FRONTESPIZI
LAVORI SCIENTIFICI
2012

“CENTRO DINO FERRARI”

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

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The Progranulin (GRN) Cys157LysfsX97 Mutation is Associated with Nonfluent Variant of Primary Progressive Aphasia Clinical Phenotype

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Handling Associate Editor: Benedetta Nacmias

Abstract. The progranulin gene (GRN) g.10325_10331delCTGCTGT (relative to nt1 in NG_007886.1), alias Cys157LysfsX97, has been so far reported only once in a patient with frontotemporal dementia. Here, we describe a 63-year old patient carrying the same mutation, presenting with a 3-year history of language disorder, and diagnosed clinically with nonfluent variant of primary progressive aphasia according to current criteria. This patient’s description expands the spectrum of clinical presentations of frontotemporal lobar degeneration caused by the GRN Cys157LysfsX97 mutation.

Keywords: Alzheimer’s disease, Dementia, frontotemporal lobar degeneration, GRN mutation, nonfluent variant of primary progressive aphasia, progranulin

Supplementary data available online: http://www.j-alz.com/issues/28/vol28-4.html#supplementarydata02

INTRODUCTION

Since the discovery of mutations in progranulin gene (GRN) associated with autosomal dominant frontotemporal lobar degeneration (FTLD) [1, 2], a wide number of different mutations have been described (http://www.molgen.ua.ac.be/). The majority of them leads to haploinsufficiency and is associated with a wide clinical heterogeneity (see [3] for review). Whereas the Arg493X still remains the most common GRN mutation worldwide [4], increasing evidence confirms the high frequency of the g.11019_11022delCACT mutation, particularly in Northern Italy [5–8]. Conversely, the frequency of other mutations is quite rare. Among these, the
Early Onset Behavioral Variant Frontotemporal Dementia due to the C9ORF72 Hexanucleotide Repeat Expansion: Psychiatric Clinical Presentations

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Handling Associate Editor: Andreas Reif

Accepted 15 April 2012

Abstract. A hexanucleotide repeat expansion in the first intron of C9ORF72 has been shown to be responsible for a high number of familial cases of amyotrophic lateral sclerosis or frontotemporal lobar degeneration with or without concomitant motor neuron disease phenotype and TDP-43 based pathology. Here, we report on three cases carrying the hexanucleotide repeat expansion with an atypical presentation consisting in the development of psychiatric symptoms. Patient #1, a 53 year old man with positive family history for dementia, presented with mood deflection, characterized by apathy, social withdraw, and irritability in the last two years. He was diagnosed with “mild cognitive impairment due to depressive syndrome” six months later and subsequently with Alzheimer’s disease. Patient #2, a woman with positive family history for dementia, developed behavioral disturbances, aggressiveness, and swearing at 57 years of age. Patient #3 presented, in the absence of brain atrophy, with mystical delirium with auditory hallucinations at 44 years of age, and did not present neurological symptoms over a 7-year follow up. The description of these cases underlines that the hexanucleotide repeat expansion in chromosome 9 could be associated with early onset psychiatric presentations.

Keywords: C9ORF72 hexanucleotide repeat expansion, delirium, dementia, frontotemporal lobar degeneration, psychosis

INTRODUCTION

A hexanucleotide repeat expansion in the C9ORF72 gene has been shown to be responsible for many cases of autosomal dominant inherited amyotrophic lateral sclerosis (ALS) or frontotemporal lobar degeneration (FTLD) with or without concomitant motor neuron disease phenotype and TAR DNA binding Protein (TDP)-43 pathology [1, 2].

This mutation causes the loss of one or more alternatively spliced transcript(s) of unknown function and the formation of nuclear RNA foci, suggesting multiple disease mechanisms [1, 3]. Wild-type alleles contain no more than 23–30 repeats, whereas mutated alleles have hundreds to thousands repeats. These studies thus demonstrated that C9ORF72 mutation could represent a major cause of both familial FTLD (11.7%) and ALS cases (38.1%), with a higher prevalence in
Research Article

The Impact of Osteopontin Gene Variations on Multiple Sclerosis Development and Progression

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Received 15 June 2012; Revised 3 August 2012; Accepted 6 August 2012

Academic Editor: Timothy B. Niewold

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Osteopontin is a proinflammatory molecule, modulating TH1 and TH17 responses. Several reports suggest its involvement in multiple sclerosis (MS) pathogenesis. We previously reported that OPN gene variations at the 3′ end are a predisposing factor for MS development and evolution. In this paper, we extended our analysis to a gene variation at the 5′ end on the −156G > GG single nucleotide polymorphism (SNP) and replicated our previous findings at the 3′ end on the +1239A > C SNP. We found that only +1239A > C SNP displayed a statistically significant association with MS development, but both +1239A and −156G had an influence on MS progression, since patients homozygous for both +1239A and −156G alleles displayed slower progression of disability and slower switch to secondary progression than those carrying +1239C and/or −156G and those homozygous for +1239A only. Moreover, patients homozygous for +1239A also displayed a significantly lower relapse rate than those carrying +1239C, which is in line with the established role of OPN in MS relapses.

1. Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterized by an autoimmune response against the myelin sheaths and axons, resulting in progressive neurological dysfunction [1]. Patients with MS display variable clinical course; at onset, approximately 10% of patients display a primary progressive form (PP), whereas the remainder start out with a relapsing remitting form (RR), and most of them switch to a secondary progressive form (SP) within 10–30 years [2]. Both genetic and environmental factors are involved in the development/progression of MS, and several studies point to a complex inheritance involving interactions between combinations of loci that may influence the immune response [3, 4]. An increasing bulk of data suggest that osteopontin (OPN) may play a role in the pathogenesis of MS [5]. OPN is a 60 kDa-secreted phosphoprotein functioning as a free cytokine in body fluids or as an immobilized extracellular matrix molecule in mineralized tissue [6]. OPN serum levels are increased in several autoimmune diseases and may influence development of these diseases through the OPN immunoregulatory effects enhancing the proinflammatory T helper type 1 (TH1) and TH17 cell responses and inhibiting the TH2 responses [7].

OPN transcript is abundant in plaques dissected from brains of patients with MS, whereas it is absent in control
Genetics and Expression Analysis of the Specificity Protein 4 Gene (SP4) in Patients with Alzheimer’s Disease and Frontotemporal Lobar Degeneration

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Handling Associate Editor: Emilio Di Maria

Accepted 19 April 2012

Abstract. Transcription factor Sp4 (Specificity protein 4) levels are increased in the brain of patients with Alzheimer’s disease (AD), and Sp4 colocalizes with neurofibrillary tangles. Moreover, SP4 is a susceptibility gene for bipolar disorder and schizophrenia, which share many clinical features with frontotemporal lobar degeneration (FTLD). The distribution of three tagging single nucleotide polymorphisms (SNPs)—rs9639379, rs10272006, and rs6461569—has been determined in a population of 352 patients diagnosed clinically with AD, 290 patients with FTLD, and 341 age-matched controls. Expression analysis of SP4 was performed in peripheral blood mononuclear cells (PBMC). No significant differences in either allelic or genotypic frequency of the three SNPs were found (p > 0.05), even stratifying according to gender and to the apolipoprotein E status. Significantly increased SP4 relative expression levels were observed in PBMC from patients with AD as compared with controls.

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Epigenetic Regulation of Fatty Acid Amide Hydrolase in Alzheimer Disease

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Abstract

Objective: Alzheimer disease (AD) is a progressive, degenerative and irreversible neurological disorder with few therapies available. In search of new potential targets, increasing evidence suggests a role for the endocannabinoid system (ECS) in the regulation of neurodegenerative processes.

Methods: We have studied the gene expression status and the epigenetic regulation of ECS components in peripheral blood mononuclear cells (PBMCs) of subjects with late-onset AD (LOAD) and age-matched controls (CT).

Results: We found an increase in fatty acid amide hydrolase (faah) gene expression in LOAD subjects (2.30±0.48) when compared to CT (1.00±0.14; *p<0.05) and no changes in the mRNA levels of any other gene of ECS elements. Consistently, we also observed in LOAD subjects an increase in FAAH protein levels (CT: 0.75±0.04; LOAD: 1.11±0.15; *p<0.05) and activity (pmol/min per mg protein CT: 103.80±8.73; LOAD: 125.10±4.00; *p<0.05), as well as a reduction in DNA methylation at faah gene promoter (CT: 55.90±4.60%; LOAD: 41.20±4.90%; *p<0.05).

Conclusions: Present findings suggest the involvement of FAAH in the pathogenesis of AD, highlighting the importance of epigenetic mechanisms in enzyme regulation; they also point to FAAH as a new potential biomarker for AD in easily accessible peripheral cells.

Introduction

Alzheimer disease (AD) is the most frequent form of dementia in the elderly, affecting more than 25 million people worldwide; it is characterized by progressive deterioration of cognition and memory as a result of selective neuronal loss in the hippocampus and cerebral cortex.

Current treatments for AD provide only palliative approaches [1], and in the search for new therapeutic targets the “endocannabinoid system” (ECS) recently emerged as a promising candidate, due to its role in neuroinflammatory and neurodegenerative diseases [2,3]. In the past centuries, cannabinoids have been used for the treatment of various diseases [4], but only recently the mechanisms by which these compounds exert their effects began to be understood. G-protein-coupled type-1 and type-2 cannabinoid receptors (CB1 and CB2), located in both the central nervous system and the periphery, have been characterized along with their two main endogenous ligands, the ethanolamine of arachidonic acid [“anandamide” (AEA)], and 2-arachidonoyl-glycerol (2-AG) [4,5]. AEA and 2-AG are endocannabinoids (eCBs) able to activate also non-CB1/non-CB2 receptors and/or a purported “CB3” (or GPR55) receptor. Furthermore AEA, but not 2-AG, behaves as a ligand to type-1 vanilloid receptor (TRPV1) channels (for a detailed review see Pertwee [6]). AEA is synthesized through multiple pathways, of which the best characterized is catalyzed by N-acyl-phosphatidylethanolamines-hydrolyzing phospholipase D (NAPE-PLD). The biological activity of AEA through its receptors is terminated upon intracellular degradation by fatty acid amide hydrolase (FAAH). Instead, 2-AG is mainly synthesized by an sn-1-specific diacylglycerol lipase (DAGL), and is degraded by a specific monoacylglycerol lipase (MAGL) (for detailed review see Di Marzo [7]). AEA, 2-AG and congeners, their target receptors and the respective metabolic enzymes form the ECS [8,9,10]. Accumulated evidence shows that both exogenous plant-derived and endogenous cannabinoids are neuroprotective [11]. Additional...
Clinical, Neuropathological, and Genetic Characteristics of the Novel IVS9+1delG
GRN Mutation in a Patient with Frontotemporal Dementia

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Handling Associate Editor: Beatrice Arosio

Accepted 22 January 2012

Abstract. Frontotemporal lobar degeneration (FTLD) refers to a clinically, pathologically, and genetically heterogeneous group of dementias that arises from the degeneration of the frontal and temporal lobes. Mutations in the progranulin gene (GRN) are a major cause of FTLD with TDP-43 inclusions. Herein, we describe the clinical, neuropathological, and genetic findings in a case of autosomal dominant behavioral variant of frontotemporal dementia (bvFTD) with asymmetrical parkinsonism and prominent visuospatial deficits that carries a novel GRN mutation. This case highlights important clinical characteristics that seem to be common in FTLD GRN-associated patients, such as asymmetrical parkinsonism and parietal symptoms, and that are correlated to the pathological involvement of striatum (rather than substantia nigra in our case) and parietal lobe. We also emphasize that plasma progranulin level can be useful to infer about the pathogenicity of new GRN mutations.

Keywords: Frontotemporal lobar degeneration (FTLD), parkinsonism, parietal lobe, progranulin, TDP43

INTRODUCTION

Frontotemporal lobar degeneration (FTLD) refers to a clinically, pathologically, and genetically heterogeneous group of dementias, with onset of illness usually before 65 years of age, that arises from the degeneration of the frontal and temporal lobes. Mutations in the progranulin gene (GRN) are a major cause of FTLD with TDP-43 inclusions [1]. Since the discovery of mutations in GRN gene associated with autosomal dominant FTLD [2, 3], 69 different mutations in more than 231 families have been described (http://www.molgen.ua.ac.be/). The majority of them leads to haploinsufficiency and are associated with a wide clinical heterogeneity [4]. More recently, plasma...
Plenary Article

MicroRNA and mRNA expression profile screening in multiple sclerosis patients to unravel novel pathogenic steps and identify potential biomarkers

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A R T I C L E   I N F O

Article history:
Received 20 September 2011
Received in revised form 18 October 2011
Accepted 2 November 2011

Key words:
Multiple sclerosis
MicroRNA
Gene expression

A B S T R A C T

Identification of novel targets and biomarkers, such as microRNAs, is extremely helpful to understand the pathogenic mechanisms in a disease like multiple sclerosis (MS). We tested the expression profile of 1145 microRNAs in peripheral blood mononuclear cells (PBMCs) of 19 MS patients and 14 controls, and we further explored their function by performing a whole-genome mRNA profiling in the same subjects and using bioinformatic prediction tool. A total of 104 miRNAs have been identified as deregulated in MS patients; 2/10 which ranked highest (let-7g and miR-150) have been validated in a replication sample, leading to the identification of putative target genes.

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1. Introduction

In the last few years, a number of gene expression studies have been a useful tool to discover novel factors and molecular pathways involved in the pathogenesis of multiple sclerosis (MS) [5,13,14,25,26]. Nevertheless, mRNA translation into protein can be modulated by specific microRNAs (miRNAs), which are small non-coding RNA molecules recently identified in several species, ranging from worms to humans and representing more than 95% of the total human cellular RNAs. MiRNAs are single-stranded molecules of 20–25 nucleotides, partially complementary to one or more mRNA sequences, encoded by nuclear genes and postulated to be functional in the cytoplasm. Their function is to bind to specific 3′UTR sequences, down-regulating their target mRNAs via translational repression in case of imperfect sequence match or mRNA cleavage in case of perfect sequence match [4].

There is increasing evidence that miRNAs represent key regulators of several biological phenomena, including cell proliferation and differentiation, apoptosis, signal transduction and organ development [1,4]. Several papers have been published in the last years exploring the role of miRNAs in multiple sclerosis [9–12,12,15–17,23], suggesting an involvement of miRNAs in the pathogenesis and course of MS. In this study, we tested the expression profile of 1145 miRNAs in PBMCs of 19 patients with MS as compared with 14 controls (discovery sample) and validated results on a second MS sample. The potential impact of deregulated miRNAs has been tested experimentally by performing a whole-genome mRNA profiling on the same discovery sample as well as by looking at predicted targets according to online database. In this study we attempt to compare two different approaches, one experimental and one in silico, to identify targets of miRNA using a whole-genome unbiased design.

2. Materials and methods

2.1. Subjects

As regards the discovery sample, 19 patients with MS, 8 males and 11 females, have been consecutively recruited at the Department of Neurology of the Scientific Institute San Raffaele (HSR) and at the Fondazione Ca’ Granda, IRCCS Ospedale Maggiore Policlinico in Milan. All patients underwent a standard battery of examinations, including medical history, physical and neurological examination, screening laboratory test, and brain magnetic resonance imaging (MRI). Diagnosis was made in accordance to McDonald criteria and further revision [20,24]. The clinical course of MS was described as relapsing remitting (RRMS; 2 males and 5 females, mean age ± SEM: 43.7 ± 5.4), secondary progressive (SPMS; 2 males and 4 females, mean age ± SEM: 45.7 ± 2.9) or...
Progress in Alzheimer’s disease

Daniela Galimberti · Elio Scarpini

Received: 27 April 2011 / Revised: 8 June 2011 / Accepted: 9 June 2011 / Published online: 25 June 2011
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Abstract After more than one century from Alois Alzheimer and Gaetano Perusini’s first report, progress has been made in understanding the pathogenic steps of Alzheimer’s disease (AD), as well as in its early diagnosis. This review discusses recent findings leading to the formulation of novel criteria for diagnosis of the disease even in a preclinical phase, by using biological markers. In addition, treatment options will be discussed, with emphasis on new disease-modifying compounds and future trial design suitable to test these drugs in an early phase of the disease.

Keywords Alzheimer’s disease · Amyloid · Tau · Inflammation · Genetics · Biomarkers · Diagnosis · Disease-modifying drugs

Alzheimer’s disease: clinical aspects and pathogenesis

Alzheimer’s disease (AD) is the most common cause of dementia in the elderly, with prevalence of 5% after 65 years of age, increasing to about 30% in people aged 85 years or older. It is characterized clinically by progressive cognitive impairment, including impaired judgment, decision-making and orientation, often accompanied, in later stages, by psychobehavioural disturbances as well as language impairment.

The two major neuropathological hallmarks of AD are extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). The production of Aβ, which is considered a crucial step in AD pathogenesis, is the result of cleavage of a larger peptide, named amyloid precursor protein (APP), which is overexpressed in AD [1]. Aβ forms highly insoluble and proteolysis-resistant fibrils known as senile plaques (SP). NFTs are composed of the tau protein. In healthy subjects, tau is a component of microtubules, which represent the internal support structures for transport of nutrients, vesicles, mitochondria and chromosomes within the cell. Microtubules also stabilize growing axons, which are necessary for the development and growth of neurites [1]. In AD, tau protein is abnormally hyperphosphorylated and forms insoluble fibrils, originating deposits within the cell.

A number of additional pathogenic mechanisms, possibly overlapping with Aβ plaques and NFT formation, have been described, including inflammation [2], oxidative damage [3], iron deregulation [4], mitochondrial dysfunction [5] and a number of amyloid-independent hypotheses [6].

In about 95% of cases, the disease is sporadic (caused by the interaction between genetic and environmental factors). Autosomal dominant mutations in APP, presenilin 1 (PSEN1) and presenilin 2 (PSEN2) account for about 5% of cases, often characterized by early onset (before 65 years of age). To date, 32 different mutations, causing amino acid changes at putative sites for cleavage of the protein, have been described in the APP gene in 89 families, together with 182 mutations in PSEN1 and 13 in PSEN2.

The amyloid hypothesis

The human APP gene was first identified in 1987 by several laboratories independently [7–9]. The two APP homologues, APLP1 and APLP2, were discovered several
Clinical phenotypes and genetic biomarkers of FTLD

Daniela Galimberti · Elio Scarpini

Abstract Frontotemporal lobar degeneration (FTLD) is the most frequent neurodegenerative disorder with a pre-senile onset. It presents with a spectrum of clinical manifestations, ranging from behavioural and executive impairment to language disorders and motor dysfunction. New diagnostic criteria identified two main cognitive syndromes: behavioural variant frontotemporal dementia (bvFTD) and primary progressive aphasia (PPA). Regarding bvFTD, new criteria that include the use of biomarkers have been proposed. According to them, bvFTD can be classified in “possible” (clinical features only), “probable” (inclusion of imaging biomarkers) and “definite” (in the presence of a known causal mutation or at autopsy). Concerning autosomal dominant mutations, microtubule associated protein tau gene mutations have been the first ones identified and are generally associated with early onset bvFTD phenotype. More recently, progranulin gene mutations were recognized in association with familial form of FTLD. In addition, other genes are linked to rare cases of familial FTLD, primarily the newly discovered C9ORF72 hexanucleotide expansion repeats. As regards PPA, new consensus criteria identify three syndromes: primary non-fluent aphasia, semantic variant of PPA and logopenic aphasia, which seems to be associated, in the majority of cases, with underlying Alzheimer’s disease pathology. In this review, new criteria, including MRI, cerebrospinal fluid and genetic biomarkers, will be presented and discussed.

Keywords Frontotemporal lobar degeneration · Behavioural variant front temporal dementia · Primary progressive aphasia · Genetics · Biomarker

Clinical features of frontotemporal lobar degeneration: new diagnostic criteria

Frontotemporal lobar degeneration (FTLD) represents a common cause of dementia in subjects under 65 years. The age at onset is typically 45–65 years, with a mean average in the 50s, and the prevalence is equal among men and women. It is associated with frontal and temporal lobes atrophy, involving the right and left hemispheres, in some cases asymmetrically (Rosen et al. 2006). It can be classified into two main cognitive syndromes (Neary et al. 1998): behavioural variant frontotemporal dementia (bvFTD) and primary progressive aphasia (PPA), whose diagnostic criteria have been recently revised including neuroimaging and genetic findings (Gorno-Tempini et al. 2011; Rascovsky et al. 2011). In addition, other phenotypes such as progressive supranuclear palsy (PSP), corticobasal syndrome (CBS) and bvFTD with motor neuron disorders (MND) are part of the clinical manifestation within the FTLD spectrum. Overall, these different phenotypes well reflect the clinical heterogeneity of FTLD and the underlying clinico-pathological spectrum of FTLD.

bvFTD is the most frequent FTLD phenotype. It is primarily characterised by behavioural changes and progressive deterioration of personality. Throughout the disease, patients show a wide spectrum of symptoms, including behavioural alterations, such as disinhibition, overeating and impulsiveness, and impairment of cognitive functions, with relative sparing of memory (Hou et al. 2004). Despite recent advances in the characterization of this disorder, the
Possible Influence of a Non-Synonymous Polymorphism Located in the NGF Precursor on Susceptibility to Late-Onset Alzheimer’s Disease and Mild Cognitive Impairment

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Accepted 30 December 2011

Abstract. The complex network of neurotrophic factors is proposed to play a role in neurodegeneration, but the effect of variations in their coding genes on susceptibility to sporadic Alzheimer’s disease is not established. The mature form of nerve growth factor (NGF) derives from a precursor, proNGF, which was recently discovered to exert crucial functions in brain. We designed a case-control association study to test the hypothesis as to whether polymorphisms located in the proNGF genomic region influence the liability to Alzheimer’s disease and its prodromal form, mild cognitive impairment. Three independent case-control samples, with individuals aged >60 years, were collected in Italian Alzheimer Units. One polymorphism located in the proNGF region, rs6330, demonstrated a minor allele frequency >5% and was used in the association study. The minor allele of rs6330 was more frequent in patients from the three sample series as compared to respective normal controls. Multivariate logistic regression showed a significant association under the dominant model in one cohort (OR 1.83, 95% CI 1.00–3.54) and in the pooled case-control sample (OR 1.47, 95% CI 1.03–2.08). These findings further suggest that proNGF may play a role in Alzheimer-type neurodegeneration and that genetic variations in the NGF locus may influence the occurrence of sporadic, late-onset Alzheimer’s disease.

Keywords: Alzheimer’s disease, APOE, association study, mild cognitive impairment, NGF, NGFB, proNGF
Progress in multiple sclerosis research in the last year

Daniela Galimberti · Elio Scarpini

Herein, we summarize the main articles describing novel findings in multiple sclerosis published in the *Journal of Neurology* over the last year, including clinical, therapeutic and research issues.

**Keywords** Multiple sclerosis · Demyelination · Relapse · Acute phase · MRI · Fatigue · Progression · Therapy

**Abstract** Herein, we summarize the main articles describing novel findings in multiple sclerosis published in the *Journal of Neurology* over the last year, including clinical, therapeutic and research issues.

**Introduction**

Herein, main articles describing novel findings in multiple sclerosis published in the *Journal of Neurology* over the last year are summarized. Relevant findings in each topic are described in Table 1.

**Clinical aspects**

Fatigue

Fatigue, defined as "subjective lack of physical or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities" [1], is one of the most common symptoms in patients with multiple sclerosis (MS), leading to a severe level of disability and impaired quality of life. Yaldizli et al. [2] investigated the relationship between progression of corpus callosum (CC) atrophy at magnetic resonance imaging (MRI) and fatigue in 70 patients with relapsing-remitting (RR) MS over a 4.8 year follow-up study. Patients with fatigue (representing 40% of the cohort) had higher expanded disability status scale (EDSS) scores, as well as a more pronounced CC atrophy [2]. Morgante et al. [3] tested the hypothesis that central fatigue in MS might be correlated with a dysfunction in the cortical areas upstream of the pyramidal tract involved in motor planning and preparation. To investigate this hypothesis, they recruited 33 patients with RRMS, who underwent MRI and transcranial magnetic stimulation (TMS). Results showed a significant increase in the burden of lesion load in frontal areas correlating with the degree of fatigue, expressed by the fatigue severity scale (FSS) score, thus demonstrating that frontal lobe impairment is associated with fatigue in MS [3].

Besides, Horowski et al. [4] raised the question whether sonographic changes of the substantia nigra (SN), brainstem raphe, lenticular nucleus (LN) or caudate nucleus are related to nonmotor symptoms of MS. They demonstrated that sonographic alterations of the LN correlated with cognitive dysfunctions, and that combined alteration of both LN and SN was associated with cognitive dysfunction and cognitive fatigue [4].

Regarding the rehabilitative approach for fatigue, Judica et al. [5] investigated whether an intensive, short-term inpatient rehabilitation program is able to improve fatigue in MS, and if fatigue is able to negatively influence the clinical and functional outcome of rehabilitation in MS. Fatigue symptoms were measured before and after rehabilitation with the FSS, while EDSS and functional independence measure (FMI) were used to measure clinical outcomes, in terms of the efficacy of the program. Results showed that the short-term rehabilitation program resulted in a significant reduction in fatigue symptoms compared with untreated MS patients; however, the presence of
Short Communication

Replication Study to Confirm the Role of CYP2D6 Polymorphism rs1080985 on Donepezil Efficacy in Alzheimer’s Disease Patients

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Handling Associate Editor: Patrizia Mecocci

Accepted 11 March 2012

Abstract Alzheimer’s disease (AD) is a neurodegenerative disorder often treated with donepezil, an acetylcholinesterase inhibitor. Response to donepezil is variable, probably based on patients’ genetic background in donepezil metabolizing enzymes,

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Frontotemporal lobar degeneration: current knowledge and future challenges

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Received: 30 January 2012 / Accepted: 29 March 2012 / Published online: 25 April 2012
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Abstract Frontotemporal lobar degeneration (FTLD) is one of the most frequent neurodegenerative disorders with a presenile onset. It presents with a spectrum of clinical manifestations, ranging from behavioral and executive impairment to language disorders and motor dysfunction. New diagnostic criteria identified two main cognitive syndromes: behavioral variant frontotemporal dementia (bvFTD) and primary progressive aphasia. Regarding bvFTD, new criteria include the use of biomarkers. According to them, bvFTD can be classified in “possible” (clinical features only), “probable” (inclusion of imaging biomarkers) and “definite” (in the presence of a known causal mutation or at autopsy). Familial aggregation is frequently reported in FTLD, and about 10% of cases have an autosomal dominant transmission. Microtubule-associated protein tau gene mutations have been the first ones identified, and are generally associated with early onset (40–50 years) and with the bvFTD phenotype. More recently, progranulin gene mutations were recognized in association with the familial form of FTLD and a hexanucleotide repetition in C9ORF72 has been shown to be responsible for familial FTLD and amyotrophic lateral sclerosis. In addition, other genes are linked to rare cases of familial FTLD. Lastly, a number of genetic risk factors for sporadic forms have also been identified.

Keywords Frontotemporal lobar degeneration · Tau · Progranulin (GRN) · C9ORF72 · Genetics · Risk factor

Introduction

Frontotemporal lobar degeneration (FTLD) is a rare, often misdiagnosed neurodegenerative disorder, representing a common cause of dementia in subjects <65 years. The age at onset is typically 45–65 years, with a mean average in the 50s, and the prevalence is equal among men and women. It is associated with frontal and temporal lobe atrophy, involving the right and left hemispheres, in some cases asymmetrically [1]. It can be classified into two main cognitive syndromes [2]: behavioral variant frontotemporal dementia (bvFTD) and primary progressive aphasia (PPA), whose diagnostic criteria have been recently revised including neuroimaging and genetic findings [3, 4]. In addition, other phenotypes, such as progressive supranuclear palsy (PSP), corticobasal syndrome (CBS), and bvFTD with motor neuron disorders—MND, are part of the clinical manifestation within the FTLD spectrum. Overall, these different phenotypes well reflect the clinical heterogeneity of FTD and the underlying clinico-pathological spectrum of FTLD.

Diagnostic criteria of bvFTD

Behavioral variant FTD is the most frequent FTLD phenotype. It is primarily characterized by a long phase of subclinical behavioral changes and progressive deterioration of personality. Throughout the disease, patients show a wide spectrum of symptoms, including behavioral alterations, such as apathy, disinhibition, overeating and
Frontotemporal lobar degeneration (FTLD), the most frequent neurodegenerative disorder with a presenile onset, presents with a spectrum of clinical manifestations, ranging from behavioral and executive impairment to language disorders and motor dysfunction. Familial aggregation is frequently reported, and about 10% of cases have an autosomal dominant transmission. Microtubule associated protein tau (MAPT) gene mutations have been the first ones identified and are associated with early onset behavioral variant frontotemporal dementia phenotype. More recently, progranulin gene (GRN) mutations were recognized in association with familial form of FTLD. In addition, other genes are linked to rare cases of familial FTLD. Lastly, a number of genetic risk factors for sporadic forms have also been identified. In this review, current knowledge about mutations at the basis of familial FTLD will be described, together with genetic risk factors influencing the susceptibility to FTLD.

Keywords: genetics, frontotemporal lobar degeneration, autosomal dominant, mutation, risk factor

NEW DIAGNOSTIC CRITERIA OF FRONTOTEMPORAL LOBAR DEGENERATION

Frontotemporal lobar degeneration (FTLD) represents a common cause of dementia in subjects under 65 years. The age at onset is typically 45–65 years, with a mean average in the 50s, and the prevalence is equal among men and women. It is associated with frontal and frontal lobe atrophy, involving the right and left hemispheres, in some cases asymmetrically (Rosen et al., 2006). It can be classified into two main cognitive syndromes (Neary et al., 1998): behavioral variant frontotemporal dementia (bvFTD) and primary progressive aphasia (PPA), whose diagnostic criteria have been recently revised including neuroimaging and genetics (Gorno-Tempini et al., 2011; Rascovsky et al., 2011).

Behavioral variant frontotemporal dementia is the most frequent FTLD phenotype, characterized by behavioral alterations, such as disinhibition, overeating, and impulsiveness, and impairment of cognitive functions, with relative sparing of memory (Hou et al., 2004). Changes in social behavior, loss of empathy, and impairment of social insight are early and consistent symptoms of bvFTD, whose importance and role for the early diagnosis has been emphasized in the new consensus criteria (Rascovsky et al., 2011). According to these criteria, bvFTD main feature is the progressive deterioration of behavior and/or cognition by observation or history. If this criterion is satisfied, there are three further levels of certainty for bvFTD: possible, probable, or definite. “Possible” bvFTD requires three out of six clinically discriminating features (disinhibition, apathy/inertia, loss of empathy/empathy, perseverative/compulsive behaviors, hyperorality, and dysexecutive neuropsychological profile). “Probable” bvFTD meets the criteria of “possible” bvFTD plus (1) a significant functional decline (by caregiver report or evidenced at neuropsychological testing) (2) frontal and/or anterior temporal atrophy on MRI or CT, or frontal and/or anterior temporal hypoperfusion or hypometabolism on PET or SPECT. “Definite” bvFTD imply the histopathological evidence of FTLD on biopsy or post mortem or the presence of a known pathogenic mutation. These new criteria have a flexible structure to account for the high heterogeneity at initial presentation.

Early and progressive changes in language functions represent the alternative presentation of FTLD. Progressive loss of speech, with hesitant, non-fluent speech output with phono-phonological errors, and distortions and/or agrammatism is typical of primary non-fluent aphasia (PNFA) subtype (Scarpini et al., 2006), whereas loss of knowledge about words and objects, anomia and single-word comprehension deficits are core features of the semantic variant of PPA, named semantic dementia (SD; Gorno-Tempini et al., 2011). A third subtype of PPA has been recently described as logopenic or phonological variant (LPA). It is characterized by phonological disorders, defective word retrieval, and sentence repetition deficits. This PPA subtype seems to be associated with underlying Alzheimer’s disease (AD) pathology (Rabinovici et al., 2008).

GENETICS: AUTOSOMAL DOMINANT MUTATIONS

The presence of familial aggregation and the autosomal dominant transmission of the disease suggested so far a genetic cause (Snowden et al., 2002; Bird et al., 2003; Goldman et al., 2005). Up to 40% of patients have a family history suggesting FTLD in at least one extra family member (Goldman et al., 2005; Pickering-Brown, 2007), with a percentage of autosomal dominant cases accounting for 13.4% of the total (Goldman et al., 2005).

New criteria for bvFTD diagnosis (Rascovsky et al., 2011) include the presence of a known mutation as a biomarker. The demonstration of an autosomal dominant mutation is
 requested for the diagnosis of “definite” bvFTD, and is the only criterion existing so far to make a definite diagnosis during life. Genes demonstrated to be responsible for familial FTLD include: microtubule associated protein tau (MAPT) gene, progranulin (GRN), valosin-containing protein (VCP)-1, chromatin-modifying 2B (CHMP2B), TAR-DNA binding protein 43 encoding gene (TARBDP), and, very recently, a novel hexanucleotide expansion in chromosome 9 (Dejesus-Hernandez et al., 2011; Renton et al., 2011).

MICROTUBULE ASSOCIATED PROTEIN TAU GENE

The first evidence of a genetic cause for familial FTLD came from the demonstration of a linkage with chromosome 17q21.2 in autosomal dominantly inherited form of FTD with parkinsonism (Lynch et al., 1994) resulting in the label of “frontotemporal dementia and parkinsonism linked to chromosome 17” (FTDP-17). The gene responsible for such association, named MAPT gene, was discovered few years later (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). MAPT encodes the microtubule associated protein Tau, which is involved in microtubule stabilization and assembly. To date, more than 40 pathogenic MAPT mutations have been described in 134 families (http://www.molgen.ua.ac.be/). MAPT mutations can be non-synonymous, deletions, or intronic mutations located close to the splice-donor site of the intron after the alternatively spliced exon 10 (Rademakers et al., 2004). They are mainly clustered in exons 9–13, which contain the microtubule binding regions (Rademakers et al., 2002) and affect the normal function of tau, i.e., the stabilization of microtubules promoting their assembly by binding tubulin. Some mutations increase the free cytoplasmic portion of the protein promoting tau aggregation, whereas others lead to an aberrant phosphorylation of tau protein, which damages microtubule stabilization (Buee and Delacourte, 1999; Goedert and Jakes, 2005). Otherwise, many other mutations affect the alternative splicing, thus producing altered ratios of the different isoforms (3R/4R tau; Goedert et al., 1989). At autopsy, patients with MAPT mutations show tau-positive inclusions (Rademakers et al., 2004).

The clinical presentation in MAPT mutation carriers in mainly consistent with bvFTD, with a mean onset in the 50s (Yancopoulou and Spillantini, 2003; Villa et al., 2011). Nevertheless, cases of PNFA have been reported as well, with an onset even in the sixth decade of life (Villa et al., 2011).

PROGRANULIN GENE

After the discovery of MAPT as causal gene for FTDP-17, there were still numerous autosomal dominant FTLD cases genetically linked to the same chromosomal region of MAPT (chr17q21), in which no pathogenic mutations had been identified. A small region rich of genes, localized approximately 6.2 Mb in physical distance to MAPT locus, had been recognized as that one containing the gene responsible for the disease in these families. Systematic sequencing of candidate genes within this minimal region was performed and the first mutation in progranulin gene (GRN) was identified. It consists of a 4-bp insertion of CTGC between coding nucleotides 90 and 91, causing a frameshift and premature termination in progranulin (C31LinsX34; Baker et al., 2006). Cruts et al. (2006), analyzing other families with a FTLD pathology without MAPT mutation, found at the same time another mutation of five base pairs into the intron following the first non-coding exon of the gene (IVS0 + 5G–C). This mutation causes the splicing out of the intron 0, leading the retention of mRNA within the nucleus and its degradation.

GRN gene encodes for the growth regulation factor progranulin, belonging to a family of proteins involved in many biological functions including development, wound repair, and inflammation by activating signaling cascades that control cell cycle progression (He and Bateman, 2003). Progranulin is a 593 amino acid protein, rich of cysteine with a molecular weight of 68.5 kDa, subjected to proteolysis by elastase in a process regulated by a secretory leukocyte protease inhibitor (SLPI; Zhu et al., 2002). It is expressed not only in neurons but also is the activated microglia (Baker et al., 2006).

Since the original identification of null-mutations in FTLD in 2006, 69 different mutations have been described so far (http://www.molgen.ua.ac.be/) in 231 families. Most of the known pathogenic GRN mutations, including frameshift, splice-site, and nonsense mutations, are predicted to result in a premature stop codon. The resulting aberrant mRNA is degraded through the process of nonsense mediated decay, leading to haploinsufficiency (Gass et al., 2006).

Clinically, mutations in GRN are associated with extremely heterogeneous phenotypes, including, besides the classical FTLD presentations, AD (Carecchio et al., 2009), corticobasal syndrome (CBS; Carecchio et al., 2011), or Mild Cognitive Impairment (Pietroboni et al., 2011). Age at disease onset is extremely wide, even in the same family (Pietroboni et al., 2011). In addition, the demonstration of the clinical overlap between psychiatric disorders and genetically determined FTLD comes from the recent description of a patient with heterosexual pedophilia (Rainero et al., 2011), who was a carrier of a GRN mutation and developed bvFTD over time, and from a second description reporting two clinically different, apparently sporadic FTLD cases sharing the previously described Thr272fs GRN mutation, who had had a premorbid bipolar disorder history (Cerami et al., 2011).

A major contribution to achieve a correct diagnosis independent of the phenotypic presentation is the demonstration that progranulin plasma levels are extremely low in GRN mutation carriers, even in asymptomatic subjects (Ghidoni et al., 2008; Finch et al., 2009; Carecchio et al., 2011; Pietroboni et al., 2011).

Notwithstanding the striking proximity of MAPT and GRN on chromosome 17, at this time, there is no clear link between these two genes, suggesting that their closeness is just a coincidence.

GRN-mutated FTLD cases at the neuropathological examination presented ubiquitin immunoreactive cytoplasmic and intranuclear neuronal inclusions similar to the microvacuolarty type still observed in a large proportion of apparently sporadic FTLD, and differing from the tau-positive inclusions typical of MAPT mutated cases. Soon after the identification of GRN mutation, truncated, and hyperphosphorylated isoforms of the TAR–DNA binding protein (TDP)-43 were recognized as main components of the ubiquitin-positive inclusions typical of the GRN-mutated families, as well as of idiopathic FTLD and of a proportion of amyotrophic lateral sclerosis (ALS) cases (Neumann et al., 2006).
Identification of a new susceptibility variant for multiple sclerosis in OAS1 by population genetics analysis

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Received: 18 March 2011 / Accepted: 21 June 2011 / Published online: 7 July 2011 © Springer-Verlag 2011

Abstract Contrasting results have been reported concerning the association of a splice-site polymorphism (rs10774671) in OAS1 with multiple sclerosis (MS). We analysed two OAS1 regions encompassing alternatively spliced exons. While the region carrying the splice-site variant is neutrally evolving, a signature of long-standing balancing selection was observed across an alternative exon 7. Analysis of variants in this exon identified an insertion/deletion polymorphism (rs11352835, A/−) that originates predicted products with distinct C termini. This variant is located along the major branch of the haplotype genealogy, suggesting that it may represent the selection target. A case/control study for MS indicated that rs11352835 is associated with disease susceptibility (for an allelic model with the deleted allele predisposing to MS, OR 1.27, 95% CI 1.072–1.513, p = 0.010). No association was found between rs10774671 and MS. As the two SNPs are in linkage disequilibrium in Europeans, the previously reported association between rs10774671 and MS susceptibility might be driven by rs11352835, possibly explaining the contrasting results previously observed for the splice-site polymorphism. Thus, we describe a novel susceptibility variant for MS in OAS1 and show that population genetic analyses can be instrumental to the identification of selection targets and, consequently, of functional polymorphisms with an effect on phenotypic traits.

Introduction

In humans four 2′,5′-oligoadenylate synthetase genes (OAS1, OAS2, OAS3 and OASL) are located on the long arm of chromosome 12 and play a central role in the innate immune response against viruses. These enzymes are activated either by the presence of double strand RNA or by single strand RNA with secondary structure, and
Dear Sirs,

Many causes of traumatic sciatic neuropathy have been described in the literature [1], but extrapelvic endometriosis of the gluteal region presenting as sciatica remains difficult to recognize. Here, we present the case of a 45-year-old woman with a history of lower back pain and lower limb stiffness in whom hip MRI showed multiple loci of endometriosis localized in both iliac muscles and in the right gluteus.

The woman presented with a 15-day history of lower back pain and impaired ambulation. Neurological examination showed lower limb stiffness with left leg flexure and right leg hyperextension, right foot dorsiflexion deficit, positive Lasègue sign, gait disturbance and severe pain with apparent (L4)-L5-S1 distribution. An EMG and a spinal cord MRI scan had previously been performed without any pathological findings. Intravenously administered myorelaxants and NSAIDs were tried without any benefit. Her past medical history was consistent only with diabetes. Routine blood tests showed only mild anaemia and high cancer antigen-125 (CA-125) levels. Brain and spinal cord MRI, backbone CT, and total-body PET scans did not show any pathological findings. Orthopaedic examination showed groin pain, irradiating to the knee and worsened by hip movement.

On MRI T2-weighted images both the iliac and psoas muscles and the gluteus maximus and minimus appeared markedly bright. In the iliac muscles two T2-hyperintense formations, without contrast enhancement but with a hypervascular rim, were evident; the right gluteus showed similar findings (Fig. 1a). On the 4th day of hospitalization her period started and the patient reported worsening pain and severe abdominal cramps. Gynaecological examination showed a 2-cm fibrotic nodule in the pouch of Douglas, suggestive of endometriosis. CA-125 was 49.3 IU/ml (cut-off 35 IU/ml). A cycle of leuprorelin (a gonadotropin-releasing hormone agonist which downregulates the secretion of gonadotropin luteinizing hormone and follicle-stimulating hormone leading to hypogonadism) was started, and thereafter symptoms slowly resolved. A follow-up MRI scan performed 3 months later showed a reduction in size and number of the lesions; neurological examination was normal (Fig. 1b). In accordance with previous report [1], CA-125 was elevated in our patient. The marked T2 brightness in both the iliac and gluteus muscles seen on the MRI scan was consistent with oedema and hyperaemia or, as described previously [2], neurogenic muscular injury. This finding may be suggestive of an endometriotic lesion localized at or near the sciatic notch. The T2-hypentense formations were consistent with foci of endometriosis localized in the muscle tissues. The cyclicity of the symptoms and their regression after a cycle of leuprorelin further supports our hypothesis [3, 4].

Endometriosis-induced cyclic sciatica was firstly reported by Schlincke in 1946 [5]. Since then, approximately 60 cases have been described. Lesions of the ipsilateral sciatic nerve are reported as the primary cause of almost all the endometriosis-induced cyclic sciatica [6]. The commonest localization is the sciatic notch where fibrosis, organized haematoma and endometrial tissue

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A. Esposito
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A Functional Variant in *ERAP1* Predisposes to Multiple Sclerosis

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**Abstract**

The *ERAP1* gene encodes an aminopeptidase involved in antigen processing. A functional polymorphism in the gene (rs30187, Arg528Lys) associates with susceptibility to ankylosing spondylitis (AS), whereas a SNP in the interacting *ERAP2* gene increases susceptibility to another inflammatory autoimmune disorder, Crohn’s disease (CD). We analysed rs30187 in 572 Italian patients with CD and in 517 subjects suffering from multiple sclerosis (MS); for each cohort, an independent sex- and age-matched control group was genotyped. The frequency of the 528Arg allele was significantly higher in both disease cohorts compared to the respective control population (for CD, OR = 1.20 95%CI: 1.01–1.43, p = 0.036; for RRMS, OR = 1.26; 95%CI: 1.04–1.51, p = 0.01). Meta-analysis with the Wellcome Trust Cases Control Consortium GWAS data confirmed the association with MS (Pmeta = 0.005), but not with CD. In AS, the rs30187 variant has a predisposing effect only in an HLA-B27 allelic background. It remains to be evaluated whether interaction between *ERAP1* and distinct HLA class I alleles also affects the predisposition to MS, and explains the failure to provide definitive evidence for a role of rs30187 in CD. Results herein support the emerging concept that a subset of master-regulatory genes underlay the pathogenesis of autoimmunity.

**Introduction**

Antigen processing and presentation by MHC class I molecules is essential for assuring immune surveillance and for establishing immunodominance. The process initiates with the transport of proteasome-generated antigenic peptides to the endoplasmic reticulum (ER), where they are customized to optimal size for MHC class I loading by resident enzymes. In humans, two ER-aminopeptidases, encoded by *ERAP1* and *ERAP2*, trim imported peptides at their N-terminus and contribute to the shaping of the antigenic repertoire presented by class I MHC molecules [1]. Studies in humans and mice have shown that, depending on peptide length and sequence composition, ERAP1 has the ability to both destroy and create peptide cargos for MHC class I [2]. Therefore, in mice lacking the enzyme the presentation of some peptides is dramatically reduced, whereas other peptides are much more abundant than what is observed in wild-type animals [3]. This applies to both proteolytic fragments of pathogen-derived proteins and to endogenous peptides. As a consequence, immunodominance is disrupted in *Erap1−/−* mice and these animals display a distinct repertoire of antigenic peptides [3]. Because ERAP1 also contributes to shedding the membrane-bound receptor for inflammatory cytokines including IL1R2, TNFR1, and IL6R [4], ERAP1 is likely to play a pivotal role in protection from infectious diseases, in maintaining immunotolerance, and in controlling inflammation. A single nucleotide polymorphism (SNP) in *ERAP1* (rs30187), which changes a highly conserved residue (Arg528Lys), is maintained at intermediate frequency in human populations by natural selection [5] and affects the enzyme catalytic activity [6]. This SNP has been associated with susceptibility to ankylosing spondylitis (AS) [7], and variants in linkage disequilibrium (LD) with it increase association with susceptibility to ankylosying spondylitis (AS) [7], and variants in linkage disequilibrium (LD) with it increase association with susceptibility to ankylosying spondylitis (AS) [7], and variants in linkage disequilibrium (LD) with it increase association with susceptibility to another inflammatory autoimmune disorder, Crohn’s disease (CD). Additional shared variants between CD and AS have recently been described [10,11], and provide genetic evidence to the clinical observation that the two diseases have frequent co-occurrence and co-symptomatology [12].


* Editor: Pablo Villalobos, Institute of Biomedical Research August Pi i Sunyer (IDIBAPS) - Hospital Clinic of Barcelona, Spain

* Received September 14, 2011; Accepted December 7, 2011; Published January 12, 2012

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* Funding: This work was supported by the Broad Medical Research Program of The Broad Foundation (grant IBD-0294), by 2010 Ricerca Corrente [Italian Ministry of Health], and Fondazione CARIPLO. The funding sources had no involvement in study design, collection, analysis and interpretation of data, in the writing of the report as well as in the decision to submit the paper for publication. No additional external funding received for this study.

* Competing Interests: The authors have declared that no competing interests exist.

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A Trans-Specific Polymorphism in ZC3HAV1 Is Maintained by Long-Standing Balancing Selection and May Confer Susceptibility to Multiple Sclerosis


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Abstract

The human ZC3HAV1 gene encodes an antiviral protein. The longest splicing isoform of ZC3HAV1 contains a C-terminal PARP-like domain, which has evolved under positive selection in primates. We analyzed the evolutionary history of this same domain in humans and in Pan troglodytes. We identified two variants that segregate in both humans and chimpanzees; one of them (rs3735007) does not occur at a hypermutable site and accounts for a nonsynonymous substitution (Thr851Ile). The probability that the two trans-specific polymorphisms have occurred independently in the two lineages was estimated to be low (P = 0.0054), suggesting that at least one of them has arisen before speciation and has been maintained by selection. Population genetic analyses in humans indicated that the region surrounding the shared variants displays strong evidences of long-standing balancing selection. Selection signatures were also observed in a chimpanzee population sample. Inspection of 1000 Genomes data confirmed these findings but indicated that search for selection signatures using low-coverage whole-genome data may need masking of repetitive sequences. A case–control study of more than 1,000 individuals from mainland Italy indicated that the Thr851Ile SNP is significantly associated with susceptibility to MS, possibly via the interaction with environmental factors.

Key words: ZC3HAV1, trans-specific polymorphism, balancing selection, multiple sclerosis.

Introduction

The zinc-finger antiviral protein (ZAP) was originally identified from a rat cDNA library due to its conferring resistance to murine leukemia viruses (Gao et al. 2002). Subsequent studies indicated that ZAP also inhibits several viruses of the alphaviridae (Bick et al. 2003; Zhang et al. 2007) and filoviridae (Muller et al. 2007) families. ZAP acts through direct binding to the viral RNA and recruits the processing exosome, eventually leading to viral RNA degradation (Guo et al. 2004, 2007). The human ortholog of ZAP is encoded by ZC3HAV1, an interferon-inducible gene located on chromosome 7 (7q34). The gene codes for two major isoforms generated by alternative splicing (Kerns et al. 2008). The products share a common N-terminus carrying four CCCH zinc-finger motifs, although only the longest isoform displays a carboxy-terminal poly(ADP-ribos) polymerase (PARP)–like domain. The CCCH zinc-finger motifs are directly involved in viral RNA binding; conversely, the precise function of the PARP-like domain is unknown, but it enhances the activity of ZC3HAV1 against alphaviruses (Kerns et al. 2008).
Selective DNA Methylation of BDNF Promoter in Bipolar Disorder: Differences Among Patients with BDI and BDII

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The etiology of bipolar disorder (BD) is still poorly understood, involving genetic and epigenetic mechanisms as well as environmental contributions. This study aimed to investigate the degree of DNA methylation at the promoter region of the brain-derived neurotrophic factor (BDNF) gene, as one of the candidate genes associated with major psychoses, in peripheral blood mononuclear cells isolated from 94 patients with BD (BD I = 49, BD II = 45) and 52 healthy controls. A significant BDNF gene expression downregulation was observed in BD II 0.53 ± 0.11%; P < 0.05, but not in BD I (1.13 ± 0.19%) patients compared with controls (CONT: 1 ± 0.2%). Consistently, an hypermethylation of the BDNF promoter region was specifically found in BD II patients (CONT: 24.0 ± 2.1%; BD I: 20.4 ± 1.7%; BDII: 33.3 ± 3.5%; P < 0.05). Of note, higher levels of DNA methylation were observed in BD subjects on pharmacological treatment with mood stabilizers plus antidepressants (34.6 ± 4.2%, predominantly BD II) compared with those exclusively on mood-stabilizing agents (21.7 ± 1.8%; P < 0.01, predominantly BD I). Moreover, among the different pharmacological therapies, lithium (20.1 ± 3.8%, P < 0.05) and valproate (23.6 ± 2.9%, P < 0.05) were associated with a significant reduction of DNA methylation compared with other drugs (35.6 ± 4.6%). Present findings suggest selective changes in DNA methylation of BDNF promoter in subjects with BD type II and highlight the importance of epigenetic factors in mediating the onset and/or susceptibility to BD, providing new insight into the mechanisms of gene expression. Moreover, they shed light on possible mechanisms of action of mood-stabilizing compounds vs antidepressants in the treatment of BD, pointing out that BDNF regulation might be a key target for their effects.

Neuropsychopharmacology advance online publication, 22 February 2012; doi:10.1038/npp.2012.10

Keywords: brain-derived neurotrophic factor (BDNF); peripheral blood mononuclear cells (PBMCs); DNA methylation; gene expression; bipolar disorder (BD); mood stabilizers and antidepressants

INTRODUCTION

Bipolar disorder (BD) is a prevalent, recurring, and highly disabling mood disorder determined by the interplay of genes and environmental factors. Despite consistent evidence from genetic studies supporting the role for genes in BD, the precise molecular bases of the disorder remain to be unraveled. Actually, genetic investigation has clearly pointed out that no specific gene is incontrovertibly related to the development of BD which, likely, represents a complex condition in which pathological behaviors, and ultimately, patients’ symptoms, result from the combination of numerous susceptibility genes, each of which is not necessarily uncommon (Gershon, 2000). Among genes potentially implicated in the pathophysiology of BD, the brain-derived neurotrophic factor (BDNF) gene has been extensively investigated over the last few years and associated with neural adaptations to stress, synaptic plasticity, and antidepressant response, and with an influence on serotonergic system and mood regulation.
SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis
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Neurology 2012;79;1556; Published online before print September 12, 2012;
DOI 10.1212/WNL.0b013e31826e25df

This information is current as of December 21, 2012

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://www.neurology.org/content/79/15/1556.full.html

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SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis

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ABSTRACT

Objective: There is increasing evidence that common genetic risk factors underlie frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). Recently, mutations in the sequestosome 1 (SQSTM1) gene, which encodes p62 protein, have been reported in patients with ALS. P62 is a multifunctional adapter protein mainly involved in selective autophagy, oxidative stress response, and cell signaling pathways. The purpose of our study was to evaluate the frequency of SQSTM1 mutations in a dataset of unrelated patients with FTLD or ALS, in comparison with healthy controls and patients with Paget disease of bone (PDB).

Methods: Promoter region and all exons of SQSTM1 were sequenced in a large group of subjects, including patients with FTLD or ALS, healthy controls, and patients with PDB. The clinical characteristics of patients with FTLD or ALS with gene mutations were examined.

Results: We identified 6 missense mutations in the coding region of SQSTM1 in patients with either FTLD or ALS, none of which were found in healthy controls or patients with PDB. In silico analysis suggested a pathogenetic role for these mutations. Furthermore, 7 novel noncoding SQSTM1 variants were found in patients with FTLD and patients with ALS, including 4 variations in the promoter region.

Conclusions: SQSTM1 mutations are present in patients with FTLD and patients with ALS. Additional studies are warranted in order to better investigate the role of p62 in the pathogenesis of both FTLD and ALS. Neurology® 2012;79:1556-1562

GLOSSARY

AD = Alzheimer disease; ALS = amyotrophic lateral sclerosis; FTLD = frontotemporal lobar degeneration; HD = Huntington disease; PD = Parkinson disease; PDB = Paget disease of bone; SQSTM1 = sequestosome 1 gene.

In recent years, there has been a growing body of clinical, pathologic, and genetic evidence supporting the idea that frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) belong to the same clinicopathologic spectrum of disease.1–3

FTLD and ALS are genetically heterogeneous disorders. Mutations in the CHMP2B, FUS, OPTN, PGRN, TARDBP, UBQLN2, and VCP genes and a repeat expansion in the C9orf72 gene have been reported to be associated with both diseases.4–11 Therefore, genes linked to both diseases may converge into a common pathogenetic pathway, explaining the overlap of clinical symptoms.

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Study funding: Ministero dell’Istruzione, dell’Università e della Ricerca Scientifica (MIUR) of Italy, Regione Piemonte, Ministero della Salute of Italy, Fondazione Mondino, W. Garfield Weston Foundation, the Canadian Institutes of Health Research, Ontario Research Fund, the Howard Hughes Medical Institute, The Wellcome Trust, the Alzheimer Society of Ontario, the Canada Foundation for Innovation, the Ontario Mental Health Foundation, Genome Canada and the Alzheimer Society of Canada.

Preliminary data of the present study were presented at the annual congress of the Italian Society of Neurology (SIN) in 2010 and at the annual congress of the Italian Society for the Study of Dementia (SINDEM) in 2011.

Go to Neurology.org for full disclosures. Disclosures deemed relevant by the authors, if any, are provided at the end of this article.
Association between DPP6 polymorphism and the risk of progressive multiple sclerosis in Northern and Southern Europeans

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HIGHLIGHTS

► 19 SNPs on DPP6 were genotyped in 244 Italian PrMS patients and 225 controls.
► 5 SNPs resulted significantly associated with PrMS in the Italian sample.
► These 5 SNPs have been tested and not replicated in 197 PPMS cases from Northern Europe.
► Meta-analysis approach has been used confirming association for 1 SNP (p = 2.5 × 10−3).
► Expression level of DPP6 were different between Italian PrM cases and controls.

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form 24 September 2012
Accepted 2 October 2012

Keywords:
Multiple sclerosis
DPP6
Single nucleotide polymorphism
Genetics
Association

ABSTRACT

Background: In this study, we investigated the role of the dipeptidyl-peptidase-6 (DPP6) gene in the etiopathogenesis of progressive forms of multiple sclerosis (PrMS).

This gene emerged as a candidate gene in a genome-wide association study (GWAS) performed in an Italian sample of PrMS and controls in which two SNPs located in the gene (rs6956703 and rs11767658) showed evidence of association (nominal p-value < 10−8) [18]. Moreover, the gene is highly expressed in the central nervous system, and it has been found to be associated with sporadic cases of amyotrophic lateral sclerosis which shares some feature with PrMS.

Methods: We genotyped 19 SNPs selected using a direct and tagging approach in 244 Italian PrMS and 225 controls, and we measured the expression levels of the gene in 13 PrMS cases and 25 controls.

Results: Five out of 19 SNPs were found to be associated with the disease (adjusted p < 0.05), and they have been tested in an independent sample of 179 primary progressive MS and 198 controls from Northern Europe. None of the SNPs was replicated, but combined analysis confirmed the presence of association for rs2046748 (p = 2.5 × 10−3, OR = 1.82, 95% CI = 1.24–2.69).

Conclusions: These results, inflated by the limited sample size determined by the rarity of this condition, suggest a possible role of this gene in the susceptibility to PrMS, at least in Southern Europeans. Moreover, DPP6 was over-expressed in PrMS patients compared to controls.

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Does Vascular Burden Contribute to the Progression of Mild Cognitive Impairment to Dementia?

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Key Words
Alzheimer’s disease · Cerebrovascular disease · Hypertension · Mild cognitive impairment · Vascular burden · Vascular risk factors

Abstract
Aims: To investigate the contribution of vascular risk factors (VRFs), vascular diseases (VDs) and white matter lesions (WMLs) to the progression of mild cognitive impairment (MCI) to dementia and Alzheimer’s disease (AD). Methods: Two hundred forty-five consecutive subjects with MCI (age 74.09 ± 6.92 years) were followed for an average of 2.4 years. The Hachinski Ischemic Score and the Framingham Stroke Risk Profile were used to summarize VRFs and VDs. WMLs were graded using the Age-Related White Matter Changes Scale. Results: One hundred twenty-nine (52.6%) out of 245 subjects at risk converted to dementia, including 87 cases of AD. When hypertension occurred in MCI with deep WMLs, a 1.8-fold increased risk of dementia was observed (95% CI = 1.0–3.4). When deep WMLs occurred in MCI with high scores (≥4) on the Hachinski scale, a 3.5-fold (95% CI = 1.6–7.4) and 3.8-fold (95% CI = 1.2–11.5) risk of progression to dementia and AD was observed, respectively. Analogously, the joint effect of WMLs and high scores (≥14) on the Framingham scale nearly doubled the risk of dementia (hazard ratio = 1.9, 95% CI = 1.1–3.3). Conclusions: Accelerated progression of MCI to dementia and AD is to be expected when VRFs and VDs occur together with WMLs.

Introduction
Identification of individuals at risk of developing dementia is a clinical priority. Mild cognitive impairment (MCI) is a state characterized by mild cognitive deficits that do not fulfill a diagnosis of dementia [1] and is, to date, the strongest predictor of dementia and Alzheimer’s disease (AD) [2]. Even if MCI may sometimes revert to normal cognition [2], it has been estimated that people with MCI have a 9-fold and a 14-fold annual increased risk of progressing to dementia and AD as compared to cognitively healthy elderly.
Abstract  Alzheimer’s disease (AD) is the most common cause of dementia in the elderly, and is typically characterized by memory loss. In addition, during the disease progression, most patients develop behavioural and psychiatric symptoms of dementia (BPSD). Frontotemporal Lobar Degeneration (FTLD) is the most frequent neurodegenerative disorder with a presenile onset. It is characterized mainly by behavioural disturbances, whereas memory is conserved. The two major neuropathologic hallmarks of AD are extracellular Amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs). Conversely, in FTLD the deposition of tau has been observed in a number of cases, but in several brains there is no deposition of tau but instead a positivity for ubiquitin. In some families these diseases are inherited in an autosomal dominant fashion. Genes responsible for familial AD include the Amyloid Precursor Protein (β–APP), Presenilin 1 (PS1) and Presenilin 2 (PS2). The majority of mutations in these genes are often associated with a very early onset (40–50 years of age). Regarding FTLD, the first mutations described are located in the Microtubule Associated Protein Tau gene (MAPT). Tau is a component of microtubules, which represent the internal support structures for the transport of nutrients, vesicles, mitochondria and chromosomes within the cell. Mutations in MAPT are associated with an early onset of the disease (40–50 years), and the clinical phenotype is consistent with Frontotemporal Dementia (FTD). Recently, mutations in a second gene, named progranulin (GRN), have been identified in some families with FTLD. The pathology associated with these mutations is most frequently characterized by the immunostaining of TAR DNA Binding Protein 43 (TDP-43), which is a transcription factor.
Observational clinical study in juvenile-adult glycogenosis type 2 patients undergoing enzyme replacement therapy for up to 4 years

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Received: 19 August 2011 / Revised: 7 October 2011 / Accepted: 12 October 2011 / Published online: 12 November 2011
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Abstract  The objective of this study was to describe a large Italian cohort of patients with late-onset glycogen storage disease type 2 (GSDII) at various stages of disease progression and to evaluate the clinical effectiveness of alglucosidase alpha enzyme replacement therapy (ERT). Previous studies showed in late-onset patients ERT efficacy against placebo and variable response in uncontrolled studies. Seventy-four juvenile or adult GSDII patients were treated with ERT in a multicenter open label, non-randomized study, from 12 months up to 54 months. Recombinant human alpha glucosidase (rh-GAA) was injected by intravenous route at 20 mg/kg every second week. Patients were divided into three groups according to ERT duration: Group A received treatment for 12–23 months (n = 16), Group B for 24–35 months (n = 14), and Group C for more than 36 months (n = 21).

C. Angelini, T. Mongini, A. Toscano: Coordinators of the Italian Group on GSDII.

The members of the Italian GSDII Group are listed in the Appendix.

Electronic supplementary material  The online version of this article (doi:10.1007/s00415-011-6293-5) contains supplementary material, which is available to authorized users.

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Importance of SPP1 genotype as a covariate in clinical trials in Duchenne muscular dystrophy

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ABSTRACT

Objective: To test the effect of the single nucleotide polymorphism –66 T>G (rs28357094) in the osteopontin gene (SPP1) on functional measures over 12 months in Duchenne muscular dystrophy (DMD).

Methods: This study was conducted on a cohort of ambulatory patients with DMD from a network of Italian neuromuscular centers, evaluated longitudinally with the North Star Ambulatory Assessment (NSAA) and the 6-Minute Walk Test (6MWT) at study entry and after 12 months. Genotype at rs28357094 was determined after completion of the clinical evaluations. Patients were stratified in 2 groups according to a dominant model (TT homozygotes vs TG heterozygotes and GG homozygotes) and clinical data were retrospectively compared between groups.

Results: Eighty patients were selected (age 4.1–19.3 years; mean 8.3 ± 2.7 SD). There were no differences in age or steroid treatment between the 2 subgroups. Paired t test showed a significant difference in both NSAA (p = 0.013) and 6MWT (p = 0.03) between baseline and follow-up after 12 months in patients with DMD carrying the G allele. The difference was not significant in the T subgroup. The analysis of covariance using age and baseline values as covariate and SPP1 genotype as fixed effect showed that these parameters are significantly correlated with the 12-month values.

Conclusions: These data provide evidence of the role of SPP1 genotype as a disease modifier in DMD and support its relevance in the selection of homogeneous groups of patients for future clinical trials. Neurology. 2012;79:159-162

GLOSSARY

6MWT = 6-Minute Walk Test; ANCOVA = analysis of covariance; DMD = Duchenne muscular dystrophy; NSAA = North Star Ambulatory Assessment.

Osteopontin (secreted phosphoprotein, SPP1), a 35–60 kDa secreted glycoprotein, functions as a pleiotropic cytokine in several pathologic and reparative processes. Lately, evidence has emerged that in the mdx mouse model SPP1 modulates muscle inflammation and regeneration, and that SPP1 genetic ablation (“double mutant” mouse) induces a milder phenotype. We recently found that the polymorphic G genotype at position –66 in the SPP1 promoter (rs28357094) is associated with earlier loss of ambulation and more rapid weakness progression in Duchenne muscular dystrophy (DMD). This association needs further confirmation and assessment of magnitude and statistical power.2

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Study funding: Supported by grants from NIH (U54HD053177 – Wellstone Muscular Dystrophy Center), Italian Telethon U1LDM grant (GUP07009), the Eurobiobank network (QLRT2001-027769 to C.A.), Telethon Bank (GTF05003), and NHGMBB (Second University of Napoli), member of Eurobiobank and Telethon Network of Genetic Biobanks.

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Review Article

Neurological features of Fabry disease: clinical, pathophysiological aspects and therapy


Fabry disease is a multisystem, X-linked, lysosomal storage disorder caused by a mutation in the GLA gene on chromosome Xq22 resulting in alpha-galactosidase A enzyme (α-Gal A) deficiency. Neurological manifestations other than cerebrovascular accidents include small fibre neuropathy and dysautonomic disorders, which may be the presenting clinical features in a proportion of patients. An atypical disease onset may be misdiagnosed until the emergence of a more typical clinical picture, characterized by chronic renal and cardiac failure. Thus, neurologists should consider Fabry disease in differential diagnosis and provide an appropriate diagnostic work up. This review focuses on central and peripheral nervous system involving available diagnostic tools and diagnostic work up in Fabry disease. It also covers the most recent evidence regarding enzyme replacement therapy.

Introduction

Fabry disease (FD: Anderson-Fabry disease, Online Mendelian Inheritance in Man OMIM 301500) is a multisystem, X-linked lysosomal storage disorder caused by a mutation in the GLA gene on chromosome Xq22 resulting in lysosomal alpha-galactosidase A enzyme (α-Gal A) deficiency (1, 2).

Alpha-galactosidase is widely expressed and its deficiency results in deposition of glycosphingolipids, particularly globotriaosylceramide (Gb3), in different tissues and organs (peripheral and central nervous system neurons, skin, eyes, heart and kidneys). Lipid accumulation occurs preferentially within the vascular endothelium and smooth muscle cells leading to progressive vessel occlusion, ischaemia and, ultimately, organ dysfunction.

To date, hundreds of mutations, mainly missense and nonsense, but also small and large deletions, have been identified in the GLA gene (3, 4). The severity of FD is related to residual α-Gal A enzymatic activity. Even in the absence of a clear genotype–phenotype correlation, mutations leading to a complete loss of function generally result in the classic phenotype.

The disease primarily occurs among hemizygous males. However a significant proportion of heterozygous (carrier) females also develop symptoms, but they have an older age at onset (5, 6). Although an X inactivation mechanism is probably involved, it cannot fully explain the presence of symptomatic heterozygous females; therefore, the clinical variability is probably due to other genetic or environmental factors.

Fabry disease is a relatively rare condition with a reported worldwide incidence ranging from 1:40,000 to 1:117,000 live male births (7). However, in view of its heterogeneous phenotype, it is probably underdiagnosed (8), a suggestion strengthened by a recent study in which, in FD screening of 37,104 newborn males, deficient α-Gal A activity was identified in 1:3100 subjects (9). A wide inter...
Next-generation sequencing reveals DGUOK mutations in adult patients with mitochondrial DNA multiple deletions

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The molecular diagnosis of mitochondrial disorders still remains elusive in a large proportion of patients, but advances in next generation sequencing are significantly improving our chances to detect mutations even in sporadic patients. Syndromes associated with mitochondrial DNA multiple deletions are caused by different molecular defects resulting in a wide spectrum of predominantly adult-onset clinical presentations, ranging from progressive external ophthalmoplegia to multi-systemic disorders of variable severity. The mutations underlying these conditions remain undisclosed in half of the affected subjects. We applied next-generation sequencing of known mitochondrial targets (MitoExome) to probands presenting with adult-onset mitochondrial myopathy and harbouring mitochondrial DNA multiple deletions in skeletal muscle. We identified autosomal recessive mutations in the DGUOK gene (encoding mitochondrial deoxyguanosine kinase), which has previously been associated with an infantile hepatocerebral form of mitochondrial DNA depletion. Mutations in DGUOK occurred in five independent subjects, representing 5.6% of our cohort of patients with mitochondrial DNA multiple deletions, and impaired both muscle DGUOK activity and protein stability. Clinical presentations were variable, including mitochondrial myopathy with or without progressive external ophthalmoplegia, recurrent rhabdomyolysis in a young female who had received a liver transplant at 9 months of age and adult-onset lower motor neuron syndrome with mild cognitive impairment. These findings reinforce the...
Variants in SNAP25 are targets of natural selection and influence verbal performances in women

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Received: 1 September 2011 / Revised: 21 November 2011 / Accepted: 22 November 2011 / Published online: 23 December 2011 © Springer Basel AG 2011

Abstract Descriptions of genes that are adaptively evolving in humans and that carry polymorphisms with an effect on cognitive performances have been virtually absent. SNAP25 encodes a presynaptic protein with a role in regulation of neurotransmitter release. We analysed the intra-specific diversity along SNAP25 and identified a region in intron 1 that shows signatures of balancing selection in humans. The estimated TMRCA (time to the most recent common ancestor) of the SNAP25 haplotype phylogeny amounted to 2.08 million years. The balancing selection signature is not secondary to demographic events or to biased gene conversion, and encompasses rs363039. This SNP has previously been associated to cognitive performances with contrasting results in different populations. We analysed this variant in two Italian cohorts in different age ranges and observed a significant genotype effect for rs363039 on verbal performances in females alone. Post hoc analysis revealed that the effect is driven by differences between heterozygotes and both homozygous genotypes. Thus, heterozygote females for rs363039 display higher verbal performances compared to both homozygotes. This finding was replicated in a cohort of Italian subjects suffering from neuromuscular diseases that do not affect cognition. Heterozygote advantage is one of the possible reasons underlying the maintenance of genetic diversity in natural populations. The observation that heterozygotes for rs363039 display higher verbal abilities compared to homozygotes perfectly fits the underlying balancing selection model. Although caution should be used in inferring selective pressures from observed signatures, SNAP25 might represent the first description of an adaptively evolving gene with a role in cognition.

Keywords SNAP25 · Balancing selection · Intelligence quotient · Verbal performances

Abbreviations

SNP Single nucleotide polymorphism
EAS East Asians

Electronic supplementary material The online version of this article (doi:10.1007/s00018-011-0896-y) contains supplementary material, which is available to authorized users.

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Identification of a new susceptibility variant for multiple sclerosis in OAS1 by population genetics analysis

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Received: 18 March 2011 / Accepted: 21 June 2011 / Published online: 7 July 2011 © Springer-Verlag 2011

Abstract Contrasting results have been reported concerning the association of a splice-site polymorphism (rs10774671) in OAS1 with multiple sclerosis (MS). We analysed two OAS1 regions encompassing alternatively spliced exons. While the region carrying the splice-site variant is neutrally evolving, a signature of long-standing balancing selection was observed across an alternative exon 7. Analysis of variants in this exon identified an insertion/deletion polymorphism (rs11352835, A/) that originates predicted products with distinct C termini. This variant is located along the major branch of the haplotype genealogy, suggesting that it may represent the selection target. A case/control study for MS indicated that rs11352835 is associated with disease susceptibility (for an allelic model with the deleted allele predisposing to MS, OR 1.27, 95% CI 1.072–1.513, \( p = 0.010 \)). No association was found between rs10774671 and MS. As the two SNPs are in linkage disequilibrium in Europeans, the previously reported association between rs10774671 and MS susceptibility might be driven by rs11352835, possibly explaining the contrasting results previously observed for the splice-site polymorphism. Thus, we describe a novel susceptibility variant for MS in OAS1 and show that population genetic analyses can be instrumental to the identification of selection targets and, consequently, of functional polymorphisms with an effect on phenotypic traits.

Introduction

In humans four 2',5'-oligoadenylate synthetase genes (OAS1, OAS2, OAS3 and OASL) are located on the long arm of chromosome 12 and play a central role in the innate immune response against viruses. These enzymes are activated either by the presence of double strand RNA or by single strand RNA with secondary structure, and
**Introduction**

Mitochondrial myopathies are a large, heterogeneous group of disorders that frequently present with multisystem dysfunction and have a broad variety of phenotypes and genetic etiologies.

Mitochondria play important roles in cellular energy metabolism, free-radical generation, and apoptosis. They are small, semiautonomous organelles involved in cellular metabolism and the regulation of cell death. Mitochondria contain their own genetic material (mitochondrial DNA [mtDNA]) along with their own transcription, translation, and protein-assembly machinery and also encode 13 structural proteins that are all subunits of the respiratory chain. Mitochondria are genomically independent of the cell nucleus, although most mitochondrial proteins are encoded by the nuclear genome (nuclear DNA) and imported into mitochondria. It is interesting to note that the replication of mitochondria does not require the presence of mtDNA because mitochondrial biosynthesis continues even when mtDNA is deleted. The human mitochondrial genome has been completely sequenced, and each gene within it has been identified and characterized.1,2

Mitochondrial disorders are due to altered mitochondrial functions, and although each of the various mitochondrial functions may be affected, mitochondrial disorders are most frequently due to disturbances of energy production via the respiratory chain and oxidative phosphorylation.

Genetically, mitochondrial disorders are due to mutations of either mtDNA or nuclear DNA. In this setting, the phenomenon of genetic heteroplasmy arises (ie, some genomes contain the mutation although the remaining genomes are wild-type genomes). mtDNA mutations may lead to the coexistence of wild-type and mutated mitochondrial genomes within a mitochondrion, cell, or tissue (heteroplasmy) or the presence of exclusively mutated mtDNA or wild-type mtDNA (homoplasmy). Heteroplasmic mtDNA mutations become symptomatic only if a certain threshold mutation load (usually 60% to 70%) is exceeded, at which point they behave as recessive-like traits.

The transmission of mtDNA mutations is complex and incompletely understood; women with mtDNA mutations pass their mutations on at a level of heteroplasmy that is unpredictable and apparently random.

Because mitochondria are the main source of energy production in mammalian cells, clinical manifestations of disorders of these organelles typically involve tissues with the highest energy requirements. Furthermore, the presence of mtDNA in all human tissues means that a dysfunction affects multiple organ systems. The most commonly affected organs are the nervous system (CNS and peripheral and autonomic systems as well as the optic nerve and retina), muscles (and, in particular, extraocular muscles), the cardiac apparatus, and the endocrine system. The clinical presentation is highly variable with regard to age at onset, symptoms, signs, severity, and prognosis.

Mitochondrial defects have long been suspected to play an important role in the development and progression of cancer. Some mutations in mtDNA have been identified in various types of human cancer such as breast cancer, ovarian cancer, colorectal cancer, renal cell carcinoma, and lung cancer.7-13 Moreover, pathogenic mtDNA mutations seem to promote tumors by preventing apoptosis.14

However, it is unclear whether there are interactions between mitochondrial myopathies and cytotoxic drugs. Theoretically, and according to preclinical data, cytotoxic drugs could cause damage to mitochondria. In preclinical studies, cisplatin and carboplatin were shown to reduce L-carnitine and cause mtDNA mutations,15,16 and doxorubicin was also found to cause mtDNA mutations.15,16 Cyclophosphamide decreases the activity of enzymes of the citric acid cycle but also decreases the activity of respiratory chain complexes.17 There are also indications that the nephrotoxicity of ifosfamide is due to the inhibition of respiratory chain complexes.18

To our knowledge, there are no reports on the feasibility and safety of systemic chemotherapy in patients with mitochondrial myopathies. As a consequence, when discussing the treatment possibilities for a patient with an advanced tumor and concomitant mitochondrial myopathy, oncologists are forced to consider the option of systemic chemotherapy without being able to predict the risk of toxicity.

**Case Report**

In this article, we describe the clinical case of a 61-year-old man who was a former smoker and affected by a mitochondrial myopathy who developed stage IV lung adenocarcinoma with synchronous cerebral metastases. The diagnosis of the adenocarcinoma was made from transbronchial needle aspiration; molecular analyses, such as epidermal growth factor receptor (EGFR) mutation analysis, were not performed because of the lack of adequate tumor samples.

Early signs of the mitochondrial myopathy appeared at 20 years of age with the onset of palpebral ptosis, which was surgically corrected when the patient was 36 years old. The diagnosis of mitochondrial myopathy was first made after a biopsy of the left biceps when the man was 34 years old and subsequently confirmed by two more muscle biopsies performed during the next 12 years.

Histopathologic findings were an increased amount of ragged-red fibers (RRFs) as detected by the modified Gomori trichrome stain, increased hyperactive fibers demonstrated by the succinate dehydrogenase stain, failure of both RRFs and some non-RRFs to stain with the histochemical reaction for cytochrome c-oxidase, and Southern blot demonstration of a heteroplasmic 8,288 to 14,430 mtDNA deletion.

At the time the cancer of the patient was diagnosed, the mitochondrial myopathy-related symptoms of the patient were dysphagia, dysarthria, ophthalmoplegia, myopathy, sensory ataxia, diabetes, and
toxicity was observed, and no treatment delay was required. Mitochondrial myopathy (paresthesia of lower extremities). No grade 3 or 4 neuropathic pain assessment was performed on day 1 of each treatment. Physical examination, including motor and sensory neuropathy and hypomotility of the diaphragm (the patient had been receiving respiratory support with bilevel positive airway pressure overnight since he was 55 years old). No renal impairment or cardiopathy was found; the left-ventricular ejection fraction of the patient was 55%.

In August 2011, the patient was admitted to our department to start systemic chemotherapy. In the absence of clinical information about interactions between systemic chemotherapy and mitochondrial myopathy, the treatment was chosen according to standard guidelines and preference given to drugs with limited neurotoxicity.

Four cycles of carboplatin combined with pemetrexed were planned. Dosages of carboplatin given were calculated according to the Calvert formula, with an area under the curve of 2 in the first cycle and 3 in the next three cycles. The dose of pemetrexed was 500 mg/m² although, precautionally, 75% of the full dose was administered in the first cycle. All four treatment cycles were given in an outpatient setting.

Grade 1 to 2 toxicities observed were grade 1 asthenia and grade 1 neurotoxicity (paresthesia of lower extremities). No grade 3 or 4 toxicity was observed, and no treatment delay was required. Mitochondrial myopathy-related symptoms remained stable throughout the treatment period. In the absence of new symptoms related to the mitochondrial myopathy, no changes in daily activities or physical, mental, and social functioning were observed.

Plasma levels of glucose and of markers of muscle damage (ie, lactate dehydrogenase [LDH], aldolase, and creatine phosphokinase [CK]) were measured on day 1 of each treatment cycle and did not increase significantly over time (Fig 1; AUC, area under the curve). Tumor assessment after three cycles of chemotherapy showed stable disease (tumor reduction, 10%). The planned four cycles of chemotherapy were given and whole brain radiation was started.

In conclusion, to our knowledge, this is the first report on the safety of chemotherapy in a patient with mitochondrial myopathy. In our experience, the administration of carboplatin and pemetrexed in the patient with lung cancer and mitochondrial myopathy was well tolerated, and no changes in either mitochondria myopathy-related symptoms or plasma levels of muscle enzymes were observed. The carboplatin and pemetrexed combination can be considered a treatment option in patients affected by lung adenocarcinoma and mitochondrial myopathy. Different chemo-therapeutic agents could still have an unexpected interplay with mitochondrial metabolic pathways.

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Authors’ disclosures of potential conflicts of interest
The author(s) indicated no potential conflicts of interest.

References

First cycle: carboplatin AUC2 + pemetrexed 500 mg/m² (75% full dose)
Second cycle: carboplatin AUC3 + pemetrexed 500 mg/m² (100% full dose)
Third cycle: carboplatin AUC3 + pemetrexed 500 mg/m² (100% full dose)
Fourth cycle: carboplatin AUC3 + pemetrexed 500 mg/m² (100% full dose)
SHORT REPORT

Riboflavin transporter 3 involvement in infantile Brown-Vialetto-Van Laere disease: two novel mutations

Mariana Ciccolella, Stefania Corti, Michela Catteruccia, Stefania Petrini, Giulia Tozzi, Teresa Rizza, Rosalba Carrozzo, Monica Nizzardo, Andreina Bordoni, Dario Ronchi, Adele D’Amico, Cristiano Rizzo, Giacomo Pietro Comi, Enrico Bertini

ABSTRACT

Background Brown-Vialetto-Van Laere (BVVL) syndrome is a rare disorder characterised by progressive pontobulbar palsy and sensorineural deafness. Causative mutations in genes encoding human riboflavin transporter 2 (hRFT2) and 3 (hRFT3) have been identified in BVVL patients.

Methods and results We report the clinical and molecular features of a severe BVVL patient in whom screening of SLC52A3/hRFT2 was negative. Sequence analysis identified two novel compound heterozygous mutations in SLC52A2/hRFT3, namely c.155C>T and c.1255G>A, leading to the amino acid changes p.S52F and p.G419S, respectively. Functional studies show that these defects impair the gene expression of the corresponding transporter, resulting in a significant reduction of riboflavin transport.

Conclusions These findings support the pathogenetic role of SLC52A2/hRFT3 in BVVL with important clinical and therapeutic implications.

INTRODUCTION

Brown-Vialetto-Van Laere (BVVL) syndrome (OMIM 211530) is a rare childhood neurological disorder characterised by progressive pontobulbar palsy associated with sensorineural deafness. This syndrome was first described by Charles Brown in 1894 in a 15-year-old German boy, and then further documented by Vialetto and Van Laere. To date, more than 60 cases have been described. Sensorineural deafness usually precedes neurological symptoms, followed by the involvement of the motor components of the 7th and 9th to 12th cranial nerves. BVVL patients show respiratory impairment, upper and lower limb weakness and wasting resembling that of amyotrophic lateral sclerosis (ALS). Motor neuron diseases similar to BVVL syndrome have been reported, including Madras motor neuron disease, Boltshauser syndrome, Nathalie syndrome, Fazio-Londe syndrome (possibly an allelic condition to BVVL syndrome), and other conditions of very early onset ALS. No effective therapy is available for these disorders.

Inheritance of BVVL is often autosomal recessive, although autosomal dominant forms and variable penetrance has been reported. Recently, SLC52A3 and SLC52A2 (solute carrier family 52, encoding for human riboflavin transporters (hRFT2), formerly named C20orf54, and hRFT3) were found to be mutated in some recessive BVVL cases. These findings led to the proposal of riboflavin (RF) supplementation as a possible therapeutic strategy, with positive results. However, other patients with BVVL have been found to be negative for mutations in SLC52A3 and SLC52A2, suggesting the existence of other genetic defects.

Here we describe the case of a boy with an early onset, severe fatal form of BVVL in which the analysis of SLC52A3/hRFT2 was negative. We thus performed sequence analysis of SLC52A1/hRFT1 and SLC52A2/hRFT3 genes disclosing two heterozygous mutations in SLC52A2/hRFT3, which were absent in control subjects. We also performed functional studies addressing their effects on the expression and activity of the transporter. Because this is a retrospective analysis, the effect of RF therapy could not be assessed. Our data support the causative role of hRFT3 in the pathogenesis of BVVL, with relevant therapeutic implications.

PATIENTS AND METHODS

Case report

The proband was the only male child of healthy unrelated parents. He was born after an uncomplicated pregnancy by normal delivery at 40 weeks of gestation. Weight at birth was 3.2 kg. He had normal early psychomotor development and was able to walk at 12 months. At age 2 years he developed progressive dysphonia and notable exercise intolerance with dyspnoea and cyanosis. At age 3 years brainstem auditory evoked responses (BAERs) documented bilateral sensorineural hearing loss. Ophthalmological evaluation revealed reduced visual acuity. In the following 3 months he developed progressive shoulder and axial muscle weakness with sloping and adducted shoulders and kyphosis, wasting and weakness of hand muscles, and walking difficulties with foot drop. Neurophysiological studies showed a neurogenic pattern, and brain MRI was unremarkable. Clinical conditions rapidly deteriorated. He was hospitalised for acute respiratory failure and aspiration pneumonia. Urinary organic acids and plasma acylcarnitines were normal (see online supplementary file for methods and details). The patient died 5 days after admission to hospital.

Research Article

Direct reprogramming of human astrocytes into neural stem cells and neurons

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ARTICLE INFORMATION

Abstract

Generating neural stem cells and neurons from reprogrammed human astrocytes is a potential strategy for neurological repair. Here we show dedifferentiation of human cortical astrocytes into the neural stem/progenitor phenotype to obtain progenitor and mature cells with a neural fate. Ectopic expression of the reprogramming factors OCT4, SOX2, or NANOG into astrocytes in specific cytokine/culture conditions activated the neural stem gene program and induced generation of cells expressing neural stem/precursor markers. Pure CD44+ mature astrocytes also exhibited this lineage commitment change and did not require passing through a pluripotent state. These astrocyte-derived neural stem cells gave rise to neurons, astrocytes, and oligodendrocytes and showed in vivo engraftment properties. ASCL1 expression further promoted neuronal phenotype acquisition in vitro and in vivo. Methylation analysis showed that epigenetic modifications underlie this process. The restoration of multipotency from human astrocytes has potential in cellular reprogramming of endogenous central nervous system cells in neurological disorders.

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Keywords:
Astrocytes
Reprogramming
Neural stem cells
Induced pluripotent stem cells

Introduction

Replacement of neurons after degeneration or damage is absent in the vast majority of the mammalian central nervous system (CNS), and neuronal loss is thought to be definitive in neurological diseases such as stroke or Alzheimer’s disease [1]. In apparent contrast, several studies have demonstrated that some cells resembling glial cells function as neural stem cells (NSCs) or progenitors in specific areas of the adult brain, such as the ventricular subependymal zone and the subgranular zone of the hippocampus [1]. These data pose the questions of [1] why glia in the vast majority of the CNS, such as the cortical regions, apparently cannot produce new neurons and [2] if these cells can be redirected towards neurogenesis when supplied with the appropriate transcriptional factors and/or environmental signals.

It has been demonstrated that astrocytes from murine cerebral cortex can be directly differentiated into neurons by the forced expression of a single transcription factor, such as PAX6, Neurog2 or
Editor's Summary

Engineering iPSC-Derived Motor Neurons for Cell Therapy

Spinal muscular atrophy (SMA) is an autosomal recessive disorder caused by mutations in the gene encoding the survival motor neuron 1 (SMN1) protein. The mutant protein causes loss of spinal cord motor neurons, and there is no effective therapy. Humans have a paralogous gene, SMN2, that differs from SMN1 by a single nucleotide variant within exon 7 that results in the production of an incomplete and nonfunctional protein. Now, Corti et al. investigate the feasibility of genetically engineering induced pluripotent stem cells (iPSCs) derived from SMA patients to generate motor neurons that do not show the disease phenotype. The authors generated human SMA-iPSCs using nonviral, nonintegrating episomal vectors and then performed genetic editing with oligonucleotides to modify SMN2 to produce a functional SMN1-like protein. Uncorrected SMA-iPSC-derived motor neurons reproduced disease-specific features, whereas motor neurons derived from genetically corrected SMA-iPSCs showed rescue of the disease phenotype. Upon direct transplantation into a severe SMA mouse model, corrected SMA-iPSC–derived motor neurons engrafted in the spinal cord and improved the disease phenotype. This study demonstrates the feasibility of generating patient-specific iPSCs and their motor neuron progeny that are genetically corrected and free of exogenous sequences and suggests the potential of this approach for clinical translation.

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Genetic Correction of Human Induced Pluripotent Stem Cells from Patients with Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is among the most common genetic neurological diseases that cause infant mortality. Induced pluripotent stem cells (iPSCs) generated from skin fibroblasts from SMA patients and genetically correct have been proposed to be useful for autologous cell therapy. We generated iPSCs from SMA patients (SMA-iPSCs) using nonviral, nonintegrating episomal vectors and used a targeted gene correction approach based on single-stranded oligonucleotides to convert the survival motor neuron 2 (SMN2) gene into an SMN1-like gene. Corrected iPSC lines contained no exogenous sequences. Motor neurons formed by differentiation of uncorrected SMA-iPSCs reproduced disease-specific features. These features were ameliorated in motor neurons derived from genetically corrected SMA-iPSCs. The different gene splicing profile in SMA-iPSC motor neurons was rescued after genetic correction. The transplantation of corrected motor neurons derived from SMA-iPSCs into an SMA mouse model extended the life span of the animals and improved the disease phenotype. These results suggest that generating genetically corrected SMA-iPSCs and differentiating them into motor neurons may provide a source of motor neurons for therapeutic transplantation for SMA.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive genetic disorder caused by a genetic defect in the survival motor neuron 1 (SMN1) gene resulting from deletions or other mutations (1, 2). SMA arises when there are no functional copies of SMN1, and the affected individual therefore must fully rely on the protein produced from SMN2, a gene paralogous to SMN1 (1, 2). A decrease in full-length SMN protein results in the selective degeneration of spinal cord motor neurons (3). Patients with SMA typically show generalized muscle weakness and paralysis that can progress very rapidly to early childhood death (4). There is no cure.

SMN2 shows a high sequence homology to SMN1, and the only critical difference is the C-to-T base change 6 base pairs (bp) inside exon 7. This alteration, but not other variations in the SMN genes, affects the splicing of SMN2 (5). This splicing change yields 10% of the full-length protein and high concentrations of an unstable, truncated protein lacking exon 7 (SMNA7) (5). The disease severity inversely correlates with SMN2 copy number (6). Worms, flies, and mice lack SMN2, which can be introduced only by transgenic modifications (7–10). Several therapeutic strategies have targeted increased exon 7 inclusion in SMN2 transcripts, and a human cell–based assay is a critical tool (10).

The differentiation of viral vector–generated induced pluripotent stem cells (iPSCs) from SMA patients into motor neurons has recently been demonstrated (11). Ex vivo correction of the SMN1 mutation in SMA-iPSCs might allow the generation of disease-free motor neurons for cell therapy. The vectors used, however, can produce insertional mutations that interfere with normal cell function, and transgene expression can modify differentiation into specific lineages (12) or even lead to tumorigenesis (13), limiting their usefulness.

RESULTS

Nonviral vector generation and characterization of SMA-iPSCs

We generated iPSC lines from two type I SMA patients (SMA-iPSC-1 and SMA-iPSC-2) using a nonviral vector method based on nucleofection of adult fibroblasts with constructs encoding OCT4, SOX2, NANOG, LIN28, c-Myc, and KLF4 (14) (Fig. 1, A and B, and figs. S1 and S2). These plasmids are progressively lost from cells, leading to the generation of iPSCs free of vector and exogenous sequences. As a control, we generated iPSCs from the father of patient 1 (heterozygous, HET-iPSC-1), and we used an already generated wild-type cell line (iPSC 19.9). We isolated at least three clonal iPSC lines for each individual free from reprogramming vectors (Fig. 1V). All iPSC lines displayed embryonic stem (ES) cell morphology (Fig. 1, C to N) and expressed the pluripotency markers NANOG, SOX2, OCT4, SSEA-4, and TRA-1-60 (Fig. 1, F to H and L to N). All iPSC lines also maintained euploid karyotypes (Fig. 1O), could differentiate into all three germ layers in vitro (fig. S3), and could form teratomas in vivo (Fig. 1, P to U). DNA fingerprinting confirmed their origin from parental fibroblasts (table S1). These data and genome-wide
Metformin overdose causes platelet mitochondrial dysfunction in humans

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See related commentary by Orban et al., http://ccforum.com/content/16/5/164

Abstract

Introduction: We have recently demonstrated that metformin intoxication causes mitochondrial dysfunction in several porcine tissues, including platelets. The aim of the present work was to clarify whether it also causes mitochondrial dysfunction (and secondary lactate overproduction) in human platelets, in vitro and ex vivo.

Methods: Human platelets were incubated for 72 hours with saline or increasing doses of metformin (in vitro experiments). Lactate production, respiratory chain complex activities (spectrophotometry), mitochondrial membrane potential (flow-cytometry after staining with JC-1) and oxygen consumption (Clark-type electrode) were then measured. Platelets were also obtained from ten patients with lactic acidosis (arterial pH 6.97 ± 0.18 and lactate 16 ± 7 mmol/L) due to accidental metformin intoxication (serum drug level 32 ± 14 mg/L) and ten healthy volunteers of similar sex and age. Respiratory chain complex activities were measured as above (ex vivo experiments).

Results: In vitro, metformin dose-dependently increased lactate production (P < 0.001), decreased respiratory chain complex I activity (P = 0.009), mitochondrial membrane potential (P = 0.003) and oxygen consumption (P < 0.001) of human platelets. Ex vivo, platelets taken from intoxicated patients had significantly lower complex I (P = 0.045) and complex IV (P < 0.001) activity compared to controls.

Conclusions: Depending on dose, metformin can cause mitochondrial dysfunction and lactate overproduction in human platelets in vitro and, possibly, in vivo.

Trial registration: NCT 00942123.

Introduction

Metformin is the drug of choice for adults with type 2 diabetes [1]. It is the seventh most frequently prescribed generic drug in the US (fifty-nine million prescriptions in 2011) [2] and is currently taken by almost two per cent of the Italian population [3].

Metformin is a safe drug [4] but lactic acidosis can develop rarely, especially when renal failure leads to accidental intoxication [5-7]. Sixty-six similar cases have been reported to the Poison Control Centre of Pavia, Italy, over the last five years, resulting in seventeen deaths (Dr. Sarah Vecchio, unpublished data). Since metformin use is constantly increasing (4% to 8% rise in prescriptions per year in the US and Italy) [2,3], related episodes of lactic acidosis will possibly become less uncommon [8].

The pathogenesis of lactic acidosis during metformin therapy remains poorly understood, particularly when no other major risk factors (such as hypoxia, tissue hypoperfusion or liver failure) can be identified [9]. Nonetheless, growing evidence suggests that metformin intoxication may directly induce lactic acidosis [10], possibly by altering liver lactate metabolism. In fact, metformin readily accumulates in hepatocytes that express the Organic Cation Transporter (OCT) 1 [11] and dose-dependently inhibits
Abstract

Effects of Metformin on the Respiratory Chain Enzymes in Human Hepatocytes

Alarming increase of the number of metformin intoxications. Ten times doubled number of inquiries to the Swedish Poison Information Center since 2000. Two additional times the number of cases treated for metformin intoxications in the Toxicology Unit in University Hospital, Linköping, Sweden.

Materials and Methods

Human hepatocytes were cultured and treated with metformin or lactic acid.

Results

Metformin inhibits mitochondrial permeability transition and cell death through a mitochondrial permeability transition-dependent mechanism.

Conclusion

Metformin inhibits mitochondrial permeability transition and cell death through a mitochondrial permeability transition-dependent mechanism.

Acknowledgements

This study was supported in part by an Italian grant provided by Fondazione Fiera di Milano for Translational and Competitive Research (2007, Luciano Gattinoni).

Author details


Perspective

Nitric oxide donor and non steroidal anti inflammatory drugs as a therapy for muscular dystrophies: Evidence from a safety study with pilot efficacy measures in adult dystrophic patients

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ARTICLE INFO

Article history:
Received 13 December 2011
Received in revised form 16 January 2012
Accepted 16 January 2012

Keywords:
Muscular dystrophies
Pharmacological treatment
Nitric oxide donor
Anti-inflammatory drug
Safety

ABSTRACT

This open-label, single centre pilot study was designed to evaluate safety and tolerability of the combination of the drugs isosorbide dinitrate, a nitric oxide donor, and ibuprofen, a non steroidal anti-inflammatory drug, in a cohort of adult dystrophic patients (Duchenne, Becker and Limb-Girdle Muscular Dystrophy). Seventy-one patients were recruited: 35, treated with the drug combination for 12 months, and 36 untreated. Safety and adverse events were assessed by reported signs and symptoms, physical examinations, blood tests, cardiac and respiratory function tests. Exploratory outcomes measure, such as the motor function measure scale, were also applied.

Good safety and tolerability profiles of the long-term co-administration of the drugs were demonstrated. Few and transient side effects (i.e. headache and low blood pressure) were reported. Additionally, exploratory outcomes measures were feasible in all the disease population studied and evidenced a trend towards amelioration that reached statistical significance in one dimension of the MFM scale. Systemic administration of ibuprofen and isosorbide dinitrate provides an adequate safety margin for clinical studies aimed at assessing efficacy.

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1. Introduction

Muscular dystrophies have a complex pathogenesis since the original genetic defect leads to a host of concurrent pathogenic events. Despite substantial progress in understanding the pathophysiological bases of these diseases, no pharmacological therapies have been identified that increase muscle strength, other than corticosteroids. Several studies provide reliable data on the benefit of both prednisone/prednisolone and deflazacort [1–4].

The potential beneficial effects of corticosteroids include inhibition of muscle proteolysis, stimulation of myoblast proliferation, increase in myogenic repair, anti-inflammatory immunosuppressive effects, reduction of cytosolic calcium concentrations [5] and up regulation of utrophin [6].

Several side effects, however, limit steroids usefulness [1]. New therapies may not be able to substitute entirely the steroids but may complement them and thus limit their use and/or reduce their dosages. For muscular dystrophies in adulthood, there have been only few small clinical trials and none involving novel therapeutic drugs or drug combinations [7–13]. We recently carried out studies in the mdx and α-sarcoglycan-null mouse models of dystrophy combining nitric oxide (NO) release and non steroidal anti-inflammatory (NSAID) activity, using the NO-releasing NSAID compound HCT1026 (nitroflurbiprofen), a combination of the NO donor isosorbide dinitrate (ISDN) and the NSAID ibuprofen or a dual compound releasing NO and ibuprofen for up to 12 months [14–16]. In all studies the results show that a combination of NO and NSAID activities slows disease progression by reducing inflammation, enhancing activity of endogenous stem cells and preventing muscle wasting. The beneficial effects were persistent, while in animals treated with ISDN or ibuprofen alone

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doi:10.1016/j.phrs.2012.01.006
Dear Sir,

Late-onset Pompe disease (LOPD) is an autosomal recessive multisystemic lysosomal storage disease caused by acid alpha-glucosidase (GAA) deficiency [1–5]. Incidence of Pompe disease varies between 1:40,000 and 1:156,000 [6–8]. Except in myotonic dystrophy type 1 (DM1) [9–12], incontinence is rarely reported in myopathies [13–15]. Nevertheless, it is a disabling condition in social as well as in professional life [16]. LOPD patients generally have nonhomogeneous muscle involvement (e.g. predominance of atrophy on the posterior compartment of the femoral muscles) [17]. To the best of our knowledge, incontinence has been reported in only 4 LOPD patients (2 urinary and 2 fecal) [18, 19]. Bernstein et al. [19] reported the case of an LOPD incontinent patient with subjective improvement after 3 months of enzyme replacement therapy (ERT). Pathophysiologic hypotheses for incontinence in myopathies include striated and smooth pelvic floor muscle as well as lower motor neuron or autonomic involvement [9–11, 14]. We aimed to assess the prevalence of fecal and urinary incontinence in LOPD and to determine if incontinent patients presented a more severe phenotype. We focused our study on patients with incontinence definitely attributable to LOPD and describe in detail a patient who recovered from his incontinence after ERT. We emphasize the need to look for incontinence in LOPD and to perform the appropriate workup.

Materials and Methods

From our previously described LOPD cohort [20], we studied only patients being clinically revisited (n = 20). Inclusion criteria for LOPD diagnosis were positivity for at least 2 of the following criteria: low GAA enzyme assay (<30%) [8], vacuolar myopathy and/or increased acid-phosphatase and/or periodic acid-Schiff stain and double GAA gene mutation. We systematically asked the patients if they had urinary or fecal incontinence, diarrhea or constipation. Main causes of incontinence such as central nervous system disease, peripheral nerve disease, dysautonomia or pelvic surgery were systematically and reasonably excluded by standardized detailed anamnesis and clinical examination in each patient. Women were asked to disclose their maternity status, and in men prostatism was assessed. The main risk factors for incontinence were also assessed (as previously described) [16]. We classified patients into 3 categories: definite, possible or no incontinence related to LOPD (definite: presence of incontinence without any other potential causes such as multiple deliveries or prostatic symptoms, or improvement due only to ERT; possible: presence of incontinence with other potential etiologies and no clinical response to ERT if administered).

Quality of life (QoL) was assessed by the SF-36 questionnaire that was previously validated in QoL assessment of LOPD patients [21]. We compared the demographic and phenotypic data of the group having no incontinence with that having definite incontinence by using the Mann-Whitney test.

ERT was administered intravenously, in 4 patients, following recommendations of the manufacturer (1/2 weeks; 20 mg/kg). In a 34-year-old male patient (P1), a complete fecal incontinence workup was performed before treatment and 1 year after starting ERT; it included pelvic floor electromyography (EMG), anorectal manometry, and pelvic magnetic resonance imaging (MRI).

The study was in accordance with local ethical committee recommendations.
Results

Out of 20 patients assessed, 9 (45%) had no incontinence, 6 (30%) had incontinence possibly attributable and 5 (25%) definitely attributable to LOPD (definite incontinence subgroup) (table 1). No patients in the definite subgroup disclosed any risk factors for incontinence.

In the definite incontinence subgroup, 2 of the 3 ERT-treated patients showed an improvement of their incontinence (the other did not but had started ERT only 3 months before our assessment). No statistically significant difference was found between the latter group and the group without incontinence, using the Mann-Whitney test for sex distribution, age at assessment, duration of the disease, limb muscular strength and respiratory status. Only QoL assessed by the SF-36 questionnaire was statistically significantly better in patients with no incontinence (data not shown) (p < 0.05). Clinical data of patients with definite incontinence due to LOPD are summarized in table 2.

The patient who was fully assessed for fecal incontinence had a 4-limb proximal weakness but was still ambulatory (P1, table 2). He complained of having had fecal and urinary incontinence for several months at the time of his first assessment. Anal sphincter EMG showed a clear myogenic pattern. Pelvic floor MRI revealed diffuse fatty pelvic floor muscle infiltration and levator ani muscle atrophy but no significant atrophy of the sphincter ani externus. Anorectal manometry showed reduced pressure of both internal and external anal sphincters: internal sphincter basal pressure was 45 mm Hg (normal range: 60–70) and external sphincter maximal pressure was 52.5 mm Hg (normal range: 110–180). Perineal physiotherapy did not provide improvement. One year later he began ERT. After 3 months of treatment, he observed an improvement in walking and roughly 1 month later he was free of incontinence (improvement remaining after 4 years of follow-up with ERT). Anorectal manometry, performed 1 year after the beginning of ERT, showed significant improvement of internal and external sphincter pressures (58 and 70 mm Hg, respectively; increase of 28.9 and 33.3%), the first measurement being related to smooth muscles. The patient’s motor and respiratory conditions were stabilized with ERT.

Discussion

For the first time, to the best of our knowledge, we estimate the prevalence of incontinence reasonably due to LOPD to be as high as 25%. These patients had no other etiologies or risk factors for incontinence.

The fecal incontinence rate in the general population varies regarding age and sex. Under the age of 70 years, the maximal rate reported is 14% [22]; in our small sample (none were more than 70 years old), we observed a rate of 30% (6/20) including 2 young male patients (28 and 31 years old) without significant limb or respiratory involvement (P4 and P5, respectively, table 2).

Table 1. Findings from the LOPD patient cohort assessed for incontinence

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>20</td>
</tr>
<tr>
<td>Male, n (age at assessment, years)</td>
<td>13 (median: 37; min: 23; max: 66)</td>
</tr>
<tr>
<td>Female, n (age at assessment, years)</td>
<td>7 (median: 46; min: 34; max: 64)</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean age ± SD at assessment, years</td>
<td>44.8 ± 13.6</td>
</tr>
<tr>
<td>Definite incontinent patients due to LOPD, n (%)</td>
<td>5 (25), M: 4 (31), F: 1 (14)</td>
</tr>
<tr>
<td>ERT</td>
<td>yes</td>
</tr>
<tr>
<td>Fecal incontinence</td>
<td>no</td>
</tr>
<tr>
<td>Double incontinence (fecal and urinary)</td>
<td>no</td>
</tr>
<tr>
<td>Incontinent patients improved by ERT, n</td>
<td>2 (1M, 1F)</td>
</tr>
</tbody>
</table>

Demographic data of our cohort of LOPD patients and distribution of the subgroup with definite incontinence due to LOPD. min = minimum, max = maximum, M = male, F = female.

Table 2. Clinical data of the LOPD patients having incontinence definitely attributable to LOPD

<table>
<thead>
<tr>
<th>Definite incontinent patient subgroup</th>
<th>Type of incontinence</th>
<th>Sex</th>
<th>ERT</th>
<th>Age at assessment (years)</th>
<th>Disease duration (time between first symptom and assessment) (years)</th>
<th>Walton 10-item score</th>
<th>Forced vital capacity (% of predicted value)</th>
<th>Assisted ventilation</th>
<th>SF-36 score (/100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>double</td>
<td>M</td>
<td>yes</td>
<td>4 years</td>
<td>34</td>
<td>25</td>
<td>3</td>
<td>40.8</td>
<td>45</td>
</tr>
<tr>
<td>P2</td>
<td>fecal</td>
<td>F</td>
<td>yes</td>
<td>1 year</td>
<td>61</td>
<td>16</td>
<td>3</td>
<td>47</td>
<td>NINV</td>
</tr>
<tr>
<td>P3</td>
<td>fecal</td>
<td>M</td>
<td>yes</td>
<td>3 months no</td>
<td>60</td>
<td>25</td>
<td>6</td>
<td>52</td>
<td>NINV</td>
</tr>
<tr>
<td>P4</td>
<td>fecal</td>
<td>M</td>
<td>no</td>
<td></td>
<td>31</td>
<td>11</td>
<td>0</td>
<td>83</td>
<td>no</td>
</tr>
<tr>
<td>P5</td>
<td>fecal</td>
<td>M</td>
<td>no</td>
<td></td>
<td>28</td>
<td>8</td>
<td>0</td>
<td>86</td>
<td>no</td>
</tr>
</tbody>
</table>

NINV = Noninvasive nocturnal ventilation.
C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect

Antonia Ratti, Lucia Corrado, Barbara Castellotti, Roberto Del Bo, Isabella Fogh, Cristina Cereda, Cinzia Tiloca, Carla D’Ascenzo, Alessandra Bagarotti, Viviana Pensato, Michela Ranieri, Stella Gagliardi, Daniela Calini, Letizia Mazzini, Franco Taroni, Stefania Corti, Mauro Ceroni, Gaia D. Oggioni, Kuang Lin, John F. Powell, Gianni Soraru, Nicola Ticozzi, Giacomo P. Comi, Sandra D’Alfonso, Cinzia Gellera, Vincenzo Silani, and the SLAGEN Consortium

A hexanucleotide repeat expansion (RE) in C9ORF72 gene was recently reported as the main cause of amyotrophic lateral sclerosis (ALS) and cases with frontotemporal dementia. We screened C9ORF72 in a large cohort of 259 familial ALS, 1275 sporadic ALS, and 862 control individuals of Italian descent. We found RE in 23.9% familial ALS, 5.1% sporadic ALS, and 0.2% controls. Two cases carried the RE together with mutations in other ALS-associated genes. The phenotype of RE carriers was characterized by bulbar-onset, shorter survival, and association with cognitive and behavioral impairment. Extrapyramidal and cerebellar signs were also observed in few patients. Genotype data revealed that 95% of RE carriers shared a restricted 10-single nucleotide polymorphism haplotype within the previously reported 20-single nucleotide polymorphism risk haplotype, detectable in only 27% of nonexpanded ALS cases and in 28% of controls, suggesting a common founder with cohorts of North European ancestry. Although C9ORF72 RE segregates with disease, the identification of RE both in controls and in patients carrying additional pathogenic mutations suggests that penetrance and phenotypic expression of C9ORF72 RE may depend on additional genetic risk factors.

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Keywords: Amyotrophic lateral sclerosis; Frontotemporal dementia; C9ORF72; Repeat expansion; Mutation analysis; Haplotype analysis

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder in which the relentless degeneration of motor neurons leads to a progressive muscular weakness and, ultimately, death. The etiology is sporadic (SALS) in the majority of cases, although familial forms (FALS) account...
A novel mutation in the β-tubulin gene TUBB2B associated with complex malformation of cortical development and deficits in axonal guidance

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PUBLICATION DATA
Accepted for publication 12th January 2012.
Published online 16th May 2012.

ABBREVIATIONS
ACC Agenesis of the corpus callosum
PMG Polymicrogyria
SCH Schizencephaly

ABSTRACT
Neurological disorders characterized by abnormal neuronal migration, organization, axon guidance, and maintenance have recently been associated with missense and splice-site mutations in the genes encoding α- and β-tubulin isotypes TUBA1A, TUBB2B, TUBB3, and TUBA8. We found a novel heterozygous mutation c.419G > C in exon 4 of the gene encoding TUBB2B in a female with microcephaly, agenesis of the corpus callosum, open-lip schizencephaly of the left parietal lobe, extensive polymicrogyria, basal ganglia and thalami dysmorphisms, and vermis and right third nerve hypoplasia. The missense change results in a glycine to alanine substitution; the mutated residue falls within an invariant glycine-rich region and therefore is likely to result in impaired protein function and possibly microtubule formation. This study expands the spectrum of brain malformations associated with mutations in the β-tubulin gene TUBB2B, supporting its critical role in migration/organization and axon guidance processes. In addition, it suggests a possible genetic aetiology of schizencephaly, thus strengthening the hypothesis that there is a common pathophysiological base in polymicrogyria and schizencephaly.

A spectrum of neurological disorders characterized by abnormal neuronal migration, differentiation, organization, axon guidance, and maintenance have recently been associated with missense and splice-site mutations in the genes encoding the α-tubulin and β-tubulin isotypes TUBA1A, TUBB2B, TUBB3, and TUBA8. Defects in any of the genes encoding any of these isotypes generate a large spectrum of brain malformations including dysmorphism of the basal ganglia and brainstem, partial or complete agenesis of the corpus callosum (ACC), cerebellar vermis hypoplasia, and different types of cortical malformations.1,2 Various grades of lissencephaly, ranging from the complete loss of gyri and sulci (agyria) to the brain with simplified abnormally thick convolutions (pachygyria) and perisylvian polymicrogyria (PMG), have been associated with mutations of the α-tubulin gene TUBA1A.3–5 Bilateral asymmetrical PMG has been observed in association with mutations in the β-tubulin gene TUBB2B,6 whereas cortical defects (PMG and gyral disorganization) and axon guidance disorders (hypoplasia of the oculomotor nerves) have been found in individuals displaying defects of the β-tubulin gene TUBB3.7,8 Finally, a mutation in the α-tubulin gene TUBA8 has been described in individuals with generalized PMG and optic nerve hypoplasia.9 These findings suggest a crucial role of tubulin genes in neuronal microtubules coassembly.10 Microtubules play a key role in cellular processes that are crucial for cortical development during neuronal migration and differentiation, but also in cortical lamination organization (involving both pyramidal neurons and interneurons), in the regulation of neuronal cell proliferation, and in neuronal guidance of the radial glia (axon outgrowth and maintenance).6,10

In this study we report the case of a female with microcephaly, complex malformation of the cortex (open-lip schizencephaly [SCH] and PMG), ACC, dysmorphism of basal ganglia and thalami, and vermis and right third nerve hypoplasia. A search for mutations in the TUBA1A, TUBB2B, TUBB3, and TUBA8 genes led to the identification of a novel mutation in TUBB2B.

The study was approved by the IRCCS ethics committee.

CASE REPORT
The female examined in this report, now 7 years old, was the only child of non-consanguineous parents with no family history of genetic or neurological diseases. Delivery was...
Low abdominal contribution to breathing as daytime predictor of nocturnal desaturation in adolescents and young adults with Duchenne Muscular Dystrophy

M. Romei, M.G. D’Angelo, A. LoMauro, S. Gandossini, S. Bonato, E. Brighina, E. Marchi, G.P. Comi, A.C. Turconi, A. Pedotti, N. Bresolin, A. Aliverti

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Received 23 May 2011; accepted 19 October 2011
Available online 13 November 2011

KEYWORDS
Abdomen; Diaphragm; Duchenne Muscular Dystrophy; Nocturnal hypoxemia

Summary
In the respiratory management of DMD patients it is still under debate what parameter should indicate the correct timing for institution of nocturnal non-invasive ventilation (NIV), in addition to forced vital capacity, which is generally considered as a prognostic marker of disease progression.

The aim of this study was to determine if volume variations of rib cage and abdominal compartments measured by Opto-Electronic Plethysmography can be helpful to distinguish between those patients who are in the early stages of nocturnal oxygen desaturation development and those who do not yet.

Pulmonary function, abdominal contribution to tidal volume and to inspiratory capacity (%Abd IC) and a set of breathing pattern indexes were assessed in 40 DMD patients older than 14 years and not yet under nocturnal NIV.

ROC analysis revealed that among all the considered parameters, %Abd IC in supine position was the best discriminator between DeSat (at least 10% of the night time with SpO2 < 95%) and NonDe-Sat patients, providing an area under the curve with 95%CI equal to 0.752.

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0954-6111/S - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2011.10.010
SHORT REPORT

The novel mitochondrial tRNA\textsuperscript{Asn} gene mutation m.5709T>C produces ophthalmoparesis and respiratory impairment

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Although mutations in mitochondrial tRNAs constitute the most common mtDNA defect, the presence of pathological variants in mitochondrial tRNA\textsuperscript{Asn} is extremely rare. We were able to identify a novel mtDNA tRNA\textsuperscript{Asn} gene pathogenic mutation associated with a myopathic phenotype and a previously unreported respiratory impairment. Our proband is an adult woman with ophthalmoparesis and respiratory impairment. Her muscle biopsy presented several cytochrome associated with a myopathic phenotype and a previously unreported respiratory impairment. Our proband is an adult woman with mitochondrial myopathy.2

INTRODUCTION

Keywords: progressive external ophthalmoplegia; tRNA(Asn); mitochondrial myopathy

INTRODUCTION

Many mitochondrial disorders are associated with mutations in mitochondrial tRNAs. To date, over 200 point mutations affecting mitochondrial tRNA genes have been described.\textsuperscript{1}

Nevertheless, mutations in the tRNA\textsuperscript{Asn} gene are very rare; until now, only five pathogenic variants have been associated with clinical phenotypes ranging from chronic external ophthalmoplegia (cPEO), with or without mitochondrial myopathy, to lethal early-onset encephalomyopathy.\textsuperscript{2}

Here we present clinical and molecular features of a patient with ophthalmoparesis and respiratory impairment associated with a novel heteroplasmic mutation (m.5709T>C) disclosed in the mitochondrial tRNA\textsuperscript{Asn} gene.

CASE REPORT

The proband is a 51-year-old woman with a several year history of progressive external ophthalmoplegia (PEO) and bilateral eyelid ptosis, which was surgically corrected at age 29, but which gradually worsened again in the following years. At age 47 years, she was admitted to hospital for subacute onset of a severe respiratory insufficiency and she was diagnosed with a restrictive syndrome with indication for non-invasive ventilation (B-PAP) during the night. Her clinical history is complicated by hypothyroidism (she is taking replacement therapy) and anxious–depressive syndrome.

Neurological examination showed bilateral eyelid ptosis and bilateral, both vertical and horizontal, ophthalmoparesis without diplopia. She had mild axial and proximal upper limb weakness (bilateral sternocleidomastoid and deltoid muscles) with brisk tendon reflexes and no sensitive alterations. Neither cerebellar nor gait dysfunction were observed.

Blood tests were normal, except for mildly elevated CK (between 290 and 450 U/L, nv <185 U/L), LDH (872 U/L with nv 125–243 U/L) and basal lactate (3.9 mmol/l venous, with nv 0.90–1.70 mmol/l and 3.1 mmol/l arterial with nv 0.36–1.25 mmol/l).

EMG examination disclosed mild non-specific abnormalities in both deltoid and quadriceps muscles. Cardiological evaluation and tests were normal.

Respiratory functional tests confirmed a chronic respiratory insufficiency with restrictive syndrome.

Her younger sister, aged 42 years, has mild mental retardation and unspecified psychiatric disorders, but no eyelid ptosis or ophthalmoparesis. Her serum CK levels are normal. Both the mother and the maternal grandmother are reported affected with sarcoidosis and ophthalmoparesis (no clinical reports available).

The father is healthy and neither the proband nor the sister has children.

At age 49 years, the patient underwent left deltoid muscle biopsy, which was consistent with a mitochondrial disorder.


Keywords: progressive external ophthalmoplegia; tRNA(Asn); mitochondrial myopathy

INTRODUCTION

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At age 49 years, the patient underwent left deltoid muscle biopsy, which was consistent with a mitochondrial disorder.
Generation of skeletal muscle cells from embryonic and induced pluripotent stem cells as an in vitro model and for therapy of muscular dystrophies

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Received: July 20, 2011; Accepted: November 23, 2011

Abstract

Muscular dystrophies (MDs) are a heterogeneous group of inherited disorders characterized by progressive muscle wasting and weakness likely associated with exhaustion of muscle regeneration potential. At present, no cures or efficacious treatments are available for these diseases, but cell transplantation could be a potential therapeutic strategy. Transplantation of myoblasts using satellite cells or other myogenic cell populations has been attempted to promote muscle regeneration, based on the hypothesis that the donor cells repopulate the muscle and contribute to its regeneration. Embryonic stem cells (ESCs) and more recently induced pluripotent stem cells (iPSCs) could generate an unlimited source of differentiated cell types, including myogenic cells. Here we review the literature regarding the generation of myogenic cells considering the main techniques employed to date to elicit efficient differentiation of human and murine ESCs or iPSCs into skeletal muscle. We also critically analyse the possibility of using these cellular populations as an alternative source of myogenic cells for cell therapy of MDs.

Keywords: myoblast • embryonic stem cell • induced pluripotent stem cell • muscular dystrophy • protocol

Introduction

Muscular dystrophies (MDs) are a heterogeneous group of inherited disorders characterized by progressive skeletal muscle weakness and degeneration [1]. Of the MDs, Duchenne muscular dystrophy (DMD) and limb girdle muscular dystrophy (LGMD) are the most frequent forms.

DMD is a genetic X-linked recessive disorder that affects 1 in 3500 male births [2], caused by mutations in the gene encoding dystrophin [3], a protein normally localized in the cytoskeleton and implicated in the stability of the skeletal muscle myofibre membrane [4]. The LGMDs are a group of muscular disorders, characterized by muscle degeneration resulting from a defect in specific skeletal muscle proteins. These diseases show wide genetic and phenotypic inter- and intra-family heterogeneity, and one of their possible clinical manifestations is the involvement of limb girdles [5]. The most frequent are LGMD2B and LGMD2A. LGMD2B or dysferlinopathy is an autosomal recessive disease...
Mutant superoxide dismutase-1 indistinguishable from wild-type causes ALS

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Received March 30, 2012; Revised and Accepted May 11, 2012

A reason for screening amyotrophic lateral sclerosis (ALS) patients for mutations in the superoxide dismutase-1 (SOD1) gene is the opportunity to find novel mutations with properties that can give information on pathogenesis. A novel c.352C>G (L117V) SOD1 mutation was found in two Syrian ALS families living in Europe. The disease showed unusually low penetrance and slow progression. In erythrocytes, the total SOD1 activity, as well as specific activity of the mutant protein, was equal in carriers of the mutation and family controls lacking SOD1 mutations. The structural stabilities of the L117V mutant and wild-type SOD1 under denaturing conditions were likewise equal, but considerably lower than that of murine SOD1. As analyzed with an ELISA specific for misfolded SOD1 species, no differences were found in the content of misfolded SOD1 protein between extracts of fibroblasts from wild-type controls and from an L117V patient. In contrast, elevated levels of misfolded SOD1 protein were found in fibroblasts from ALS patients carrying seven other mutations in the SOD1 gene. We conclude that mutations in SOD1 that result in a fully stable protein are associated with low disease penetrance for ALS and may be found in cases of apparently sporadic ALS. Wild-type human SOD1 is moderately stable, and was found here to be within the stability range of ALS-causing SOD1 variants, lending support to the hypothesis that wild-type SOD1 could be more generally involved in ALS pathogenesis.

INTRODUCTION

The amyotrophic lateral sclerosis (ALS) syndrome is characterized by adult-onset progressive loss of upper and lower motor neurons, resulting in paralysis and inevitable death from respiratory failure. Although most of the cases appear to be sporadic amyotrophic lateral sclerosis (SALS), ~10% of patients report a familial predisposition (denoted FALS). The 15 identified ALS-associated genes explain half of the familial cases, and mutations in them have occasionally also been reported in apparently SALS cases (1).

Mutations in the gene encoding superoxide dismutase-1 (SOD1) are found in ~6% of all ALS cases (2,3). Some 167 mutations have been found (http://alsod.iop.kcl.ac.uk/) and...
Negative results

Screening of the PFN1 gene in sporadic amyotrophic lateral sclerosis and in frontotemporal dementia

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A R T I C L E   I N   P R E S S

Neurobiology of Aging xxx (2012) 1–2

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

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

ARTICLE INFO

Article history:
Received 13 August 2012
Received in revised form 15 September 2012
Accepted 16 September 2012

Keywords:
Amyotrophic lateral sclerosis
Frontotemporal dementia
PFN1

ABSTRACT

Mutations in the profilin 1 (PFN1) gene, encoding a protein regulating filamentous actin growth through its binding to monomeric G-actin, have been recently identified in familial amyotrophic lateral sclerosis (ALS). Functional studies performed on ALS-associated PFN1 mutants demonstrated aggregation propensity, alterations in growth cone, and cytoskeletal dynamics. Previous screening of PFN1 gene in sporadic ALS (SALS) cases led to the identification of the p.E117G mutation, which is likely to represent a less pathogenic variant according to both frequency data in control subjects and cases, and functional experiments. To determine the effective contribution of PFN1 mutations in SALS, we analyzed a large cohort of 1168 Italian SALS patients and also included 203 frontotemporal dementia (FTD) cases because of the great overlap between these 2 neurodegenerative diseases. We detected the p.E117G variant in 1 SALS patient and the novel synonymous change p.G15G in another patient, but none in a panel of 1512 control subjects. Our results suggest that PFN1 mutations in sporadic ALS and in FTD are rare, at least in the Italian population.

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disease mainly caused by the loss of upper and lower motor neurons, resulting in progressive muscle atrophy and paralysis. Although most ALS cases are sporadic (SALS), approximately 5% of them are familial (FALS), usually with an autosomal-dominant inheritance pattern. Approximately 5% of all ALS cases exhibit signs of frontotemporal dementia (FTD), supporting the increasing evidence of a pathophysiologic and genetic link between these 2 disorders (Orr, 2011). Repeat expansions in C9ORF72 gene and SOD1 mutations represent the most frequent causes of FALS, accounting overall for 40%–50% of cases (Ratti et al., 2012), and...
Investigation of C9orf72 in 4 Neurodegenerative Disorders

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Objective: To estimate the allele frequency of C9orf72 (G4C2) repeats in amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), Alzheimer disease (AD), and Parkinson disease (PD).

Design: The number of repeats was estimated by a 2-step genotyping strategy. For expansion carriers, we sequenced the repeat flanking regions and obtained APOE genotypes and MAPT H1/H2 haplotypes.

Setting: Hospitals specializing in neurodegenerative disorders.

Subjects: We analyzed 520 patients with FTLD, 389 patients with ALS, 424 patients with AD, 289 patients with PD, 602 controls, 18 families, and 29 patients with PD with the LRRK2 G2019S mutation.

Main Outcome Measure: The expansion frequency.

Results: Based on a prior cutoff (>=30 repeats), the expansion was detected in 9.3% of patients with ALS, 5.2% of patients with FTLD, and 0.7% of patients with PD but not in controls or patients with AD. It was significantly associated with family history of ALS or FTLD and age at onset of FTLD. Phenotype variation (ALS vs FTLD) was not associated with MAPT, APOE, or variability in the repeat flanking regions. Two patients with PD were carriers of 39 and 32 repeats with questionable pathological significance, since the 39-repeat allele does not segregate with PD. No expansion or intermediate alleles (20-29 repeats) were found among the G2019S carriers and AD cases with TAR DNA-binding protein 43-positive inclusions. Surprisingly, the frequency of the 10-repeat allele was marginally increased in all 4 neurodegenerative diseases compared with controls, indicating the presence of an unknown risk variation in the C9orf72 locus.

Conclusions: The C9orf72 expansion is a common cause of ALS and FTLD, but not of AD or PD. Our study raises concern about a reliable cutoff for the pathological repeat number, which is important in the utility of genetic screening.


MYOTROPHIC LATERAL SCLEROSIS (ALS) AND FRONTOTEMPORAL LOBAR DEGENERATION (FTLD) ARE FATAL NEURODEGENERATIVE SYNDROMES THAT BELONG TO THE SAME CLINICOPATHOLOGICAL SPECTRUM.1,2 FRONTOTEMPORAL LOBAR DEGENERATION IS A PRIMARY DEMENTIA CHARACTERIZED BY EARLY BEHAVIORAL, LANGUAGE, AND EXTRAPYRAMIDAL CHANGES, WHILE SYMPTOMS OF ALS ARE THE RESULT OF THE DEGENERATION OF MOTOR NEURONS. BOTH SYNDROMES MAY OCCUR WITHIN THE SAME FAMILY OR-even the same patient.

Previously, linkage analyses revealed a 3.7-Mb region on 9p21 associated with familial ALS/FTLD,3-10 and genome-wide association studies suggested a major risk factor in the same locus for sporadic ALS and FTLD.11-15 Recently, 2 research groups independently explained this locus by a pathological noncoding hexanucleotide (G4C2)10-1600 repeat expansion in the chromosome 9 open reading frame 72 (C9orf72) gene of unknown function.16,17 Based on the allele frequencies in cases vs controls, the first studies suggested that expansions with more than 30 repeats should be considered pathological, while alleles with less than 20 repeats are wild type.16 However, a reliable cutoff for the pathological alleles remains to be established by additional studies (eg, segregation, neuropathological, or functional studies). Fur-
Our results did not suggest that the expansion or intermediate alleles are associated with AD. In contrast, there was a report of 6 expansion carriers in a familial AD cohort (<1%), 4 of whom were from the same family. However, autopsy indicated that 3 carriers actually had amnestic FTLD. Whether the remaining carriers were also clinically misdiagnosed as having AD remains to be seen. In addition to typical AD pathology, 15 of our patients with AD had TDP-43 inclusions, which are known to be associated with the brain pathology of the expansion, but, however, all of these patients with AD have genotypes within the normal range (2-12 repeats).

Our case-control studies also assessed the frequency of alleles within the normal range (<30 repeats) and observed a trend toward an association between the 10-repeat allele and risk for all 4 disorders (odds ratio, 1.72-2.14). It is tempting to speculate that this allele is in linkage disequilibrium with an unknown C9orf72 risk variation. Further genetic work has to validate this observation, including follow-up case-control studies and sequencing of 10-repeat carriers.

Accepted for Publication: May 30, 2012.

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Financial Disclosure: Dr Masellis has received speaker honoraria from Novartis and EMD Serono Inc; serves as an associate editor for Current Pharmacogenomics and Personalized Medicine; receives publishing royalties from Henry Stewart Talks; has served as a consultant for Bioscape Medical Imaging CRO; and receives research support from the Canadian Institutes of Health Research, Parkinson Society Canada, an Early Researcher Award from the Ministry of Economic Development and Innovation of Ontario, Teva Pharmaceutical Industries Ltd, and the Department of Medicine, Sunnybrook Health Sciences Centre.

Funding/Support: This work was supported by grants from the Ontario Research Fund, the Weston Foundation (Drs Rogaeva and St George-Hyslop), the Howard Hughes Medical Institute, The Wellcome Trust and Medical Research Council, the Alzheimer Society of Ontario (Dr St George-Hyslop), the Canadian Institutes of Health Research (Drs St George-Hyslop, Rogaeva and Black), a Center of Excellence grant from the National Parkinson Foundation (Drs Marras and Lang), and the James Hunter Family ALS Initiative (Drs Robertson and Zinman).
Mutational analysis of VCP gene in familial amyotrophic lateral sclerosis

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Abstract

Mutations in valosin-containing protein (VCP) gene, already known to be associated with the multisystemic disorder, inclusion body myopathy with Paget’s disease and frontotemporal dementia (IBMPFD), have been recently found also in familial cases of amyotrophic lateral sclerosis (ALS). To further define the frequency of VCP mutations in ALS Italian population, we screened a cohort of 166 familial ALS and 14 ALS-frontotemporal dementia (FTD) individuals. We identified a previously reported synonymous mutation (c.2093A>C; p.Q568Q), 2 intronic variants (c.1749-14C>T; c.2085-3C>T), and 1 nucleotide change (c.2814G>T) in the 3 untranslated region (UTR). Bioinformatical analyses predicted no changes in splicing process or microRNA binding sites. Our results do not confirm a main contribution of VCP gene to familial ALS in the Italian population.

Keywords: ALS (Amyotrophic lateral sclerosis); VCP (valosin-containing protein); Genetics

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disorder characterized by the progressive and selective loss of motor neurons in the cerebral cortex, brainstem, and spinal cord, resulting in muscle atrophy and paralysis. The disease is fatal within 2–5 years after onset and death is often caused by respiratory failure. Most cases of ALS are sporadic (SALS), but approximately 5%–10% of patients are classified as familial forms (FALS), commonly occurring with an autosomal dominant pattern of inheritance. Mutations in SOD1 gene represent the most commonly identified cause of FALS, accounting for about 20% of cases, while mutations in TARDBP encode 43-kDa transactive response (TAR) DNA-binding protein (TDP-43) and in FUS/TLS are responsible overall for approximately an additional 10% FALS cases. Other genes, including ALS2, SETX, VAPB, ANG, FIG4, SPG11 and, more recently, DAO and OPTN, have been associated with some rare and/or atypical FALS forms (Ticozzi et al., 2011).
Atypical adult onset complicated spastic paraparesis with thin corpus callosum in two patients carrying a novel FA2H mutation

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Keywords: FA2H, fatty acid hydroxylase-associated neurodegeneration, leukodystrophy, mutation, spastic paraparesis, SPG35

LETTER TO THE EDITOR

Atypical adult onset complicated spastic paraparesis with thin corpus callosum in two patients carrying a novel FA2H mutation

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Keywords: FA2H, fatty acid hydroxylase-associated neurodegeneration, leukodystrophy, mutation, spastic paraparesis, SPG35

Received 7 May 2012 Accepted 4 July 2012

Sirs,

The FA2H gene encodes a fatty acid 2-hydroxylase catalysing the 2-hydroxylation of myelin galactolipids representing one-third of the lipid content of the myelin sheath [1]. Homozygous FA2H mutations are associated with different neurodegenerative disorders such as leukodystrophies [2], a complicated form of spastic paraparesis with leukodystrophy (SPG35) [3], a form of neurodegeneration with brain iron accumulation (NBIA) [4], and clinical pictures partly overlapping all three disorders [5, 6]. Recently, these diseases were collectively referred to as fatty acid hydroxylase-associated neurodegeneration [6]. This is characterized by childhood onset of spasticity, dystonia, seizures, axonal neuropathy (in one family) [6], optic atrophy, cognitive decline, cerebellar atrophy, leukodystrophy, and brain iron accumulation [2–6]. Here we report a novel mutation in FA2H gene in two sibs (patients 1 and 2) presenting with adult onset complicated spastic paraparesis and thin corpus callosum (cHSP-TCC).

These patients were born to healthy consanguineous parents (first cousins) after normal gestation, delivery and psychomotor development.

In patient 1 (a 50-year-old man), symptoms onset was at age 40 years, with mild dysarthria and unsteadiness during walking. Brain MR1 performed at that time was normal. At age 47 years, he showed spastic paraplegia with unsteady but still autonomous ambulation, dysarthria, lateral beating nystagmus, and dysmetria. Wechsler Intelligence Scale for Adult (WAIS-R) showed an intelligence quotient (IQ) within the normal range (full IQ: 106). At age 50 years, the patient was still ambulant, with severe unsteadiness and frequent falls, dysarthria, moderate cerebellar signs, and mild cognitive decline (MMSE: 25/30; Wechsler Memory: 83). Family members also reported behavioral disturbances (mainly aggressiveness) progressively increasing in frequency and severity.

Abnormalities in acoustic evoked potentials were found. EMG showed mild neurogenic signs whilst the ENG was normal.

The 3T-MR scan at age 50 years (Fig. 1a–f) showed a severe whole cerebellar and cerebral atrophy with enlarged ventricles and TCC. No signs of leukodystrophy were found. Hypointensity on T2-TSE and FLAIR images was detected at the level of red nuclei bilateral cerebral peduncles, pulvinar nuclei and pallidi, with possible iron deposits only in cerebral peduncles and in central portion of pallidi (Fig. 1d). Optic nerves were atrophic.

The clinical features of the proband’s sister (patient 2, a 47-year-old woman) largely overlapped the proband’s features with symptoms onset at age 38 years. However, differently from the brother, no signs of mineralization were found at the 3T-MR scan at age 47 years, and the cognitive decline is slightly more severe (MMSE: 23/30; Wechsler Memory: 79).

Patient 1 had been previously screened for mutations in SPG11, SPG15, SPG7 and SPG21 genes (with informed consent approved by the local Institution Ethics Committee) with negative results. Complete mutation analysis of FA2H revealed the presence of a homozygous change c.509A>G (exon 4) leading to the p. Y170C substitution (Fig. 1g). This change (absent in the 1000 Genomes database and in 500 Italian adult controls) segregated also in patient 2. A nonsense mutation involving the same residue (p.Y170X) had been previously described in an NBIA family [4]. The mutant residue is located in the transmembrane domain I of FA2H (Fig. 1g), a membrane protein of the endoplasmic reticulum with four transmembrane regions in the C-terminal domain thought to be the catalytic site (Fig. 1g). The conserved N-terminus accounts for the FA2H-redox activity. The p.Y170C mutation is predicted to have deleterious effects on protein function by different software (see Data S1); in particular, TMHMM predicts the loss of the first transmembrane domain of the protein.

This might lead to either protein delocalization or destabilization by weakening protein binding to or insertion into the membrane. Based on these findings, we screened a cohort of 55 patients with cHSP-TCC negative for mutations in SPG11, SPG15, SPG4, and SPG7 with negative results.

Overall, the mutated patients herein described differ from the published cases mainly in the mild late onset phenotype, with no signs of leukodystrophy and intrafamilial variability for brain iron accumulation. These features along with the lack of white matter hyperintensity fit with the hypothesis that the destabilized mutant protein may retain a partial fatty acid hydroxylase function to generate precursors for the synthesis of lipid components of myelin [7]. These data thus widen the spectrum of FA2H-related clinical phenotypes whilst suggesting the need for further investigation to better define the involvement of FA2H in the genetically heterogeneous group of eHSP-TCC besides its role in the SPG35 subtype [3]. To this aim, follow-up of the neuroradiological profile may provide with potential elements for differential diagnosis in these patients.

Indeed, at this stage, FA2H mutations screening is suggested in eHSP-TCC cases displaying cognitive decline regardless of the age at onset, with neuroradiological features suggesting brain iron deposition even in the absence of leukodystrophy.

Acknowledgements

The authors wish to thank the patients for participating in the study. The work...
Myotonia congenita: Novel mutations in CLCN1 gene and functional characterizations in Italian patients

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A R T I C L E   I N F O
Article history:
Received 17 February 2012
Received in revised form 27 March 2012
Accepted 29 March 2012
Available online 21 April 2012

Keywords:
Skeletal muscle
Channelopathy
CLCN1 gene
Myotonia congenita
Thomson’s disease
Becker’s generalized myotonia

A B S T R A C T
Myotonia congenita is an autosomal dominantly or recessively inherited muscle disorder causing impaired muscle relaxation and variable degrees of permanent muscle weakness, abnormal currents linked to the chloride channel gene (CLCN1) encoding the chloride channel on skeletal muscle membrane. We describe 12 novel mutations: c.1606G>C (p.Val536Leu), c.2533G>A (p.Gly845Ser), c.2434C>T (p.Gln812X), c.1499T>G (p.E500X), c.1012C>T (p.Arg337X), c.2403+1G>A, c.2840T>A (p.Val947Glu), c.1598C>T (p.Thr533Ile), c.1110delC, c.590T>A (p.Lle197Arg), c.2276insA Fs800X, c.490T>C (p.Trp164Arg) in 22 unrelated Italian patients. To further understand the functional outcome of selected missense mutations (p.Trp164Arg, p.Lle197Arg and p.Gly845Ser), and the previously reported p.Gly190Ser we characterized the biophysical properties of mutant ion channels in tsA cell model. In the physiological range of muscle membrane potential, all the tested mutations, except p.Gly845Ser, reduced the open probability, increased the fast and slow components of deactivation and affected pore properties. This suggests a decrease in macroscopic chloride currents impairing membrane potential repolarization and causing hyperexcitability in muscle membranes. Detailed clinical features are given of the 8 patients characterized by cell electrophysiology. These data expand the spectrum of CLCN1 mutations and may contribute to genotype–phenotype correlations. Furthermore, we provide insights into the fine protein structure of ClC-1 and its physiological role in the maintenance of membrane resting potential.

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1. Introduction

Myotonia congenita (MC) belongs to the group of non-dystrophic myotonias and can be inherited either by an autosomal dominant (Thomsen’s disease OMIM 160800) or recessive manner (Becker’s disease, OMIM 255700). It is characterized by impaired muscle relaxation after voluntary contraction and variable degrees of muscle weakness. It is caused by mutations in the CLCN1 gene (RefSeq NC_000007.13) on chromosome 7q35 encoding the major skeletal muscle chloride channel CLC-1 (RefSeq NM_000074.2). It is well established that chloride channels play a role in the regulation of the muscle membrane and thus participate in the maintenance of the resting potential. Their inactivation by mutations modifies the cycle of excitability of the muscle membrane, shifting it towards hyperexcitability by slowing the return of the membrane to the resting potential after depolarization. Myotonia is directly correlated to the repetitive activation of sodium channels caused by this state of hyperexcitability [1–3].

Each muscle chloride channel comprises two identical protein molecules, each constituting a separate ion conductance pathway, the so-called protopore. In autosomal recessive myotonia congenita, both subunits have a disease-causing mutation. This results in chloride channel reduction to 40% or less, which is sufficient to cause myotonic contractions. Autosomal dominant myotonia congenita is believed to result from the presence of one dominant-negative mutation that modifies either the gating of both protopores or the selectivity of one of the two protopores [4–6]. However, some mutations have been found to lead to autosomal dominant myotonia congenita in some patients, and to a homozygous recessive form in others.
Absence of T and B lymphocytes modulates dystrophic features in dysferlin deficient animal model

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ARTICLE INFORMATION

Article Chronology:
Received 28 December 2011
Revised version received 12 March 2012
Accepted 15 March 2012
Available online 23 March 2012

Keywords:
Dysferlin
Lymphocytes
Scid/A/J mice

Abstract

Dysferlin mutations cause muscular dystrophy (dysferlinopathy) characterized by adult onset muscle weakness, high serum creatine kinase levels, attenuation of muscle regeneration and a prominent inflammatory infiltrate. In order to verify the role of lymphocytes and immune cells on this disease, we generated the Scid/A/J transgenic mice and compared these animals with the age-matched A/J mice. The absence of T and B lymphocytes in this animal model of dysferlinopathy resulted in an improvement of the muscle regeneration. Scid/A/J mice showed increased specific force in the myosin heavy chain 2A-expressing fibers of the diaphragm and abdominal muscles. Moreover, a partial reduction in complement deposition was observed together with a diminution in pro-inflammatory M1 macrophages. Consistent with this model, T and B lymphocytes seem to have a role in the muscle damaging immune response. The knowledge of the involvement of immune system in the development of dysferlinopathies could represent an important tool for their rescuing. By studying Scid/baJ mice, we showed that it could be possible to modulate the pathological symptoms of these diseases by interfering with different components of the immune system.

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Introduction

Limb Girdle Muscular Dystrophies (LGMDs) are a group of muscular diseases characterized by predominant weakness and wasting of muscles of the pelvic and shoulder girdle. LGMD-2B and Miyoshi Myopathy (MM) were found to arise from defects in the dysferlin gene, located on chromosome 2p13 [1]. Both syndromes present at late teens, progress slowly; they differ in the distribution of muscle affection at onset. In LGMD-2B muscle affection predominates in proximal muscles, whereas in MM it concerns mainly distal muscles. Dysferlin is a 237 kDa protein, localized at the muscle cell membrane and associated with...
Autophagy as a new therapeutic target in Duchenne muscular dystrophy

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A resolutive therapy for Duchenne muscular dystrophy, a severe degenerative disease of the skeletal muscle, is still lacking. Because autophagy has been shown to be crucial in clearing dysfunctional organelles and in preventing tissue damage, we investigated its pathogenic role and its suitability as a target for new therapeutic interventions in Duchenne muscular dystrophy (DMD). Here we demonstrate that autophagy is severely impaired in muscles from patients affected by DMD and mdx mice, a model of the disease, with accumulation of damaged organelles. The defect in autophagy was accompanied by persistent activation via phosphorylation of Akt, mammalian target of rapamycin (mTOR) and of the autophagy-inhibiting pathways dependent on them, including the translation-initiation factor 4E-binding protein 1 and the ribosomal protein S6, and downregulation of the autophagy-inducing genes LC3, Atg12, Gabarapl1 and Bnip3. The defective autophagy was rescued in mdx mice by long-term exposure to a low-protein diet. The treatment led to normalisation of Akt and mTOR signalling; it also reduced significantly muscle inflammation, fibrosis and myofibre damage, leading to recovery of muscle function. This study highlights novel pathogenic aspects of DMD and suggests autophagy as a new effective therapeutic target. The treatment we propose can be safely applied and immediately tested for efficacy in humans.

Muscular dystrophies are a group of genetic, hereditary muscle diseases characterised by defects in muscle proteins. These defects result in progressive skeletal muscle damage accompanied by myofibre necrosis and chronic local inflammation, leading to substitution of myofibres by connective and adipose tissue. In Duchenne muscular dystrophy (DMD), the most severe form of these diseases, the continuous and progressive skeletal muscle damage leads to complete paralysis and death of patients, usually by respiratory and/or cardiac failure.

The therapeutic protocols currently in use, based on corticosteroid administration, provide some delay in the progression of the disease, but they are associated with severe side effects. Therapies that substitute corticosteroids or at least may act as corticosteroid-sparing drugs are thus being actively pursued, and biological mechanisms relevant to skeletal muscle homeostasis are explored, in order to identify new targets.

Autophagy is emerging as an important process that limits muscle damage. Inhibition/alteration of autophagy contributes to myofibre degeneration leading to accumulation of abnormal organelles. Mutations that inactivate Jumpy, a phosphatase that counteracts the activation of VPS34 for autophagosome formation and reduces autophagy, are associated with a centronuclear myopathy. This observation suggests that unbalanced autophagy is pathogenic in muscle degeneration. Likewise, hyperactivation of Akt as a consequence of muscle-specific deletion of the mammalian target of rapamycin (mTOR) leads to inhibition of autophagy and to a muscle phenotype resembling the one observed in muscular dystrophy.

The validity of autophagy modulation as a therapeutic strategy has been shown in a mouse model of Ulrich myopathy characterised by defective autophagy and accumulation of dysfunctional organelles. Forced reactivation of autophagy in these animals yielded a beneficial therapeutic response. Indirect evidence indicates a possible role of deficient autophagy also in DMD pathogenesis. Higher levels of activation and phosphorylation of Akt are observed in muscles and primary myoblasts of the mdx mouse model of muscular dystrophy; therapy

**Abbreviation:** DAPI, 4',6-diamidino-2-phenylindole; DMD, Duchenne muscular dystrophy; mTOR, mammalian target of rapamycin; WT, wild-type; TA, tibialis anterior; LC3, microtubule-associated protein-1 light chain 3; 4E-BP1, eukaryotic translation-initiation factor 4E-binding protein 1; SD, standard diet; LPD, low-protein diet; CSA, cross-sectional area; TUNEL, TdT-mediated dUTP-biotin nick end labelling; EBD, Evans blue dye; H&E, haematoxylin and eosin.

Received 25.7.12; revised 07.9.12; accepted 25.9.12; Edited by G Melino
Quantitative muscle strength assessment in duchenne muscular dystrophy: longitudinal study and correlation with functional measures

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Abstract

Background: The aim of this study was to perform a longitudinal assessment using Quantitative Muscle Testing (QMT) in a cohort of ambulant boys affected by Duchenne muscular dystrophy (DMD) and to correlate the results of QMT with functional measures. This study is to date the most thorough long-term evaluation of QMT in a cohort of DMD patients correlated with other measures, such as the North Star Ambulatory Assessment (NSAA) or the 6 min walk test (6MWT).

Methods: This is a single centre, prospective, non-randomised, study assessing QMT using the Kin Com® 125 machine in a study cohort of 28 ambulant DMD boys, aged 5 to 12 years. This cohort was assessed longitudinally over a 12 months period of time with 3 monthly assessments for QMT and with assessment of functional abilities, using the NSAA and the 6MWT at baseline and at 12 months only. QMT was also used in a control group of 13 healthy age-matched boys examined at baseline and at 12 months.

Results: There was an increase in QMT over 12 months in boys below the age of 7.5 years while in boys above the age of 7.5 years, QMT showed a significant decrease. All the average one-year changes were significantly different than those experienced by healthy controls. We also found a good correlation between quantitative tests and the other measures that was more obvious in the stronger children.

Conclusion: Our longitudinal data using QMT in a cohort of DMD patients suggest that this could be used as an additional tool to monitor changes, providing additional information on segmental strength.

Background

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder and is the most common muscular dystrophy in children [1-6]. The increasing number of possible therapeutic strategies ready to enter in phase 2 or 3 clinical trials has highlighted the need for reliable and reproducible outcomes measures. There has also been increasing evidence of the need to collect natural history data in order to establish the rate of progression and the variability of muscle strength and functional changes in a disease that does not have a linear progression with increasing age. The North Star Ambulatory Assessment (NSAA) and the 6 min walking test (6MWT), a measure previously used in other disorders, have been recently proposed as possible measures for ambulant DMD [7-9]. The choice of these measures reflects the need for assessing aspects of function that are clinically meaningful for patients. In a research setting, however, the trial design may also need objective and sensitive measures of individual muscles, as opposed to functional scales that often measure movements involving many muscles in different muscle groups. The QMT (Quantitative Muscle Testing) is a sensitive tool to
The Role of Stem Cells in Muscular Dystrophies

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Abstract: Muscular dystrophies are heterogeneous neuromuscular disorders of inherited origin, including Duchenne muscular dystrophy (DMD). Cell-based therapies were used to promote muscle regeneration with the hope that the host cells repopulated the muscle and improved muscle function and pathology. Stem cells were preferable for therapeutic applications, due to their capacity of self-renewal and differentiative potential. In the last years, encouraging results were obtained with adult stem cells to treat muscular dystrophies. Adult stem cells were found into various tissues of the body and they were able to maintain, generate, and replace terminally differentiated cells within their own specific tissue because of cell turnover or tissue injury. Moreover, it became clear that these cells could participate into regeneration of more than just their resident organ. Here, we described multiple types of muscle and non muscle-derived myogenic stem cells, their characterization and their possible use to treat muscular dystrophies. We also underlined that most promising possibility for the management and therapy of DMD is a combination of different approaches, such as gene and stem cell therapy.

Keywords: Adult stem cells, cell therapy, DMD, gene therapy, iPS cell, muscular dystrophies.

1. INTRODUCTION

Numerous types of muscular dystrophy exist depending on their degree of severity and the muscle types affected [1]. Dystrophin is the major component of the dystrophin–glycoprotein complex (DGC) and it is responsible for the maintenance of cell integrity and muscle cell function [2]. Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy, is an X-linked genetic disorder due to either spontaneous mutations or inherited nonsense point mutations in the dystrophin gene [3]. Following the loss of a functional dystrophin protein, the muscles of DMD patients progressively degenerate as a result of mechanical stress during contraction that lead to death into the second second/third decade of the patient life [4, 5]. Even if it’s characterized by similar patterns of mutations, Becker muscular dystrophy (BMD) shows a milder phenotype than DMD patients due to the expression of shorter dystrophin mRNA transcripts that maintain the coding reading frame. Limb girdle muscular dystrophy is also caused by genetic mutations in proteins that are associated with the muscle cell membrane [6, 7]. Congenital muscular dystrophy (CMD) is a heterogeneous group of inherited muscle disorders due to defects into proteins of the extracellular matrix – laminin 2, alpha dystroglycan and collagen VI [8, 9]. Muscle histology was characterized by significant dystrophic features such as fiber size variability, presence of increased endomysial fibrosis, and variably necrotic and/or regenerative fibers. Sometimes, muscle biopsy only showed fiber size variation with absence of or only mild manifestations of fibrosis, necrosis, or regeneration [10]. A lack of these proteins caused mechanical fragility and contraction-induced damage of the muscle fibres [11], which leads to infiltration of inflammatory cells into the muscle, as well as activation of satellite cells that take part in muscle regeneration [12]. More or less all these pathologies are characterized by continuous cycles of muscle fibre degeneration and regeneration, until, in the late stages of the disease, the endogenous satellite cell pool becomes exhausted and, due to genetic mutations in several proteins, muscle fibres are replaced by fibrotic and adipose tissues, compromising normal muscle function [2]. The identification and characterization of dystrophin gene and of the other genes that cause these pathologies led to the development of potential treatments for these disorders [13]. Gene therapy for muscular disorders embraced several concepts, including replacing/repairing a defective gene or modifying/enhancing cellular performance, using genes that are not directly related to the underlying defect [14]. However, no effective therapies are available for these diseases.

Cellular therapy could be an ideal treatment for recessive muscular dystrophies in which muscle fibres are lost as a result of a genetic mutation. Stem cells can replenish their numbers for long periods through cell division and, after receiving some chemical signals, they can produce through asymmetric cell division, a progeny that can differentiate into multiple cell lineages with specific functions. Moreover, from a clinical point of view, they could survive, proliferate and migrate upon arrival within host muscle; they could differentiate into muscle fibres to repair damaged fibres or to replace fibres that have already been lost; finally they could reconstitute the satellite cell pool with functional stem cells [15].

In this work, we will examine different stem cell populations, investigating how they could ameliorate the pathological phenotypes of muscular dystrophies. Moreover, we will...
Fatigue and exercise intolerance in mitochondrial diseases. Literature revision and experience of the Italian Network of mitochondrial diseases


Abstract

Fatigue and exercise intolerance are common symptoms of mitochondrial diseases, but difficult to be clinically assessed. New methods to quantify these rather common complaints are strongly needed in the clinical practice. Coenzyme Q10 administration and aerobic exercise may improve exercise intolerance, but more definite studies are still pending. Herein, we have revised “how to measure” and “how to treat” these symptoms of mitochondrial patients. Subsequently, we reviewed the clinical data of the 1164 confirmed mitochondrial patients present in the Italian nation-wide database of mitochondrial disease, with special regard to exercise intolerance. We observed that more of 20% of mitochondrial patients complain of exercise intolerance. This symptom seems to be frequently associated with specific patient groups and/or genotypes. Ragged red fibers and COX-negative fibers are more often present in subjects with exercise intolerance, whereas lactate levels could not predict this symptom. Multicenter efforts are strongly needed for rare disorders such as mitochondrial diseases, and may represent the basis for more rigorous longitudinal studies.

Keywords: Disease registry; Exercise intolerance; Fatigue; Mitochondrial myopathies; mtDNA

1. Introduction

The most crucial task of the mitochondrion is the generation of energy as adenosine triphosphate, by means of the electron transport chain [1]. This pathway is under control...
Frequency and characterisation of anoctamin 5 mutations in a cohort of Italian limb-girdle muscular dystrophy patients

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Received 28 February 2012; received in revised form 30 April 2012; accepted 3 May 2012

Abstract

Limb-girdle muscular dystrophy (LGMD) 2L, caused by mutations in the anoctamin 5 (AN05) gene, is the third most common LGMD in Northern and Central Europe, where the c.191dupA mutation causes the majority of cases. We evaluated data from 228 Italian LGMD patients to determine the prevalence of LGMD2L and the c.191dupA mutation, and to describe the clinical, muscle biopsy, and magnetic resonance imaging findings in these patients. Forty-three patients who lacked molecular diagnosis were studied for AN05 mutations, and four novel mutations were found in three probands. Only one proband carried the c.191dupA mutation, which was compound heterozygous with c.2516T>G. Two probands were homozygous for the c.1627dupA and c.397A>T mutations, respectively, while a fourth proband had a compound heterozygous status (c.220C>T and c.1609T>C). Therefore occurrence and molecular epidemiology of LGMD2L in this Italian cohort differed from those observed in other European countries. AN05 mutations accounted for ~2% of our sample. Affected patients exhibited benign progression with variable onset and an absence of cardiac and respiratory impairment; muscle biopsy generally showed mild signs, except when performed on the quadriceps muscles; MRI showed predominant involvement of the posterior thigh. Overall these common clinical, morphological and imaging findings could be useful in differential diagnosis.

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Keywords: Limb girdle muscular dystrophy 2L; Quadriceps myopathy; Anoctamin 5; Chloride channel; Membrane repair

1. Introduction

Limb-girdle muscular dystrophy (LGMD) 2L is a recessive form of LGMD described for the first time in 2010 [1]. The disease is caused by mutations in the anoctamin 5 (AN05) gene, which contains 22 exons and maps to chromosome 11. Recessive mutations in this gene have also been reported to cause a distal non-dysferlin Miyoshi-like myopathy (MM3) [2], whereas dominant variations can determine gnathodiaphyseal dysplasia (GDD) [3]. Anoctamin 5 is a member of the anoctamin family, which comprises at least ten proteins, each consisting of eight transmembrane domains and a DUF590 domain of...
Hmgb3 Is Regulated by MicroRNA-206 during Muscle Regeneration

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Abstract

Background: MicroRNAs (miRNAs) have been recently involved in most of human diseases as targets for potential strategies to rescue the pathological phenotype. Since the skeletal muscle is a spread-wide highly differentiated and organized tissue, rescue of severely compromised muscle still remains distant from nowadays. For this reason, we aimed to identify a subset of miRNAs major involved in muscle remodelling and regeneration by analysing the miRNA-profile of single fibres isolated from dystrophic muscle, which was here considered as a model of chronic damage.

Methodology/Principal Findings: The miRNA-signature associated to regenerating (newly formed) and remodelling (resting) fibres was investigated in animal models of muscular dystrophies and acute damage, in order to distinguish which miRNAs are primary related to muscle regeneration. In this study we identify fourteen miRNAs associated to dystrophic fibres responsible for muscle regeneration and remodelling, and confirm over-expression of the previously identified regeneration-associated myomiR-206. In particular, a functional binding site for myomiR-206 was identified and validated in the 3’ untranslated region (3’UTR) of an X-linked member of a family of sequence independent chromatin-binding proteins (Hmgb3) that is preferentially expressed in hematopoietic stem cells. During regeneration of single muscle fibres, Hmgb3 messenger RNA (miRNA) and protein expression was gradually reduced, concurrent with the up-regulation of miR-206.

Conclusion/Significance: Our results elucidate a negative feedback circuit in which myomiR-206 represses Hmgb3 expression to modulate the regeneration of single muscle fibres after acute and chronic muscle damage. These findings suggest that myomiR-206 may be a potential therapeutic target in muscle diseases.

Introduction

MiRNAs are a class of short non-coding RNAs that take part in mastering the balance of gene-regulating networks by binding to 3’UTR of target mRNAs and inhibiting their expression [1]. The disclosure of these small RNA molecules introduced a new labyrinthine dimension to gene regulation and gave the opportunity to deepen our understanding on many biological processes. MiRNAs were in fact demonstrated fundamental for muscle physiology and plasticity [9,10,11]. In line with a growing characterization of the myomiR network, researchers started to investigate the role of miRNAs in muscle degeneration, which turns out to be very challenging if considering the absence of an efficient therapy for patients affected by most of primary muscular disorders. A faithful model for studying muscle damage and regeneration, is represented by muscular dystrophies (MDs) since they are a group of diseases characterized by muscle wasting and weakness due to defects in structural proteins expressed in the skeletal muscle [12,13]. In particular MDs are proposed as two-tiered diseases with acute large amount of myofibre necrosis resulting from growth spurts or damaging exercise, superimposed upon a background of a chronic low level of damage, with different factors contributing to these two situations [14]. When Eisenberg et al. analyzed the miRNome of muscle biopsies from patients...
IMPARED EXPRESSION OF INSULIN-LIKE GROWTH FACTOR-1 SYSTEM IN SKELETAL MUSCLE OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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ABSTRACT: Introduction: Adult muscle fibers are a source of growth factors, including insulin-like growth factor-1 (IGF-1). These factors influence neuronal survival, axonal growth, and maintenance of synaptic connections. Methods: We investigated the components of the IGF system in skeletal muscle samples obtained from 17 sporadic amyotrophic lateral sclerosis patients (sALS) and 29 control subjects (17 with normal muscle and 12 with denervated muscle unrelated to ALS). Results: The muscle expression of IGF-1 and IGF-binding proteins 3, 4, and 5 (IGF-BP3, -4, and -5, respectively), assessed by immunohistochemistry, was differentially decreased in sALS compared with both control groups; conversely, IGF-1 receptor β subunit (IGF-1Rβ) was significantly increased. Western blot analysis confirmed the severe reduction of IGF-1, IGF-BP3, and IGF-BP5 with the increment of IGF-1Rβ in sALS. Conclusion: In this study we describe the abnormal expression of the IGF-1 system in skeletal muscle of sALS patients that could participate in motor neuron degeneration and should be taken into account when developing treatments with IGF-1.


Amyotrophic lateral sclerosis (ALS) is a fatal neuromuscular disorder characterized by motor neuron (MN) degeneration that leads to progressive skeletal muscle atrophy and paralysis. Most ALS cases are sporadic, but a small percentage (5–10%) are familial.

Many hypotheses have been formulated to explain the pathogenesis of sporadic ALS (sALS), including autoimmune reactions to calcium channels on MNs, glutamate-induced excitotoxic injury, exposure to toxins or latent infections, disorganization of intermediate filaments, and loss of neurotrophic support to MNs.1 The latter hypothesis is of particular interest, because adult muscle fibers produce molecules that influence MN survival, axonal growth, and maintenance of synaptic connections. Among these trophic factors, insulin-like growth factor-1 (IGF-1) has a key role; it is involved in muscle and nerve tissue anabolism and thus induces muscle hypertrophy and promotes neuronal survival.2–6 The neurotrophic effect of IGF-1 was the starting point for three clinical trials based on subcutaneous injections of human recombinant IGF-1 in ALS patients. Disappointingly, these studies did not show a significant positive effect of IGF-1 therapy on disease progression or survival in ALS patients.7–9 However, the therapeutic role of IGF-1 in ALS is still debated. Indeed, Kaspar and colleagues demonstrated that treatment with adenovirus/IGF-1 retrogradely transported from muscle to MNs of the spinal cord led to therapeutic benefits in the G93A transgenic mouse model.10 This effect was further increased with associated physical exercise.11

More recently, Dobrowolny et al. reported that muscle-restricted expression of IGF-1 isoforms maintained muscle integrity, stabilized neuromuscular junctions, reduced inflammation in the spinal cord, enhanced motor neuronal survival, and delayed the onset and slowed disease progression in sodium diniturate 1 (SOD1) G93A mice.12,13 Both these studies reappraised the potential role of the skeletal muscle and IGF-1 signaling as a target for treatment in ALS patients. Moreover, a recent study also showed that overexpression of IGF-1 in muscle attenuates disease in a mouse model of spinal and bulbar muscular atrophy.14

The IGF-1 signaling system is complex and regulated by many factors.15,16 The components of the IGF-1 system include a growth factor, IGF-1, which is a single-chain polypeptide with a molecular weight of approximately 7.5 kDa. The IGF-1 receptor (IGF-1R) is a membrane glycoprotein of...
Large-Scale Population Analysis Challenges the Current Criteria for the Molecular Diagnosis of Fascioscapulohumeral Muscular Dystrophy

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Fascioscapulohumeral muscular dystrophy (FSHD) is a common hereditary myopathy causally linked to reduced numbers (≤8) of 3.3 kilobase D4Z4 tandem repeats at 4q35. However, because individuals carrying D4Z4-reduced alleles and no FSHD and patients with FSHD and no short allele have been observed, additional markers have been proposed to support an FSHD molecular diagnosis. In particular, a reduction in the number of D4Z4 elements combined with the 4A(159/161/168)PAS haplotype (which provides the possibility of expressing DUX4) is currently used as the genetic signature uniquely associated with FSHD. Here, we analyzed these DNA elements in more than 800 Italian and Brazilian samples of normal individuals unrelated to any FSHD patients. We find that 3% of healthy subjects carry alleles with a reduced number (4–8) of D4Z4 repeats on chromosome 4q and that one-third of these alleles, 1.3%, occur in combination with the 4A161PAS haplotype. We also systematically characterized the 4q35 haplotype in 253 unrelated FSHD patients. We find that only 127 of them (50.1%) carry alleles with 1–8 D4Z4 repeats associated with 4A161PAS, whereas the remaining FSHD probands carry different haplotypes or alleles with a greater number of D4Z4 repeats. The present study shows that the current genetic signature of FSHD is a common polymorphism and that only half of FSHD probands carry this molecular signature. Our results suggest that the genetic basis of FSHD, which is remarkably heterogeneous, should be revisited, because this has important implications for genetic counseling and prenatal diagnosis of at-risk families.

Introduction

Fascioscapulohumeral muscular dystrophy (FSHD [MIM 158900]), a common myopathy, has a prevalence of 1 in 20,000.1,2 The disease is characterized by weakness of selective muscle groups and wide variability of clinical expression.1,3,4 The onset of the disease is in the second or third decade of life and usually involves the weakening of facial and limb-girdle muscles. The mode of inheritance of classical FSHD is considered to be autosomal dominant, with complete penetrance by age 20.4,5 No biochemical, histological, or instrumental markers are available to independently confirm a specific FSHD diagnosis that remains mainly clinical.

The FSHD genetic defect does not reside in any protein-coding gene.6 Instead, FSHD has been genetically linked to the reduction of an integral number of tandem 3.3-kb D4Z4 repeats located on chromosome 4q35.7,8 Although nearly identical D4Z4 sequences reside on chromosome 10q26,9 only subjects with a reduced number of D4Z4 repeats on chromosome 4, but not chromosome 10, develop FSHD.10–12 Based on these results, p13E-11 EcoRI alleles larger than 50 kb (≥11 D4Z4 repeats) originating from chromosome 4 have been considered normal, whereas alleles of 35 kb or less (≤8 D4Z4 repeats) have been considered diagnostic for the disease.8,13

Because there are individuals with reduced D4Z4 alleles that do not have clinical signs of FSHD,14,15 it has been proposed that additional DNA sequences flanking the D4Z4 repeat array are necessary for disease development.16–18 These studies concluded that D4Z4 reduction is pathogenic only in a few genetic backgrounds, which include a specific simple sequence length polymorphism (SSLP) proximal to the D4Z4 repeat and the 4qA polymorphism distal to

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DOI 10.1016/j.ajhg.2012.02.019. ©2012 by The American Society of Human Genetics. All rights reserved.
Novel insight into stem cell trafficking in dystrophic muscles

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Abstract: Recently published reports have described possible cellular therapy approaches to regenerate muscle tissues using arterial route delivery. However, the kinetic of distribution of these migratory stem cells within injected animal muscular dystrophy models is unknown. Using living X-ray computed micromotography, we established that intra-arterially injected stem cells traffic to multiple muscle tissues for several hours until their migration within dystrophic muscles. Injected stem cells express multiple traffic molecules, including VLA-4, LFA-1, CD44, and the chemokine receptor CXCR4, which are likely to direct these cells into dystrophic muscles. In fact, the majority of intra-arterially injected stem cells access the muscle tissues not immediately after the injection, but after several rounds of recirculation. We set up a new, living, 3D-imaging approach, which appears to be an important way to investigate the kinetic of distribution of systemically injected stem cells within dystrophic muscle tissues, thereby providing supportive data for future clinical applications.

Keywords: iron nanoparticles, micro-CT, CD133+ stem cells, dystrophic muscles

Introduction

Attempts to repair muscle damage in Duchenne muscular dystrophy (DMD) by transplanting myogenic progenitors directly into muscles are facing the problem of cell survival and the limited migration of these cells in the muscles. The delivery of myogenic stem cells to the sites of muscle lesions via systemic circulation is a potential alternative approach to treat this disease. However, intravenously injected cells may become trapped in other organs (eg, liver, spleen, lung), resulting in only a small portion entering the muscle microvasculature and migrating into dystrophic muscles. The authors of this paper contributed to the development of a cellular therapy to regenerate muscle tissues using arterial route delivery. The success of this protocol was mainly due to widespread distribution of donor stem cells through the muscle capillary network.

Elucidation of the kinetic of distribution of intra-arterially injected stem cells within injected muscular dystrophy animal models is unknown. Therefore, in the present paper, we focused on the stem cell trafficking of intra-arterially injected dystrophic muscle tissues, using living X-ray computed micromotography (micro-CT). Using this approach, we reached resolutions in the submicron range (up to 300 nm), which provided the opportunity to track intra-arterially injected stem cells in living DMD animal models with high single-cell sensitivity.

Micro-CT is one of the most advanced non-invasive techniques aimed at the qualitative and quantitative three-dimensional evaluation of tissues under different conditions, providing high spatial resolution images (from 10 µm to 1 µm) with high...
Short communication

Optic atrophy plus phenotype due to mutations in the OPA1 gene: Two more Italian families

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A B S T R A C T

Autosomal Dominant Optic Atrophy (ADOA) is characterized by the selective degeneration of retinal ganglion cells. The occurrence of mutations in the gene encoding the dynamin-like GTPase protein Optic Atrophy 1 (OPA1) has been observed in about 60–70% of ADOA cases. A subset of missense mutations, mostly within the GTPase domain, has recently been associated with a syndromic ADOA form called “OPA1 plus” phenotype presenting, at muscle level, mitochondrial DNA (mtDNA) instability.

In this study we disclosed two OPA1 gene mutations in independent probands from two families affected by OPA1 plus phenotype: the previously reported c.985-2A>G substitution and a novel microdeletion (c.2819-1_2821del).

The correlation between genotype and phenotype and the effects of these variants at the transcript level and in the muscle tissue were investigated, confirming the broad complexity in the phenotypic spectrum associated with these OPA1 mutations.

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1. Introduction

Autosomal Dominant Optic Atrophy (ADOA) is due in about 60–70% of cases to mutations in the nuclear gene encoding for the OPA1 protein, mapping to chromosome 3q28-29. ADOA is the most common form of hereditary optic neuropathy, with a prevalence of 1/50,000 [1,2]. Classical ADOA usually begins before 10 years of age with slowly progressive bilateral visual loss, dyschromatopsia, centrocecal scotomas and temporal optic disc atrophy [3,4]. The disease has an incomplete penetrance and variable phenotypic expression, ranging from mild visual impairment to blindness [5]. Up to 20% of OPA1-mutated patients also develop, during clinical history, additional neuromuscular complications leading to the so-called “OPA1 plus” phenotype [6,7].

More than 200 pathogenic mutations in the OPA1 gene have been so far described [8]. Half of these variants are predicted to result in a truncated protein producing haploinsufficiency and are usually associated to the classical non-syndromic form of optic neuropathy. Missense mutations within or close to the GTPase domain, preserving the expression of OPA1 transcript are responsible for both the classical and syndromic OPA1 phenotype.

The present study further extends the mutational spectrum of OPA1 with the report of a novel heterozygous deletion within the GTPase effector domain (GED). We also confirmed a previously published mutation in an Italian family suggesting the existence of a mutational hot spot in OPA1 sequence situated in the surroundings of exon 10 splice acceptor site.

2. Material and methods

2.1. Case reports

2.1.1. Family 1

The proband is an adult Italian male with a clinical history characterized by visual impairment since childhood.

He came to our attention at 48 years of age, complaining of generalized fatigue and progressive visual loss since childhood. Neurological examination showed a mild bilateral ptosis and ophthalmoplegia; he also presented pes cavus on the left side with a decreased/absent achilles tendon reflex bilaterally. Visual field revealed a peripheral concentric narrowing. Fundus oculi examination showed mild bilateral temporal pallor of the optic disc.
Expression of CD20 reveals a new store-operated calcium entry modulator in skeletal muscle

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A R T I C L E   I N F O
Article history:
Received 7 May 2012
Received in revised form 15 August 2012
Accepted 3 September 2012
Available online xxx

Keywords:
CD20
Skeletal muscle
Ca2+
SOCE

A B S T R A C T
Among the scarce available data about the biological role of the membrane protein CD20, there is some evidence that this protein functions as a store-operated Ca2+ channel and/or regulates transmembrane Ca2+ trafficking. Recent findings indicate that store-operated Ca2+ entry (SOCE) plays a central role in skeletal muscle function and development, but there remain a number of unresolved issues relating to SOCE modulation in this tissue. Here we describe CD20 expression in skeletal muscle, verifying its membrane localization in myoblasts and adult muscle fibers. Additionally, we show that inhibition of CD20 through antibody binding or gene silencing resulted in specific impairment of SOCE in C2C12 myoblasts. Our results provide novel insights into the CD20 expression pattern, and suggest that functional CD20 is required for SOCE to consistently occur in C2C12 myoblasts. These findings may contribute to future identification of mechanisms and molecules involved in the fine regulation of store-operated Ca2+ entry in skeletal muscle.

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1. Introduction
The MS4A family includes proteins that share a predicted tetra-spanning membrane topology with N- and C-terminal cytoplasmic domains. CD20, the best studied member of this family, is conventionally expressed on the surface of B cells where it is involved in lymphocyte activation, proliferation, and differentiation (Tedder and Schlossman, 1988). The primary structure of CD20 indicates a multi-transmembrane domain configuration resembling that of ion channels or transporters (Einfeld et al., 1988). Cell lines transfected with CD20 show an increased calcium conductance across the plasma membrane, strongly suggesting that CD20 functions as an important channel for regulating calcium homeostasis (Bubien et al., 1993; Li et al., 2003). Furthermore, reduced CD20 expression levels in B cell lines result in significantly decreased calcium entry across the plasma membrane (Deans et al., 2002; Li et al., 2003). Beyond these findings, knowledge about the biology of CD20 is relatively scarce, and its precise functionality is still not understood.

Some insight has been provided by studies showing that CD20 can associate with lipid raft domains of the plasma membrane, where it is probably involved in store-operated Ca2+ entry (SOCE) (Deans et al., 1998; Li et al., 2004). SOCE is an important Ca2+ influx pathway that was originally described in non-excitable cells, and that is regulated by the filling state of intracellular Ca2+ stores. Reduction of [Ca2+]i, the results in activation of plasma membrane store-operated Ca2+ (SOC) channels that mediate sustained Ca2+ influx, which is required for replenishing the Ca2+ stores and is also involved in cell signaling to the nucleus. Interestingly, recent findings indicate that SOCE exists in skeletal muscle (Kurebayashi...
Respiratory and cardiac function in congenital muscular dystrophies with alpha dystroglycan deficiency

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Received 20 March 2012; received in revised form 1 May 2012; accepted 18 May 2012

Abstract

The aim of this retrospective study was to assess respiratory and cardiac function in a large cohort of patients with congenital muscular dystrophies (CMD) with reduced glycosylation of alphadystroglycan (α-DG). Thirteen of the 115 patients included in the study died between the age of 1 month and 20 years. The age at last follow up of the surviving 102 ranged between 1 year and 68 years (median: 9.3 years). Cardiac involvement was found in 7 of the 115 (6%), 5 with dilated cardiomyopathy, 1 cardiac conductions defects and 1 mitral regurgitation. Respiratory function was impaired in 14 (12%). Ten of the 14 required non invasive nocturnal respiratory support, while the other four required invasive ventilation. Cardiac or respiratory involvement was found in patients with mutations in FKRP, POMT1, POMT2. All of the patients in whom mutation in POMGnT1 were identified had normal cardiac and respiratory function.

Published by Elsevier B.V.

Keywords: Congenital muscular dystrophy; Alpha dystroglycan; Heart; Respiratory

1. Introduction

The term “dystroglycanopathies” has been used to describe a genetically heterogeneous group of muscle disorders with a muscle biopsy showing dystrophic features and a reduction of α-dystroglycan (α-DG) with frequently associated central nervous system involvement [1].

The spectrum of clinical phenotypes ranges from the severe Walker–Warburg syndrome (WWS), muscle–eye–brain disease (MEB) and Fukuyama congenital muscular dystrophy (FCMD), all with severe muscle, eye and brain involvement, to mild cases of limb girdle muscular dystrophies with late onset and no brain or eye involvement.
Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disorder resulting from motor neuron death. Approximately 10% of cases are familial (FALS), typically with a dominant inheritance mode. Despite numerous advances in recent years1–9, nearly 50% of FALS cases have unknown genetic aetiology. Here we show that mutations within the profilin 1 (PFN1) gene can cause FALS. PFN1 is crucial for the conversion of monomeric (G)-actin to filamentous (F)-actin. Exome sequencing of two large ALS families showed different mutations within the PFN1 gene. Further sequence analysis identified 4 mutations in 7 out of 274 FALS cases. Cells expressing PFN1 mutants contain ubiquitinated, insoluble aggregates that in many cases contain the ALS-associated protein TDP-43. PFN1 mutants also display decreased bound actin levels and can inhibit axon outgrowth. Furthermore, primary motor neurons expressing mutant PFN1 display smaller growth cones with a reduced F/G-actin ratio. These observations further document that cytoskeletal pathway alterations contribute to ALS pathogenesis.

To identify causative genes for familial ALS, we performed exome capture followed by deep sequencing on two large ALS families (Fig. 1a, b) of Caucasian (family 1) and Sephardic Jewish (family 2) origin. Both display a dominant inheritance mode and are negative for known ALS-causing mutations, including the newly identified hexanucleotide repeat expansion in C9orf72 (refs 6, 8, 9; Supplementary Fig. 1). For each family, two affected members with maximum genetic distance were selected for exome sequencing. A high level of coverage (≥150x) was achieved with an average of 1.1×10^6 and 2.3×10^6 base pairs sequenced for members of families 1 and 2, respectively (Supplementary Tables 1 and 2). To identify candidate causative mutations, variants were identified and filtered, as in previous exome sequencing reports8, using several criteria: the variant is causative mutations identified was two within family 1 and three within family 2 (Supplementary Tables 3 and 4). Interestingly, the two families contain different mutations (C71G and M114T) within a single common gene: PFN1, located on chromosome 17p13.2. PFN1 is an 140-amino-acid protein and major growth regulator of filamentous (F)-actin through its binding of monomeric (G)-actin10.

![Figure 1](https://example.com/figure1.png)

**Figure 1** Exome sequencing identifies PFN1 gene mutations in familial ALS. a–c, Familial ALS pedigrees containing PFN1 mutations are shown. Asterisks indicate samples subjected to exome sequencing. To prevent identification of individual family members, the gender of each subject and information on the lower generation are withheld. Genotypes of available DNA samples for the indicated PFN1 mutation are shown (‘w’ denotes wild type, ‘m’ denotes mutant). The genotype of sample III:2 in family 2 (+) was inferred from the genotypes of spouse and progeny (not shown). d, The evolutionary conservation of PFN1 mutations is shown. For each, the mutated amino acid is coloured red.
Longitudinal Tracking of Human Fetal Cells Labeled with Super Paramagnetic Iron Oxide Nanoparticles in the Brain of Mice with Motor Neuron Disease

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Abstract

Stem Cell (SC) therapy is one of the most promising approaches for the treatment of Amyotrophic Lateral Sclerosis (ALS). Here we employed Super Paramagnetic Iron Oxide nanoparticles (SPIOn) and Hoechst 33258 to track human Amniotic Fluid Cells (hAFCs) after transplantation in the lateral ventricles of wobbler (a murine model of ALS) and healthy mice. By in vitro, in vivo and ex vivo approaches we found that: 1) the main physical parameters of SPIOn were maintained over time; 2) hAFCs efficiently internalized SPIOn into the cytoplasm while Hoechst 33258 labeled nuclei; 3) SPIOn internalization did not alter survival, cell cycle, proliferation, metabolism and phenotype of hAFCs; 4) after transplantation hAFCs rapidly spread to the whole ventricular system, but did not migrate into the brain parenchyma; 5) hAFCs survived for a long time in the ventricles of both wobbler and healthy mice; 6) the transplantation of double-labeled hAFCs did not influence mice survival.


Introduction

The evidence that Amyotrophic Lateral Sclerosis (ALS) also involves areas apart from the motor system supports the idea of a multisystemic disease, affecting multiple cell types, which requires therapeutic treatments able to provide an healthy environment for degenerating motor neurons and also capable of enhancing endogenous repair [1]. The potential of Stem Cell (SC) therapy has been widely demonstrated in different pre-clinical models of ALS [2] with a significant delay in neurological progression deriving more from the ability of SCs to produce and release several neuroprotective factors (bystander effect) than a direct replacement of degenerating neurons [3,4]. Nevertheless, no conclusive data on the optimal SC source or delivery route are currently available either in animal models [5] or in patients [1].

We have recently published a paper describing the therapeutic efficacy of human cord blood mononuclear cells, labeled with Hoechst 33258, in two murine models of ALS by direct administration into brain ventricles [6]. Similarly to intravenous delivery [7], no migration towards the spinal cord was observed, thus confirming that the beneficial role of transplanted cells is independent from their permanence in the site of administration and their distribution in degenerating host tissues.

The wobbler mouse, a model of spontaneous motor degeneration, is characterized by selective motor neuron death affecting the cervical spinal cord region with no evident features of degeneration in upper motor neurons of motor cortex [8,9,10]. The wobbler pathology is not exclusively confined to the cervical spinal cord, but spreads to bulbar motor neurons and neurons from cerebellum and thalamus [11]. In addition, a significant reduction of N-acetylaspartate, a putative neuronal marker, in whole wobbler mice brains has been reported by proton magnetic resonance spectroscopy [12]. In spite of the encouraging cell grafting results in murine models of ALS, a careful investigation into the interaction between transplanted cells and host tissues is an interesting missing part of preclinical studies. In this context, several SC labeling strategies have been proposed. Among them, Super Paramagnetic Iron Oxides nanoparticles (SPIOn) appeared particularly promising for tracking different types of SCs [13].

In the present study, SPIOn were coupled with the nuclear vital dye Hoechst 33258 to label a promising source of multipotent SCs, Amniotic Fluid Cells (hAFCs). hAFCs are an heterogeneous population, routinely cultured for genetic prenatal diagnostic testing, containing several undifferentiated and committed progenitor cells whose potential has not been extensively investigated.
TDP-43 and FUS RNA-binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells*

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Running title: TDP-43 and FUS mRNA targets in cytoplasmic RNPs

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Keywords: Neurodegenerative diseases; RNA binding protein; RNA metabolism; RNA processing; RNA-protein interaction; ALS; TDP-43; FUS; RIP-chip; post-transcriptional regulation

Background: The RNA-binding proteins TDP-43 and FUS form abnormal aggregates in patients with amyotrophic lateral sclerosis and fronto-temporal lobar dementia.

Results: We identified the mRNAs associated to these proteins in the cytoplasm of NSC-34 cells.

Conclusions: TDP-43 and FUS recognize distinct transcripts and differently regulate their fate.

Significance: Our results clarify TDP-43 and FUS role in neuronal metabolism and neurodegeneration.

SUMMARY

The RNA-binding proteins TDP-43 and FUS form abnormal cytoplasmic aggregates in affected tissues of patients with amyotrophic lateral sclerosis and frontotemporal lobar dementia. TDP-43 and FUS localize mainly in the nucleus where they regulate pre-mRNA splicing, but they are also involved in mRNA transport, stability and translation. To better investigate their cytoplasmic activities, we applied a RNA immunoprecipitation and chip analysis to define the mRNAs associated to TDP-43 and FUS in the cytoplasmic ribonucleoprotein complexes from motoneuronal NSC-34 cells. We found that they bind different sets of mRNAs although converging on common cellular pathways. Bioinformatical analyses identified the (UG)n consensus motif in 80% of 3'UTR sequences of TDP-43 targets, while for FUS the binding motif was less evident. By in vitro assays we validated binding to selected target 3'UTRs, including Vegfa and Grn for TDP-43, and Vps54, Nvl and Taf15 for FUS. We showed that TDP-43 has a destabilizing activity on Vegfa and Grn mRNA and may ultimately affect Progranulin protein content, while FUS does not affect mRNA stability/translation of its targets. We also demonstrated that three different point mutations in TDP-43 did not change the binding affinity for Vegfa and Grn mRNAs or their protein level.

Our data indicate that TDP-43 and FUS recognize distinct sets of mRNAs and
**ATAXIN2 CAG-repeat length in Italian patients with amyotrophic lateral sclerosis: risk factor or variant phenotype? Implication for genetic testing and counseling**

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Received 11 August 2011; received in revised form 2 February 2012; accepted 4 February 2012

**Abstract**

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease mainly involving cortical and spinal motor neurons. Several studies indicated that intermediate CAG expansions in ataxin-2 gene (ATXN2) are associated with increased risk of ALS. We analyzed ATXN2 CAG repeats in 658 sporadic ALS patients (SALS), 143 familial ALS cases (FALS), 231 sporadic ataxic subjects, and 551 control subjects. The frequency of ATXN2 alleles with 27–30 repeats was similar in SALS and control subjects. Fifteen SALS subjects carried \(31\) CAG repeats. This difference was statistically significant \((p = 0.0014)\). No alleles with \( \geq 34\) CAG were found. In FALS, the distribution of ATXN2 alleles was similar to control subjects. Our results further contributed in refining CAG-repeat range significantly associated with sporadic ALS. Literature data and our findings indicate that only alleles with \( \geq 31\) CAG may represent low-penetrance disease/susceptibility alleles associated with variable neurodegenerative phenotypes, including cerebellar ataxia, parkinsonism, and ALS. Overlapping phenotypes should be considered in genetic testing and counseling, both for patients and at-risk family members.

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**Keywords:** Spino-cerebellar ataxia type 2; Ataxin 2; ATXN2; Amyotrophic lateral sclerosis; Neurodegenerative disorders; Triplet repeats; CAG; Polyglutamine disorders

**1. Introduction**

Amyotrophic lateral sclerosis (ALS) is a late-onset neurodegenerative disease involving mainly cortical and spinal motor neurons, presenting with progressive and diffuse muscular weakness and wasting (Wijesekera and Leigh, 2009). However, ALS is increasingly recognized to be a multisystem disorder in which cognitive (Strong, 2008) and extrapyramidal dysfunctions can be observed (Pradat et al., 2009). The disease has an incidence ranging from 1.5 to 2.7 per 100,000 population/year and a Male/Female ratio of about 1.5:1.

Approximately 90% of the cases are sporadic (SALS), and 10% of patients present a positive familial history (FALS) (Wijesekera and Leigh, 2009). In the past years, a few disease-causing genes have been identified in FALS patients, with approximately 20% of the cases being due to mutations in the SOD1 gene encoding for the cytoplasmic superoxide dismutase protein (Eisen et al., 2008; Rosen et al., 1993).
The **C9ORF72** expansion mutation is a common cause of ALS+/-FTD in Europe and has a single founder

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A massive hexanucleotide repeat expansion mutation (HREM) in **C9ORF72** has recently been linked to amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Here we describe the frequency, origin and stability of this mutation in ALS+/-FTD from five European cohorts (total n = 1347). Single-nucleotide polymorphisms defining the risk haplotype in linked kindreds were genotyped in cases (n = 434) and controls (n = 856). Haplotypes were analysed using PLINK and aged using DMLE+. In a London clinic cohort, the HREM was the most common mutation in familial ALS+/-FTD: C9ORF72 29/112 (26%), SOD1 27/112 (24%), TARDBP 1/112 (1%) and FUS 4/112 (4%) and detected in 13/216 (6%) of unselected sporadic ALS cases but was rare in controls (3/856, 0.3%). HREM prevalence was high for familial ALS+/-FTD throughout Europe: Belgium 19/22 (86%), Sweden 30/41 (73%), the Netherlands 10/27 (37%) and Italy 4/20 (20%). The HREM did not affect the age at onset or survival of ALS patients. Haplotype analysis identified a common founder in all 137 HREM carriers that arose around 6300 years ago. The haplotype from which the HREM arose is intrinsically unstable with an increased number of repeats (average 8, compared with 2 for controls, P<10^-6). We conclude that the HREM has a single founder and is the most common mutation in familial and sporadic ALS in Europe.

*European Journal of Human Genetics* advance online publication, 13 June 2012; doi:10.1038/ejhg.2012.98

Keywords: ALS; common founder; C9ORF72

INTRODUCTION

Despite apparent differences in the clinical phenotype of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), evidence of an etiopathological link between these disorders is irrefutable. ALS due to motor neuron degeneration usually presents with focal weakness in a limb or mouth/throat muscles (bulbar) and spreads relentlessly causing widespread paralysis. FTD presents with changes in behaviour, personality and language due to degeneration of neurons in the frontal and temporal lobes. Both disorders can be familial and in a subset of these kindreds, individuals can present with either ALS or FTD, or features of both. In 2006, we reported linkage to a 11-Mb locus on chromosome 9p13.2–21.3 in Dutch and Scandanavian kindreds with autosomal-dominant ALS-FTD. Linkage was subsequently confirmed in eight other dominant kindreds defining a minimal overlapping region of ~3.6 Mb. Genome-wide association studies in sporadic and familial ALS demonstrated highly significant association with single-nucleotide polymorphisms (SNPs) across a 170-Kb region at 9p21.2.7–11

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Received 15 February 2012; revised 12 April 2012; accepted 24 April 2012
Noninvasive near-infrared live imaging of human adult mesenchymal stem cells transplanted in a rodent model of Parkinson’s disease

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Background: We have previously shown that human mesenchymal stem cells (hMSCs) can reduce toxin-induced neurodegeneration in a well characterized rodent model of Parkinson’s disease. However, the precise mechanisms, optimal cell concentration required for neuroprotection, and detailed cell tracking need to be defined. We exploited a near-infrared imaging platform to perform noninvasive tracing following transplantation of tagged hMSCs in live parkinsonian rats.

Methods: hMSCs were labeled both with a membrane intercalating dye, emitting in the near-infrared 815 nm spectrum, and the nuclear counterstain, Hoechst 33258. Effects of near-infrared dye on cell metabolism and proliferation were extensively evaluated in vitro. Tagged hMSCs were then administered to parkinsonian rats bearing a 6-hydroxydopamine-induced lesion of the nigrostriatal pathway, via two alternative routes, ie, intrastriatal or intranasal, and the cells were tracked in vivo and ex vivo using near-infrared technology.

Results: In vitro, NIR815 staining was stable in long-term hMSC cultures and did not interfere with cell metabolism or proliferation. A significant near-infrared signal was detectable in vivo, confined around the injection site for up to 14 days after intrastriatal transplantation. Conversely, following intranasal delivery, a strong near-infrared signal was immediately visible, but rapidly faded and was completely lost within 1 hour. After sacrifice, imaging data were confirmed by presence/absence of the Hoechst signal ex vivo in coronal brain sections. Semiquantitative analysis and precise localization of transplanted hMSCs were further performed ex vivo using near-infrared imaging.

Conclusion: Near-infrared technology allowed longitudinal detection of fluorescent-tagged cells in living animals giving immediate information on how different delivery routes affect cell distribution in the brain. Near-infrared imaging represents a valuable tool to evaluate multiple outcomes of transplanted cells, including their survival, localization, and migration over time within the host brain. This procedure considerably reduces the number of animal experiments needed, as well as interindividual variability, and may favor the development of efficient therapeutic strategies promptly applicable to patients.

Keywords: 6-hydroxydopamine, intranasal, intrastriatal, neurodegeneration, cell tracking

Introduction

In the past 30 years, translation of the outstanding potential of stem cells from bench to clinical trials in humans has represented a major objective for the scientific community. The therapeutic use of stem cells, in particular, has emerged as a promising branch of regenerative medicine for treating many aggressive and/or incurable diseases in humans, such as neurodegenerative disorders.1 In particular, Parkinson’s disease has been one of the first neurodegenerative diseases considered eligible for cellular therapy.
RESEARCH PAPER

Ubiquilin 2 mutations in Italian patients with amyotrophic lateral sclerosis and frontotemporal dementia

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ABSTRACT

Objectives Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease mainly involving cortical and spinal motor neurones. Molecular studies have recently identified different mutations in the ubiquilin-2 (UBQLN2) gene as causative of a familial form of X-linked ALS, 90% penetrant in women. The aim of our study was to analyse the UBQLN2 gene in a large cohort of patients with familial (FALS) and sporadic (SALS) amyotrophic lateral sclerosis, with or without frontotemporal dementia (FTD), and in patients with FTD.

Methods We analysed the UBQLN2 gene in 819 SALS cases, 226 FALS cases, 53 ALS–FTD patients, and 63 patients with a clinical record of FTD. Molecular analysis of the entire coding sequence was carried out in all FALS and ALS–FTD patients, while SALS and FTD patients were analysed specifically for the genomic region coding for the PXX repeat tract. Healthy controls were 845 anonymous blood donors and were screened for the PXX repeat region only.

Results We found five different variants in the UBQLN2 gene in five unrelated ALS patients. Three variants, including two novel ones, involved a proline residue in the PXX repeat region and were found in three FALS cases. The other two were novel variants, identified in one FALS and one SALS patient. None of these variants was present in controls, while one control carried a new heterozygous variant.

Conclusions Our data support the role of the UBQLN2 gene in the pathogenesis of FALS, being conversely a rare genetic cause in SALS even when complicated by FTD.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a late-onset disease caused by motor neurone degeneration, currently with no effective therapies.1 ALS is the third most common neurodegenerative disease in the western world,2 with an incidence of 1.5–2.7 per 100 000 population/year, and a male : female ratio of 1.5 : 1. Approximately 10% of cases are familial (FALS), mostly presenting with an autosomal dominant transmission,3 whereas the vast majority of patients are sporadic (SALS).4

A number of genes (SOD1, TARDBP, FUS, C9ORF72) have been discovered as causative for approximately 50–65% of FALS cases.5–10 Mutations in at least 10 other genes have also been reported as rare causes of ALS or ALS-like syndromes.11–13 Dementia, usually of the frontotemporal lobar type (FTD), may occur in some ALS cases. The identification of a hexanucleotide repeat expansion in the C9ORF72 gene in a high percentage of cases in both ALS and FTD disorders suggests that a common genetic cause may contribute to both phenotypes.9,10

Mutations in the PXX repeat region of the UBQLN2 gene have recently been identified in five large families with ALS and ALS/dementia presenting with a dominant X-linked transmission mode.14 Interestingly, aggregates of ubiquilin 2 protein were described in spinal motor neurones not only of patients carrying UBQLN2 mutations, but also in all SALS and FALS cases analysed. Moreover, ubiquilin 2-positive inclusions were frequently associated with TDP-43, FUS and optineurin proteins.14

In order to elucidate further the contribution of ubiquilin 2 to neurodegeneration we screened the UBQLN2 gene in a large cohort of Italian ALS and a smaller group of FTD patients.

METHODS

Patients and controls

Patients in this study were collected by six ALS centres participating in the Italian SLAGEN Consortium. All patients were of Italian descent. The diagnosis of ALS was made according to the El Escorial revised criteria.15 Familial history was considered positive if the proband had one or more first or second-degree relatives with ALS, according to the recent criteria proposed for FALS classification.1 The diagnosis of FTD was made according to the criteria proposed by Neary et al16 and Strong et al17. Our cohort included 519 SALS, 226 FALS, 58 ALS–FTD (of which five had a positive familial history) and 65 FTD patients.

ORIGINAL ARTICLE

Prevalence of Huntington’s disease gene CAG repeat alleles in sporadic amyotrophic lateral sclerosis patients

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Abstract
A higher prevalence of intermediate ataxin-2 CAG repeats in amyotrophic lateral sclerosis (ALS) patients has raised the possibility that CAG expansions in other polyglutamine disease genes could contribute to ALS neurodegeneration. We sought to determine whether expansions of the CAG repeat of the HTT gene that causes Huntington’s disease, are associated with ALS. We compared the HTT CAG repeat length on a total of 3144 chromosomes from 1572 sporadic ALS patients and 4007 control chromosomes, and also tested its possible effects on ALS-specific parameters, such as age and site of onset and survival rate. Our results show that the CAG repeat in the HTT gene is not a risk factor for ALS nor modifies its clinical presentation. These findings suggest that distinct neuronal degeneration processes are involved in these two different neurodegenerative disorders.

Key words: Huntington’s disease, amyotrophic lateral sclerosis, trinucleotide repeat, neurodegenerative disease, polyglutamine expansion

Introduction
Huntington’s disease (HD) is a progressive neurodegenerative disorder characterized by motor, cognitive and behavioral manifestations. It is caused by the expansion of an unstable polymorphic CAG repeat, in the first exon of the HTT gene, on chromosome 4p16.3 (1), resulting in an expanded polyglutamine (polyQ) tract in the huntingtin protein. Alleles with fewer than 35 CAGs are considered to produce no symptoms of HD and can be stable (alleles smaller than 27 CAGs) or prone to expansion (27–35 CAGs). A case report raises the question of whether, in rare instances, these high-end ‘normal’ alleles may lead to HD symptoms (2). A third class of alleles between 35 and 39 repeats shows incomplete HD penetrance while alleles with 40 repeats or more are fully penetrant.

Amyotrophic lateral sclerosis (ALS) is an adult onset disorder caused by the loss of motor neurons in the motor cortex, brainstem and spinal cord. The motor neuron loss results in muscle weakness, wasting, fasciculations, spasticity and hyperreflexia, leading to generalized paralysis and death (two to five years after disease onset). The disease is mostly sporadic (SALS) but ~10% of patients have a family history (FALS). The most frequent mutations are found in the superoxide dismutase-1 gene (SOD1), which accounts for 12–23% of FALS (3), in the TAR DNA binding protein 43 (TDP-43) gene (TARDBP) (4,5) and the fused-in sarcoma/translocated- in liposarcoma gene (FUS) (6,7), which equally account for ~5% of FALS. Both FUS and TDP-43 proteins have been found in HD inclusions (8–10). Mutations in the OPTN (optineurin) gene have recently been identified...
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Abstract

Aims: This study utilized proteomics, biochemical and enzymatic assays, and bioinformatics tools that characterize protein alterations in hindlimb (gastrocnemius) and forelimb (triceps) muscles in an amyotrophic lateral sclerosis (ALS) (SOD1G93A) mouse model. The aim of this study was to identify the key molecular signatures involved in disease progression. Results: Both muscle types have in common an early down-regulation of complex I. In the hindlimb, early increases in oxidative metabolism are associated with uncoupling of the respiratory chain, an imbalance of NADH/NAD+, and an increase in reactive oxygen species (ROS) production. The NADH overflow due to complex I inactivation induces TCA flux perturbations, leading to citrate production, triggering fatty acid synthase (FAS), and lipid peroxidation. These early metabolic changes in the hindlimb followed by sustained and comparatively higher metabolic and cytoskeletal derangements over time precede and may catalyze the progressive muscle wasting in this muscle at the late stage. By contrast, in the forelimb, there is an early down-regulation of complexes I and II that is associated with the reduction of oxidative metabolism, which promotes metabolic homeostasis that is accompanied by a greater cytoskeletal stabilization response. However, these early compensatory systems diminish by a later time point. Innovation: The identification of potential early- and late-stage disease molecular signatures in an ALS model: muscle albumin, complex I, complex II, citrate synthase, FAS, and phosphoinositide 3-kinase functions as diagnostic markers and peroxisome proliferator-activated receptor γ co-activator 1α (PGC1α), Sema-3A, and Rho-associated protein kinase 1 (ROCK1) play the role of disease progression markers. Conclusion: The differing pattern of cellular metabolism and cytoskeletal derangements in the hind and forelimb identifies the potential dysmetabolism/hypermetabolism molecular signatures associated with disease progression, which may serve as diagnostic/disease progression markers in ALS patients. Antioxid. Redox Signal. 17, 1333–1350.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that is characterized by progressive muscular paralysis reflecting the degeneration of motor neurons in the primary motor cortex, corticospinal tracts, brainstem, and spinal cord. Approximately two thirds of patients with ALS have a spinal form of the disease (limb onset) and present with symptoms related to focal muscle weakness and wasting, where the symptoms may start either distally or proximally in the upper and lower limbs. Gradually, spasticity may develop in the weakened atrophic limbs, affecting manual dexterity and gait. Patients with bulbar onset ALS usually present with dysarthria and dysphagia for solids or liquids, and limb symptoms develop either simultaneously with bulbar symptoms or within 1–2 years. Paralysis is progressive and leads to death due to respiratory failure within 2–3 years for bulbar onset cases and within 3–5 years for limb onset ALS cases (68). Most ALS cases are sporadic, but 5%–10% of cases are familial and of these, 20% have point mutations in the gene coding for copper-zinc (Cu-Zn) superoxide dismutase (SOD1) (63). Interestingly also, 2% of the sporadic cases have a mutation in the SOD1 gene.

Several hypotheses have been suggested for the mechanism(s) of toxicity caused by these point mutation(s) in the SOD1 gene. Interestingly, SOD1 mutations such as
Neuroprotective effects of human mesenchymal stem cells on neural cultures exposed to 6-hydroxydopamine: implications for reparative therapy in Parkinson’s disease

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Published online: 10 December 2011
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Abstract Stem cell (SC) transplantation represents a promising tool to treat neurodegenerative disorders, such as Parkinson’s disease (PD), but positive therapeutic outcomes require elucidation of the biological mechanisms involved. Therefore, we investigated human Mesenchymal SCs (hMSCs) ability to protect murine differentiated Neural SCs (mdNSCs) against the cytotoxic effects of 6-hydroxydopamine (6-OHDA) in a co-culture model mimicking the in vivo neurovascular niche. The internalization of 6-OHDA mainly relies on its uptake by the dopamine active transporter (DAT), but its toxicity could also involve other pathways. We demonstrated that mdNSCs consistently expressed DAT along the differentiative process. Exposure to 6-OHDA did not affect hMSCs, but induced DAT-independent apoptosis in mdNSCs with generation of reactive oxygen species and caspases 3/7 activation. The potential neuroprotective action of hMSCs on mdNSCs exposed to 6-OHDA was tested in different co-culture conditions, in which hMSCs

Lidia Cova, Patrizia Bosossalco, and Marie-Therese Armentero contributed equally to this work.
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Short Report

Clinical phenotype variability in patients with hereditary spastic paraplegia type 5 associated with CYP7B1 mutations


Spastic paraplegia type 5 (SPG5) is caused by mutations in CYP7B1, a gene encoding the cytochrome P-450 oxysterol 7-α-hydroxylase, CYP7B1, an enzyme implicated in the cholesterol metabolism. Mutations in CYP7B1 were found in both pure and complicated forms of the disease with a mutation frequency of 7.7% in pure recessive cases. The mutation frequency in complex forms, approximately 6.6%, is more controversial and needs to be refined. We studied in more detail the SPG5-related spectrum of complex phenotypes by screening CYPB1 for mutations in a large cohort of 105 Italian hereditary spastic paraplegias (HSPs) index patients including 50 patients with a complicated HSP (cHSP) phenotype overlapping the SPG11- and the SPG15-related forms except for the lack of thin corpus callosum and 55 pure patients. Five CYP7B1 mutations, three of which are novel, were identified in four patients, two with a complex form of the disease and two with a pure phenotype. The CYP7B1 mutation frequencies obtained in both complicated and pure familial cases are comparable to the known ones. These results obtained extend the range of SPG5-related phenotypes and reveal variability in clinical presentation, disease course and functional profile in the SPG5-related patients while providing with some clues for molecular diagnosis in cHSP.

Conflict of interest
Nothing to declare.

Hereditary spastic paraplegias (HSPs) are a group of neurodegenerative diseases presenting with wide genetic and clinical heterogeneity (1). Progressive spasticity and weakness of the lower limbs may occur in isolation (clinically pure form) or may be accompanied by additional neurological signs (complicated form), including cerebellar ataxia, visual dysfunction, mental retardation, dementia, thin corpus callosum (TCC) or by extraneurological signs (1).
Mutations in the motor and stalk domains of KIF5A in spastic paraplegia type 10 and in axonal Charcot–Marie–Tooth type 2


Spastic paraplegia type 10 (SPG10) is an autosomal dominant form of hereditary spastic paraplegia (HSP) due to mutations in KIF5A, a gene encoding the neuronal kinesin heavy chain implicated in anterograde axonal transport. KIF5A mutations were found in both pure and complicated forms of the disease; a single KIF5A mutation was also detected in a CMT2 patient belonging to an SPG10 mutant family. To confirm the involvement of the KIF5A gene in both CMT2 and SPG10 phenotypes and to define the frequency of KIF5A mutations in an Italian HSP patient population, we performed a genetic screening of this gene in a series of 139 HSP and 36 CMT2 affected subjects. We identified five missense changes, four in five HSP patients and one in a CMT2 subject. All mutations, including the one segregating in the CMT2 patient, are localized in the kinesin motor domain except for one, falling within the stalk domain and predicted to generate protein structure destabilization. The results obtained indicate a KIF5A mutation frequency of 8.8% in the Italian HSP population and identify a region of the kinesin protein, the stalk domain, as a novel target for mutation. In addition, the mutation found in the CMT2 patient strengthens the hypothesis that CMT2 and SPG10 are the extreme phenotypes resulting from mutations in the same gene.

Conflict of interest
None to declare.