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Clinical short communication

Mitochondrial dysfunction in hereditary spastic paraparesis with mutations in *DDHD1*/SPG28

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ABSTRACT

Mutations in *DDHD1* cause the SPG28 subtype of hereditary spastic paraplegia (HSP). Recent studies suggested that mitochondrial dysfunction occurs in SPG28. Here we describe two siblings with SPG28, and report evidence of mitochondrial impairment in skeletal muscle and skin fibroblasts.

Patient 1 (Pt1) was a 35-year-old man with spastic paraparesis and urinary incontinence, while his 25-year-old brother (Pt2) had gait spasticity and motor axonal neuropathy. In these patients we identified the novel homozygous c.1429C>T/p.R477* mutation in *DDHD1*, using a next-generation sequencing (NGS) approach. Histochemical analyses in muscle showed mitochondrial alterations, and multiple mitochondrial DNA (mtDNA) deletions were evident. In Pt1, respiratory chain enzyme activities were altered in skeletal muscle, mitochondrial ATP levels reduced, and analysis of skin fibroblasts revealed mitochondrial fragmentation.

It seems possible that the novel nonsense mutation identified abolishes DDHD1 protein function thus altering oxidative metabolism. Qualitative alterations of mtDNA could have a pathogenetic significance. We suggest to perform *DDHD1* analysis in patients with multiple mtDNA deletions.

1. Background

Hereditary spastic paraplegia (HSP) is a heterogeneous group of disorders causing spasticity in the lower limbs, which can be isolated (pure forms) or associated with other neurologic and systemic abnormalities (complicated forms) [1]. Mutations in *DDHD1* have been found to underlie the SPG28 subtype of HSP, responsible for both pure clinical phenotypes [2] and complicated variants with axonal neuropathy, distal sensory loss, and cerebellar impairment [3]. *DDHD1* encodes a cytosolic phosphatidic acid-preferring phospholipase A1 (PA-PLA1), and alters mitochondrial bioenergetics with increased oxidative stress in lymphoblasts from patients harboring pathogenic mutations [3]. A recent brain and muscle magnetic resonance spectroscopy (MRS) study has corroborated the notion that impairment of energy metabolism occurs in SPG28, suggesting that mitochondrial dysfunction could be a crucial mechanism in its pathogenesis [4]. Here we describe two additional SPG28 patients, and report further evidence of mitochondrial impairment in skeletal muscle.

2. Patients

Family pedigree is shown in Fig. 1a. Patient 1 (Pt1) was a 35-year-old man who complained of gait disturbances since age 23 and has manifested urinary incontinence in the last year. His 25-year-old brother (Pt2) referred walking difficulties since he was age 17. Patients were born to healthy non-consanguineous Italian parents. Their past medical history was unremarkable. Neurological examination of both sibs revealed paraparetic gait, lower limb hypertonia, markedly increased deep tendon reflexes, bilateral Babinski sign, mild distal lower limb muscle atrophy, and slight weakness in foot dorsiflexion. Sensation and coordination were normal. Neuropsychological assessment did not detect cognitive impairment. Patient 2 showed bilateral pes cavus. Spastic paraplegia rating scale [5] scores were 14/52 and 12/52 in Pt1 and Pt2, respectively. Routine laboratory tests, including serum lactate levels, were unremarkable. Conventional MRI of the brain and spinal cord was normal in both subjects. Brain MR-Spectroscopy (MRS) in Pt1 showed a mild reduction of the relative ratio N-acetyl aspartate/creatine, and quantitative MR (qMR) revealed a slight reduction of global and regional brain volumes, while in Pt2 both MRS and qMR were normal. Motor evoked potentials showed increased central motor conduction times (CMCTs) in the upper limbs and absence of responses in the legs. Nerve conduction velocity study(NCS) and electromyography

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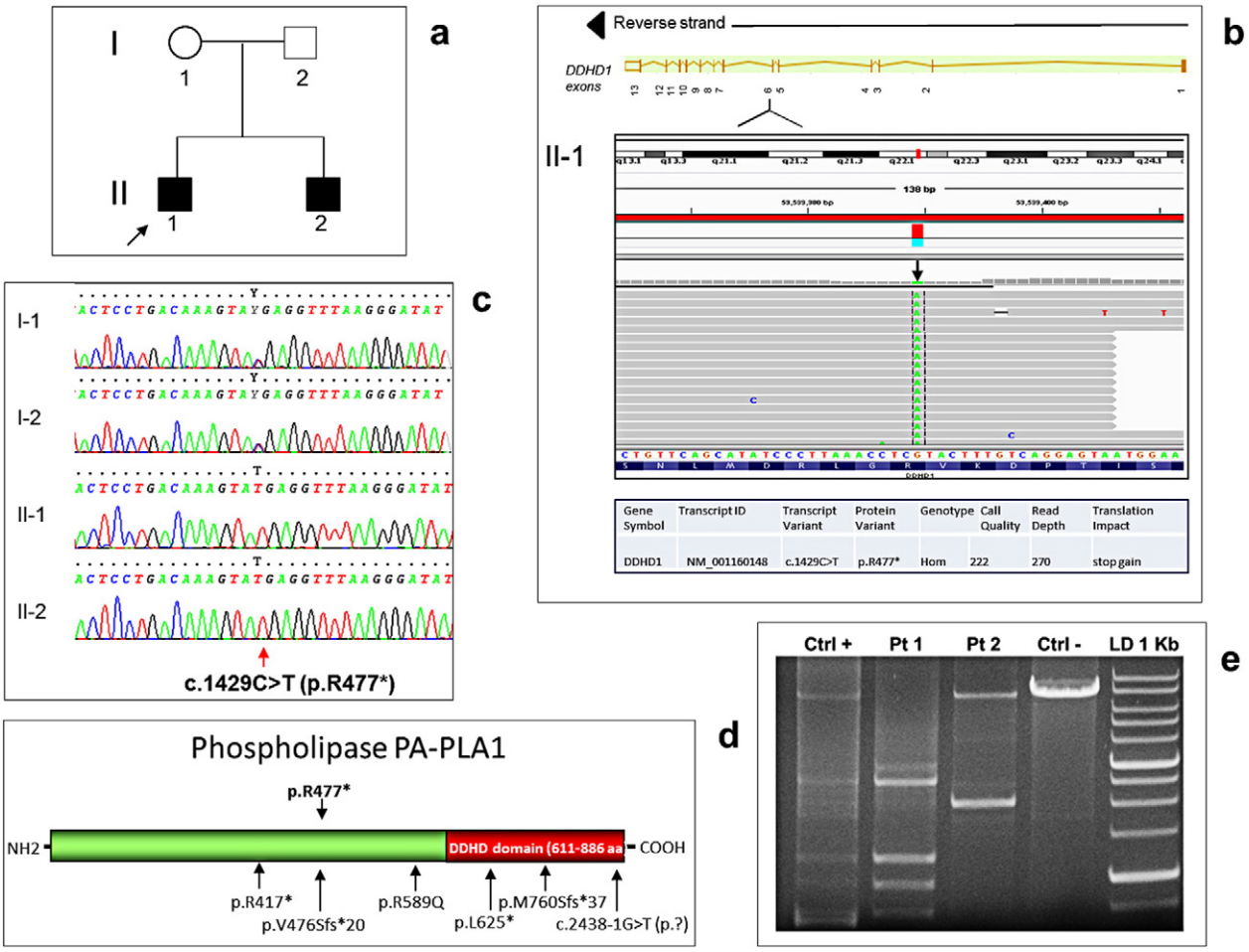

Fig. 1. a) Pedigree of the family. Squares are men and circles women. Affected individuals are in bold. b) Raw sequencing reads showing the homozygous c.1429C>T/p.R477* mutation (arrow) in the exon 6 (reverse strand) of DDHD1 visualized with Genome Viewer SureCall (Agilent Technologies). Ingenuity variant analysis table displaying the identification, call quality and read depth of c.1429C>T/p.R477*. c) Genomic sequence electropherograms displaying the nonsense c.1429C>T/p.R477* (arrow) segregating in family members (see family pedigree for reference). d) Representation of the reported mutations in DDHD1 in the scheme of the PA-PLA1 protein; the novel p.R477* mutation is in bold. e) Long range PCR demonstrating the presence of multiple mtDNA deletions in Pt1 and Pt2 muscle DNA, compared to a healthy control muscle (Ctrl−) and a control muscle positive for mtDNA deletions (Ctrl+); LD = 1Kb DNA molecular marker size.

(EMG) were normal in Pt1, and revealed a motor axonal neuropathy in Pt2.

3. Methods

After obtaining patients' informed consent, genetic analyses, muscle and skin biopsies were performed.

Mutation analysis was performed using a targeted resequencing gene panel in next-generation sequencing (NGS) (Haloplex Target Enrichment System, Agilent Technologies) that covers the coding exons and flanking intronic sequences of the 72 known HSP-related genes using standard methodologies outlined elsewhere [6].

Sequence data was generated with an Illumina MiSeq genomic platform (http://support.illumina.com/sequencing/sequencing_kits/truseq_custom_amplicon.html). Variant annotations were explored through the use of Ingenuity Variant Analysis tool (www.ingenuity.com, Qiagen). Sanger sequencing was used to validate identified variants and to perform segregation analysis.

Skeletal muscle DNA was used to investigate mitochondrial DNA (mtDNA) deletions by long-range polymerase chain reaction (PCR) using oligonucleotide primers (100F-8600R and 7400F-15000R) located in the mitochondrial genome itself, and the Expand Long Template PCR System (Roche Applied Science, Mannheim, Germany). MtDNA levels in muscle were assessed using a previously reported qPCR protocol [7]. Routine morphology, histochemical stains for oxidative metabolism enzymes in skeletal muscle biopsy, and spectrophotometric determination of respiratory electron transfer chain (RC) enzyme activities in muscle homogenate used standard methods. As controls, we tested tissue homogenate from ten healthy, age/sex-matched individuals whose skeletal muscle biopsy had already been stored in our biobank.

Levels of ATP were assayed in triplicate in cultured skin fibroblasts using the Luminescence ATP Detection Assay System (ATPlite one step, Perkin Elmer, Boston, MA, USA) according to a previously reported protocol [7]. Mitochondrial morphology was assessed in fibroblasts grown for 48 h in DMEM or DMEM-galactose using 10 nM Mitotracker Red (Life Technologies). Cellular fluorescence images were acquired with using a Zeiss AX10 inverted fluorescence microscope equipped with an AxioCam MRc5 camera. Images were collected using a 63×/1.4 oil objective and processed by AxioVision rel 4.8 acquisition software (Zeiss, Munich, Germany) [8].

Mitochondrial network analyses were performed using "Mitochondrial Morphology" in ImageJ (http://rsbweb.nih.gov/ij/) [9] and outlining three different morphologies of the mitochondrial compartment (namely, tubular, intermediate, and fragmented) as seen elsewhere [10]. For each experiment at least 20 cells derived from patients and controls were used to calculate the relative percentage of the different mitochondrial groups. The steady-state levels of proteins implicated in mitochondrial fusion and fission were assessed by Western blotting using the following monoclonal antibodies OPA1 (clone