apnea on cardiac organ damage in patients with acute ischemic stroke

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Background and purpose: Both obstructive sleep apnea (OSA) and cardiac organ damage have a crucial role in acute ischemic stroke. Our aim is to explore the relationship between OSA and cardiac organ damage in acute stroke patients.

Methods: A total of 130 consecutive patients with acute ischemic stroke were enrolled. Patients underwent full multichannel 24-h polysomnography for evaluation of OSA and echocardiography to evaluate left ventricle (LV) mass index (LV mass/BSA, LV mass/height$^{2.7}$), thickness of interventricular septum (IVS) and posterior wall (LVPW), LV ejection fraction and left atrium enlargement. Information on occurrence of arterial hypertension and its treatment before stroke was obtained from patients' history.

Results: 61.9% (70) of patients, mostly men (67.1%), with acute stroke had OSA (AHI $>$ 10). Patients with acute stroke and OSA showed a significant increase ($P < 0.05$) of LV mass index, IVS and LVPW thickness and a significant left atrial enlargement as compared with patients without OSA. LV ejection fraction was not significantly different in stroke patients with and without OSA and was within normal limits. No relationship was found among cardiac alterations, occurrence of OSA and history of hypertension.

Conclusion: Acute stroke patients with OSA had higher LV mass and showed greater left atrial enlargement than patients without OSA. This study confirms the high prevalence of OSA in stroke patients, suggesting also an association between OSA and cardiac target organ damage. Our finding of structural LV abnormalities in acute stroke patients with OSA suggests a potential role of OSA as contributing factor in determining both cerebrovascular and cardiac damage, even in absence of clear link with a history of blood pressure elevation.

Keywords: arterial hypertension, cardiac organ damage, echocardiography, left atrial enlargement, left ventricular hypertrophy, obstructive sleep apnea, stroke

Abbreviations: ABPM, ambulatory blood pressure monitoring; AHI, apnea–hypopnea index (number of episodes of apnea and hypopnea per hour of sleep); BP, blood pressure; BSA, body surface area; CHF, congestive heart failure; CSA, central sleep apnea; CT, computed tomography; CVD, cardiovascular diseases; IVS, interventricular septum; IVSTd, interventricular septal diastolic thickness; LAE, left atrial enlargement; LV, left ventricle; LVDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVM, left ventricular mass; LVM/BSA, left ventricular mass indexed to body surface area; LVM/H2.7, left ventricular mass indexed to height.2.7; LVPW, left ventricle posterior wall; LVPWTd, left ventricular posterior wall diastolic thickness; NIHSS, National Institutes of Health Stroke Scale; ODI, oxygen desaturation index; OSA, obstructive sleep apnea; PLM index, number of periodic leg movement per hour of sleep; SWS, slow waves sleep; TDI, tissue doppler imaging

INTRODUCTION

Stroke is the second most common cause of death worldwide and it is the leading cause of disability among adults [1,2]. Thus, identification and treatment of risk factors for stroke are of crucial importance to reduce the clinical burden of this condition [3].

Risk factors for ischemic stroke are multifold, including dyslipidemia, hypertension, diabetes mellitus, and obesity.

Moreover, a number of echocardiographic variables suggesting structural and functional cardiac changes have been identified as independent risk factors for stroke [4,5], including left ventricular hypertrophy (LVH) [6] and left atrial enlargement (LAE) [7].

The latter appears to play a particularly important role, with 75% of patients with first ischemic stroke having LAE [8]. Such a structural alteration may favor incidence of ischemic stroke also because it is associated with a higher

Journal of Hypertension 2018, 36:000–000

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Received 25 November 2016 **Revised** 3 January 2018 **Accepted** 24 January 2018

DOI:10.1097/HJH.0000000000001697

Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2018; 19: 387–395

RESEARCH ARTICLE

Cognitive-behavioral longitudinal assessment in ALS: the Italian Edinburgh Cognitive and Behavioral ALS screen (ECAS)

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Abstract

Objective: The study presents data on the longitudinal administration of the Italian Edinburgh Cognitive and Behavioral ALS Screen (ECAS). We investigated cognitive-behavioral performance in a group of ALS patients over time and the feasibility of repeating the ECAS longitudinally compared with standard neuropsychological tests. Finally, correlations between clinical/genetic and cognitive/behavioral data were considered. *Methods*: One hundred and sixty-eight ALS patients were tested at baseline (T_0). Among these, 48 patients performed the ECAS after 6 months (T_1), 18 patients performed it at T_2 (12 months), and five patients were assessed after 24 months (T_3). Participants were also administered two cognitive test (FAB; MoCA) and psychological questionnaires (BDI; STAI/Y). The FBI was carried out with caregivers. *Results*: No cognitive deterioration was found across follow-ups. In contrast, although scores did not change between T_0 and T_1, scores improved significantly for ECAS Total/ALS Non-specific and Memory domains when the ECAS was repeated on three occasions (T_0, T_1, T_2). Apathy/Inertia was the most common behavioral symptom, but no worsening of behavioral scores was detected over time. After 12–24 months, patients were still able to perform the ECAS in total, in contrast to FAB and MoCA, which were only partially administrable. *Conclusions*: The significant improvement of some ECAS scores over time supports the presence of possible practice effects, particularly in the memory domain, highlighting the need to accommodate for these in longitudinal assessments, through healthy controls groups or alternate versions. This work represents the first Italian ECAS follow-up study and confirms ECAS feasibility in patients with increasing physical disability.

Keywords: *ECAS*, *longitudinal assessment*, *amyotrophic lateral sclerosis*, *cognition*, *behavioral change*, *practice effect*

Introduction

Cognitive-behavioral changes in patients with amyotrophic lateral sclerosis (ALS) are now fully recognized as integral elements of the disease, along a spectrum of frontotemporal dysfunctions (1,2). In recent years, several cognitive screening tools have been developed for ALS (3–8); however, they are not designed to detect a heterogeneous cognitive involvement (9–11), nor to compensate for patients' physical disability (6,12,13). In order to overcome such limitations, Abrahams et al. (14) developed a rapid cognitive-behavioral screening tool (Edinburgh Cognitive and Behavioral ALS Screen – ECAS), specifically designed to accommodate for verbal/motor disability. The ECAS has been translated (15–17) and validated against gold standard

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(Received 28 February 2018; revised 16 April 2018; accepted 23 April 2018)

ISSN 2167-8421 print/ISSN 2167-9223 online © 2018 World Federation of Neurology on behalf of the Research Group on Motor Neuron Diseases
DOI: 10.1080/21678421.2018.1473443

REVIEW

Sexuality and intimacy in ALS: systematic literature review and future perspectives

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ABSTRACT

Several features of amyotrophic lateral sclerosis (ALS) impact on sexuality and intimate relationship; however, the issue has received poor attention so far. We performed a systematic literature review in order to provide an up-to-date account of sexuality in ALS. References were identified by searches of PubMed, Web of Science, Scopus and PsycINFO (1970–2017, English literature). The following were the key terms: 'sexual' OR 'sexuality' OR 'intimacy' OR 'marital' AND 'ALS' OR 'Amyotrophic Lateral Sclerosis' OR 'Motor Neuron Disease' OR 'MND'. Titles and abstracts were screened for relevance and a full-text analysis was performed on the selected articles. Studies were included if they referred to sexual well-being/activities/functions or intimate relationship between patients and their partners and management of such topic by clinicians. Eligibility assessment was performed independently by two reviewers. A thematic and level of evidence classification of studies was performed. Studies' design, objectives, measurements and outcomes were summarised. Thirty articles were included and four topics were identified: intimacy in the dyads; sexual activities in patients and with their partners; sexual function disturbances; and sexuality and cognitive-behavioural alterations. The quality of the studies varies, with globally poor level of evidence. Some sexuality issues have been only sparsely addressed, such as gender-related differences, same-sex relationships and sexual activities other than intercourse. Sexuality in ALS is still not adequately considered by clinicians and researchers. We present preliminary recommendations for improving sexuality and intimacy at any ALS multidisciplinary clinics.

INTRODUCTION

Sexuality in patients with amyotrophic lateral sclerosis (ALS) has received poor attention so far. One possible reason is that ALS represents a severe and progressive neurological disorder leading clinicians and researchers to focus on critical features exerting an effect on treatment and survival, such as movement, respiratory and nutritional aspects. Moreover, patients themselves often express the feeling that when everyday survival is an issue, talking about sexuality is rather embarrassing and an inappropriate subject.[1] However, even if not directly affecting survival, sexual activities are well recognised as an important component of daily life and of intimate relationships; they have significant impact on emotional well-being and therefore, indirectly, on disease-related aspects as observed in other disorders, both neurological or due to other aetiologies.[2 3] Another possible explanation for the poor consideration of sexuality issue in ALS is that this condition affects the motor system, causing skeletal muscle weakness, but it does not directly involve sexual functions. However, there are several aspects of ALS disease and progression that could influence sexuality and intimate relationship, involving both physical, cognitive-behavioural, emotional and psychosocial dimensions. In fact, both physical weakness and psychological features (ie, poor self-esteem, change in one's body image, depression) can indirectly impact sexuality. Moreover, the presence of contrasting representations of disease limitations and consequences among patients and their partners could impact on desire and willingness to engage in sexual activities.[4 5] Another reason to investigate sexuality in ALS is related to the presence of possible ALS-frontotemporal spectrum alterations, previously described and also involving disinhibition and inappropriate sexual behaviour, as observed in other neurodegenerative disorders.[6 7]

Starting from the observation that sexuality plays a crucial role in personal well-being, and from the inadequate consideration of this aspect in ALS care, we aimed to collect and summarise existing information about sexuality issues in ALS. The objectives of the present systematic review were twofold: to provide an up-to-date account of sexuality aspects and changes in ALS and to highlight both available and missing information/practices in literature and clinical care. As an outcome, we intended to provide preliminary recommendations for improving sexuality in patients with ALS and their partners within ALS multidisciplinary clinics. Specifically, the following are our questions: Is there evidence of an adequate consideration of sexuality-related topics among both researchers and clinicians in literature and clinical practice? Are patients' and spouses' needs and quality of life (QoL) issues related to sexuality and intimacy taken into account, or is most clinicians' attention focused on alterations or dysfunctions from a clinical/pathological point of view? Then, if both patients' and spouses' point of views are considered in the literature, is there any difference between them concerning sexuality interest or satisfaction?

METHODS

Eligibility criteria

Patients with ALS, together with their carers and health professionals, were considered as study participants. Both inpatients and outpatients were considered, of any age and at any disease stage. Due to the limited consideration of sexuality issues in the literature, studies of any type (experimental, observational, single-case studies and literature

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/jnnp-2018-319684).

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Received 20 September 2018
Revised 5 November 2018
Accepted 5 November 2018

To cite: Poletti B, Carelli L, Solca F, *et al*. *J Neurol Neurosurg Psychiatry* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jnnp-2018-319684

PLOS ONE

RESEARCH ARTICLE

The Arrows and Colors Cognitive Test (ACCT): A new verbal-motor free cognitive measure for executive functions in ALS

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OPEN ACCESS

Citation: Poletti B, Carelli L, Faini A, Solca F, Meriggi P, Lafronza A, et al. (2018) The Arrows and Colors Cognitive Test (ACCT): A new verbal-motor free cognitive measure for executive functions in ALS. PLoS ONE 13(8): e0200953. https://doi.org/10.1371/journal.pone.0200953

Editor: Alfonso Fasano, University of Toronto, CANADA

Received: February 2, 2018

Accepted: July 4, 2018

Published: August 9, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The presented work was partly funded by Finlombarda 16971/SAL52 within the "eBrain: BCI and ET for ALS" Lombardy Region project. The remaining part of resources involved in the study were provided by our organization, i.e. IRCCS Istituto Auxologico Italiano. There was no additional external funding received for this study. VS received the funding. The funders had no role in

Abstract

Background and objective

The presence of executive deficits in patients with Amyotrophic Lateral Sclerosis is well established, even if standardized measures are difficult to obtain due to progressive physical disability of the patients. We present clinical data concerning a newly developed measure of cognitive flexibility, administered by means of Eye-Tracking (ET) technology in order to bypass verbal-motor limitations.

Methods

21 ALS patients and 21 age-and education-matched healthy subjects participated in an ET-based cognitive assessment, including a newly developed test of cognitive flexibility (Arrows and Colors Cognitive Test–ACCT) and other oculomotor-driven measures of cognitive functions. A standard screening of frontal and working memory abilities and global cognitive efficiency was administered to all subjects, in addition to a psychological self-rated assessment. For ALS patients, a clinical examination was also performed.

Results

ACCT successfully discriminated between patients and healthy controls, mainly concerning execution times obtained at different subtests. A qualitative analysis performed on error distributions in patients highlighted a lower prevalence of perseverative errors, with respect to other type of errors. Correlations between ACCT and other ET-based frontal-executive measures were significant and involved different frontal sub-domains. Limited correlations were observed between ACCT and standard 'paper and pencil' cognitive tests.

Multiple Sclerosis and Related Disorders 25 (2018) 192–195

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

No *C9orf72* repeat expansion in patients with primary progressive multiple sclerosis

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ARTICLE INFO

Keywords:
Primary progressive multiple sclerosis
C9orf72
Amyotrophic lateral sclerosis
Frontotemporal dementia
Intermediate alleles

ABSTRACT

Pathological repeat expansion (RE) of the *C9orf72* hexanucleotide sequence is associated to amyotrophic lateral sclerosis (ALS) and frontotemporal dementia disease *continuum*, although other heterogeneous clinical phenotypes have been documented. The occurrence of multiple sclerosis (MS) in some *C9orf72* carriers with a more severe ALS disease course has suggested a possible modifying role for MS. However, *C9orf72* RE seems not to play a role in MS pathogenesis. In this study, we screened *C9orf72* in 189 Italian patients with primary progressive MS (PPMS), a rare clinical form characterized by less inflammation over neurodegenerative features. We failed to detect *C9orf72* RE, but a significant representation of intermediate alleles (≥ 20 units) was observed in our PPMS cohort (2.1%) compared to healthy controls (0%, $p < 0.05$). In the normal range, allele distribution showed a trimodal pattern (2,5,8-repeat units) in PPMS and healthy controls with no significant difference. Our findings further demonstrate that *C9orf72* RE is not genetically associated to MS spectrum, but suggest that intermediate alleles may represent risk factors as already reported for Parkinson disease.

1. Introduction

A hexanucleotide GGGGCC repeat expansion (RE) in the non-coding region of *C9orf72* gene represents the main genetic cause of familial and sporadic forms of both amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), two neurodegenerative disorders now considered part of the same disease *continuum* (Bennion Callister and Pickering-Brown, 2014; DeJesus-Hernandez et al., 2011; Renton et al., 2011). Interestingly, the occurrence of heterogeneous clinical phenotypes, including parkinsonism, psychosis, Alzheimer's disease (AD), has been reported in ALS and FTD patients carrying *C9orf72* RE and in their relatives, broadening the spectrum of possible *C9orf72*-associated diseases (Akimoto et al., 2013; Cooper-Knock et al., 2014; Harms et al., 2013; Lesage et al., 2013; Ticozzi et al., 2014).

The role of *C9orf72* RE has been also investigated in the pathogenesis of multiple sclerosis (MS), a chronic immune-mediated disorder of the central nervous system characterized by demyelination and neurodegeneration processes. Notably, the disease course of MS is highly heterogeneous, with approximately 85% of patients presenting with relapsing-remitting MS (RRMS), characterized by episodes of acute worsening of function followed by partial or complete recovery (Goodin et al., 2016). The remaining 10–15% of MS cases exhibit a continuous progression of neurological disability since disease onset without relapsing-remitting phases, a condition referred as primary progressive MS (PPMS) (Polman et al., 2011). Even if there is no evidence of genetic differences between RRMS and PPMS, there are distinctive features including a different male: female ratio (from 1:2 to 1:1.3) and age at onset (a decade later in PPMS) (Ebers, 2004). Although the concurrence of ALS and MS is extremely rare, previous epidemiological studies have reported a possible family aggregation between these two diseases, with a several-fold increase of ALS occurrence in the first degree relatives of MS patients and vice versa

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https://doi.org/10.1016/j.msard.2018.07.047
Received 20 March 2018; Received in revised form 29 May 2018; Accepted 31 July 2018

Multiple Sclerosis and Related Disorders 27 (2019) 79–80

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Commentary

Response to the commentary "The effect of C9orf72 intermediate repeat expansions in neurodegenerative and autoimmune diseases" by Biasiotto G and Zanella I.☆

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Dear Editor,

We thank Dr. Biasiotto and Dr. Zanella for their comments about our recent paper (Tiloca et al., 2018), because they address the important issue on the need to define a common cut-off value to discriminate between *C9orf72* "normal/low" and "intermediate" alleles (IA), never to be confused with pathological repeat expansions. After carefully revising literature, in our study we considered as IAs those alleles ranging from 20 to 30 repeats. The 20-units as the lower threshold value for IA was adopted in the original paper by Renton et al., based on the allele frequency observed in ALS and/or FTD cases and controls (Renton et al., 2011). Moreover, alleles in the 20–30 unit range have been reported to be rare and of uncertain clinical significance in the majority of genetic reports on *C9orf72*-related disorders, as recently revised (Ng and Tan, 2017). As Biasiotto and Zanella point out, we are aware that the definition of IA range and the role of repeat length in the non-pathological range in disease risk and/or clinical phenotype is still under debate. In few studies the lower IA repeat number was set at 7 (Cacace et al., al.,2013; Jiao et al., 2013; Wang et al., 2015), supported also by the finding that alleles with ≥ 9 repeat units show a decreased transcriptional activity by in vitro reporter gene analyses (van der Zee et al., 2013; Gijselinck et al., 2016), although no other studies on human RNA samples were available to confirm these gene expression data. Moreover, the additional effect of IAs > 7 on *C9orf72* promoter methylation degree, as stated in the Commentary, was indeed found to be very modest and significantly different in control samples and not in *C9orf72*-negative ALS/FTD patients harbouring IAs in a homozygous state (Gijselinck et al., 2016). Although a slightly differential methylation of the GGGGCC repeat itself was identified in the normal/short range versus the intermediate range-carrying individuals (both in cases and in controls) (Gijselinck et al., 2016), the epigenetic contribution of IAs need to be further confirmed on larger case-control populations given the under-representation of longer IAs.

Another important issue addressed in the Commentary regards the possibility of genetic repeat instability of *C9orf72* IAs. Intergenerational studies conducted so far seem to indicate that IAs up to 22 repeats are prone only to a small repeat instability (0,29%), but not to pathological expansion, and are stably inherited (Beck et al., 2013). The propensity to expand to a full pathological range has been documented so far only for alleles ≥ 70 units (Beck et al., 2013; Xi et al., 2015), which is far beyond the IA size. In this context of repeat instability, another important question is whether intermediate repeat size in blood could be indeed associated with full repeat expansions in CNS tissues, as *C9orf72* repeat expansion size may show high variability between neuronal and non-neuronal tissues and also among different brain areas (Van Mossevelde et al., 2017). Although more studies on post-mortem brains are needed, the few data available in literature seem to exclude this occurrence. Pamplett et al., (2013) compared differences in *C9orf72* repeats size between DNA samples from CNS and blood in sporadic ALS patients and demonstrated no instability for alleles with a repeat number in the intermediate range of 8–30 units. Similarly, another study demonstrated that *C9orf72* repeat sizes below 16 units in blood are somatically stable in brain tissues (Nordin et al., 2015).

In our PPMS cohort, the presence of two additional patients with a 19-repeat allele may be considered a borderline finding as stated by the authors in their Commentary, but here, again, a common definition for

☆Declaration of Interest: None

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https://doi.org/10.1016/j.msard.2018.10.007
Received 2 October 2018; Accepted 13 October 2018

RESEARCH PAPER

Neurofilament light chain in serum for the diagnosis of amyotrophic lateral sclerosis

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Received 25 April 2018
Revised 3 July 2018
Accepted 24 July 2018

To cite: Verde F, Steinacker P, Weishaupt JH, *et al*. *J Neurol Neurosurg Psychiatry* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jnnp-2018-318704

ABSTRACT

Objective To determine the diagnostic and prognostic performance of serum neurofilament light chain (NFL) in amyotrophic lateral sclerosis (ALS).

Methods This single-centre, prospective, longitudinal study included the following patients: 124 patients with ALS; 50 patients without neurodegenerative diseases; 44 patients with conditions included in the differential diagnosis of ALS (disease controls); 65 patients with other neurodegenerative diseases (20 with frontotemporal dementia, 20 with Alzheimer's disease, 19 with Parkinson's disease, 6 with Creutzfeldt-Jakob disease (CJD)). Serum NFL levels were measured using the ultrasensitive single molecule array (Simoa) technology.

Results Serum NFL levels were higher in ALS in comparison to all other categories except for CJD. A cut-off level of 62 pg/mL discriminated between ALS and all other conditions with 85.5% sensitivity (95% CI 78% to 91.2%) and 81.8% specificity (95% CI 74.9% to 87.4%). Among patients with ALS, serum NFL correlated positively with disease progression rate (r_s=0.336, 95% CI 0.14 to 0.506, p=0.0008), and higher levels were associated with shorter survival (p=0.0054). Serum NFL did not differ among patients in different ALS pathological stages as evaluated by diffusion-tensor imaging, and in single patients NFL levels were stable over time.

Conclusions Serum NFL is increased in ALS in comparison to other conditions and can serve as diagnostic and prognostic biomarker. We established a cut-off level for the diagnosis of ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease causing demise of motor neurons of the cerebral cortex, brainstem and spinal cord. This leads to progressive paralysis ending with death from respiratory failure after a median of 30 months. Up to 10% of patients with ALS are also affected by frontotemporal dementia (FTD),[1] while 5%–10% of ALS cases are familial and caused by mutations in known genes.[2] The diagnosis of ALS is primarily clinical; fluid biomarkers have not yet entered clinical practice, but they are urgently needed for diagnosis, prognosis, patient stratification in clinical trials and monitoring of drug effects.[3]

The most promising biomarkers in ALS are the light chain and phosphorylated heavy chain of neurofilaments (NFL and pNFH, respectively), cytoskeletal proteins of large myelinated axons of neurons and therefore axonal impairment markers.[4] NFL and pNFH are elevated in the cerebrospinal fluid (CSF) of patients with ALS relative to controls, with diagnostic sensitivities and specificities >80%; they also correlate with disease progression rate and survival.[5–7] Likewise, blood NF levels are higher in ALS in comparison to controls, but to date they have not been studied in a systematical comparison between ALS and several neurological control groups.[8–11] Therefore, we evaluated the usefulness of serum NFL in the diagnosis of ALS, as well as its role as prognostic biomarker and its stability over time.

METHODS

Participants and clinical characterisation

This prospective longitudinal study included 283 patients investigated in the Department of Neurology of Ulm University Hospital, Germany, between 2010 and 2016. Patients were subdivided as follows: 124 patients (74 (59.7%) male (M) and 50 (40.3%) female (F)) with ALS; 50 patients (23 (46%) M and 27 (54%) F) admitted to a neurological inpatient clinic but without a final diagnosis of degenerative or inflammatory central nervous system (CNS) disease (non-neurodegenerative controls); 44 patients (32 (72.7%) M and 12 (27.3%) F) with conditions included in the differential diagnosis of ALS (disease controls); 20 patients (11 (55%) M and 9 (45%) F) with FTD; 20 patients (8 (40%) M and 12 (60%) F) with Alzheimer's disease (AD); 19 patients (12 (63.2%) M and 7 (36.8%) F) with Parkinson's disease (PD); and 6 patients (4 (66.7%) M and 2 (33.3%) F) with Creutzfeldt-Jakob disease (CJD).

Patients with ALS were selected according to availability of blood samples and MRI examinations taken at the same time points. Patients of all other disease categories were selected according to a random criterion.

Patients with ALS had a diagnosis of definite or probable ALS according to the revised El Escorial criteria[12]; among them, four also had concomitant FTD. Disease severity was expressed by the score

Neurobiology of Aging 67 (2018) 21–22

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Negative results

Chromogranin A levels in the cerebrospinal fluid of patients with amyotrophic lateral sclerosis

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ARTICLE INFO

Article history:
Received 8 October 2017
Received in revised form 18 February 2018
Accepted 19 February 2018
Available online 27 February 2018

Keywords:
Amyotrophic lateral sclerosis (ALS)
Chromogranin A (CgA)
Phosphorylated neurofilament heavy chain (pNFH)
Cerebrospinal fluid (CSF)

ABSTRACT

Chromogranin A (CgA) is a protein found in large dense-core vesicles of neuroendocrine cells and neurons and regulating secretion. A relevance to amyotrophic lateral sclerosis (ALS) was suggested as its overexpression accelerates disease onset in model systems and it interacts with mutant forms of SOD1. Recently, increased cerebrospinal fluid (CSF) CgA levels have been reported in ALS patients relative to controls. With the aim of confirming this finding, we measured CgA and phosphorylated neurofilament heavy chain (pNFH), an established ALS biomarker, in the CSF of 32 ALS patients and 32 disease controls. ALS patients had clearly increased pNFH levels ($p < 0.0001$), while CgA levels were only modestly lower relative to controls ($p = 0.0265$), with wide value overlap and consequently poor discriminative performance. CgA did not correlate with any disease parameters among ALS patients. Our findings suggest that CgA is not a promising clinical biomarker for ALS.

Chromogranin A (CgA), a 439–amino acid protein found in secretory large dense-core vesicles of neuroendocrine cells and neurons, could play a role in the pathogenesis of amyotrophic lateral sclerosis (ALS) (Ezzi et al., 2010) and has recently been reported to be raised in the cerebrospinal fluid (CSF) of ALS patients relative to controls (Kaiserova et al., 2017).

We measured CSF levels of CgA and phosphorylated neurofilament heavy chain (pNFH), an established ALS biomarker (Steinacker et al., 2016), in 32 ALS patients (17 [53.1%] males and 15 [46.9%] females; median age, 64.5 years; age range, 47–80 years) and 32 disease controls (16 [50%] males and 16 [50%] females; median age, 66.5 years; age range, 49–81 years) without degenerative or inflammatory CNS diseases, who had the following diagnoses: cranial nerve palsies (N = 12), polyneuropathy (N = 2), transient global amnesia (N = 2), septic encephalopathy (N = 1), somatization disorder (N = 1), subjective memory complaints (N = 1), headache (N =1), anterior ischemic optic neuropathy (N = 1), Meige's syndrome (N = 1), cerebral arteriovenous malformation (N = 1), postherpetic neuralgia (N = 1), cervical radiculopathy (N = 1), barbiturate intoxication (N = 1), paresthesias with no evident etiology (N = 1), cataracts (N = 1), eye foreign body (N = 1), parapharyngeal abscess (N = 1), supraspinatus tendon lesion (N = 1), and arthralgias with rheumatological etiology (N = 1). Among ALS patients, disease onset was bulbar in 15 (46.9%) and spinal in 17 (53.1%); median age of onset was 63 years (range, 46–79 years). Median disease duration from onset to sampling was 11.5 months (range, 3–145 months). Two patients had concomitant frontotemporal dementia (FTD). All patients were investigated in Ulm University Hospital between 2011 and 2016 and provided written informed consent for the study. CSF sampling for ALS patients and controls occurred prospectively during in-hospital evaluation, before a clinical diagnosis was made. CSF was collected by lumbar puncture, centrifuged, and stored within 2 hours at −80 °C until analysis, according to current guidelines on biobanking for neurological biomarker research, thus ensuring long-term stability of protein biomarkers (Teunissen et al., 2009). None of the samples had previously undergone any additional freeze-thaw cycles apart from the one required for storage and measurement *per se*. The measurements were performed using sandwich ELISA kits provided by BioVendor (Brno, Czech Republic).

ALS patients had slightly lower CSF CgA levels than controls (median, 70.8 ng/mL vs. 86.7 ng/mL, respectively), with low statistical significance ($p = 0.0265$, 2-tailed unpaired Mann-Whitney U test), wide overlap between patients and controls, and consequently low discriminative performance (area under the receiver operating characteristic curve [AUC], 0.6616; 95% CI, 0.5294–0.7938; $p = 0.0263$). This contrasted with the clear increase in CSF pNFH levels in ALS

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0197-4580/$ – see front matter © 2018 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.neurobiolaging.2018.02.017