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SHORT COMMUNICATION

Digenic mutations in severe myoclonic epilepsy of infancy

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Summary The clinical features of severe myoclonic epilepsy of infancy (SMEI) resemble those of mitochondrial diseases, although most patients have the sodium channel (*SCN1A*) mutation. We describe a patient with SMEI and enlarged muscle mitochondria associated with mutations in mitochondrial polymerase gamma 1 (*POLG1*) and *SCN1A*. Due to increased risk of valproate-induced liver failure in patients with *POLG1* mutations, we recommend *POLG1* gene analysis for SMEI patients before valproate administration.

Introduction

Severe myoclonic epilepsy of infancy (SMEI, or Dravet syndrome) is a rare disease with convulsive hemiclonic or generalized drug resistant seizures, often associated with fever, and frequent status epilepticus with onset in the first year of life (Dravet, 1978). From the second year of life, the patients manifest with ataxia, clumsiness and psychomotor retardation. Initially, the electroencephalography (EEG) is normal, but later multifocal and generalized spikes and

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spikes and waves appear (Wolff et al., 2006). The first mutations in sodium channel alpha 1 subunit gene (*SCN1A*) were found in patients with the generalized epilepsy with febrile seizures plus syndrome (Escayg et al., 2000). Subsequently, SMEI, borderline SMEI and intractable childhood epilepsy with generalized tonic-clonic seizures have been associated with *SCN1A* mutations in more than 70% of patients (Marini et al., 2007).

We studied the possible digenic etiology in a boy with otherwise a typical clinical picture of SMEI except for enlarged muscle mitochondria, which suggested a mitochondrial disease. Among SMEI patients there have been occasional cases with mitochondrial changes in muscle histology and biochemical defects of respiratory chain complexes, but in these cases molecular genetic etiology has remained unknown (Castro-Gago et al., 1995, 1997; Fernández-Jaén et al., 1998). Mutations in mitochondrial polymerase gamma 1 (*POLG1*) have been associated with variable phenotypes, such as Alpers disease and epilepsy (Ferrari et al., 2005; Horvath et al., 2006). Therefore, we studied a boy with SMEI and mitochondrial aberrations for possible digenic etiology by analysis of the *SCN1A* and *POLG1* genes.

Methods

Case report

A previously healthy boy, the first child of healthy unrelated parents without family history for mitochondrial diseases or epilepsy, was admitted to hospital at age 4 months for an afebrile generalized tonic-clonic seizure lasting for almost one hour. Despite phenobarbital treatment, seizures evolved twice a month to status epilepticus. EEG was initially normal, but at age 6 months generalized spike-wave activity predominantly on the right side was found. Myoclonic jerks and atypical absences associated with spike- and polyspike-wave discharges in EEG occurred at 8 months. He was treated with various combinations of valproate, nitrazepam, clonazepam, vigabatrin, topiramate, levetiracetam and prednisolon without success. Carbamazepine and lamotrigine seemed to aggravate the seizures. His psychomotor development was initially normal, but at the age of two years he was mildly mentally retarded, ataxic and hyperkinetic. At the last follow-up visit at age 17 years, he was severely retarded, autistic, and ataxic.

At the age of three years, histological examination of lateral quadriceps femoris muscle showed normal proportions and distributions of the fiber types with a slight increase of fat. Electron microscopy revealed enlarged mitochondria with variable size and shape (Fig. 1). The respiratory chain enzyme activities, plasma and urinary levels of amino acids, urinary excretion of organic acids, and oligosaccharides were normal. During 12 months of valproate treatment serum alanine aminotransferase (ALAT) increased from 29 to 71 U/L (normal < 40 U/L), and after discontinuation of the medication, ALAT values varied between 5 and 12 U/L. Blood lactate, and pyruvate were occasionally slightly elevated lactate/pyruvate ratio varying between 13 and 22 (normal < 20). Brain magnetic resonance imaging (MRI) and positron emission tomography with 2-deoxy-2[18]fluoro-D-glucose at the age of six years were normal. Later on, the patient has moved with his family to another region of the country and clinically there has not been any indication to a control MRI imaging.

Molecular methods

Total genomic DNA was isolated from blood cells and buccal epithelial cells using a QIAamp Blood Kit (Qiagen, Hilden, Germany) or PUREGENE DNA Purification Kit (Gentra Systems Inc., Minneapolis, MN, USA) and from frozen skeletal muscle samples by the standard methods.

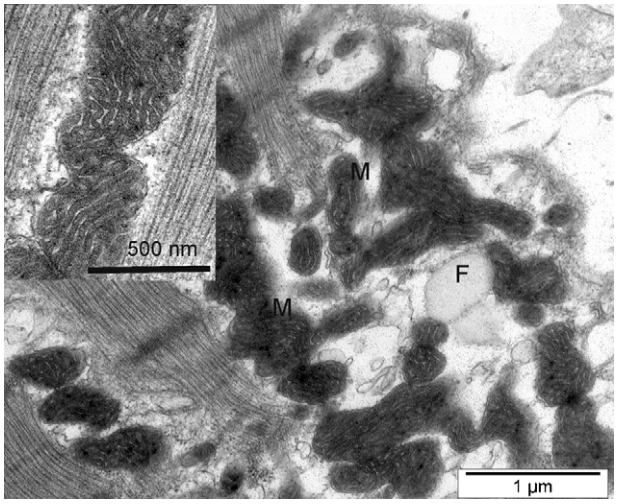

Figure 1 Transmission electron micrograph from the muscle of the patient (at the age of three years) with SMEI associated with digenic *SCN1A* and *POLG1* mutations. Increased number of mitochondria with abnormal shape and size are seen. Some giant mitochondria are seen. The number of fat droplets (F) is also moderately increased. Bar 100 nm. In the insert, mitochondria with high magnification show thick cristae. Bar 500 nm.

Patient skeletal muscle DNA was used as a template to amplify and sequence the 23 coding exons of *POLG1* (NM_002693) by automated sequencing (ABI PRISM^{TM} 3100 Sequencer) using the Dye Terminator Cycle Sequencing Ready Kit (Perkin Elmer, Foster City, CA, USA) after treatment with exonuclease I and shrimp alkaline phosphatase. *POLG1* c.1550G>T (p.G517V) and c.2447G>A (p.R722H) mutations were screened by restriction fragment length polymorphism (RFLP) (Fig. 2A and B).

MtDNA deletions were analyzed from muscle DNA of the proband by long PCR (XL-PCR) using the Expand Long Template PCR System kit (Boehringer Mannheim, Mannheim, Germany). The amount of mtDNA relative to nuclear DNA in patient and control muscle samples was determined by quantitative real-time (RT) PCR (Bio-Rad Laboratories, Hercules, CA, USA) (Uusimaa et al., 2008).

Patient blood DNA was used as a template to amplify and sequence the 26 coding exons of *SCN1A* (AB093548) with intronic primers using BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3730 DNA Analyzer (Perkin Elmer). The parents and 94 unrelated Finnish controls were analyzed for the identified mutation by sequencing.

Results

Two heterozygous mutations in *POLG1* were identified in the proband, c.1550G>T (p.G517V) in exon 8 and c.2447G>A (p.R722H) in exon 13 (Fig. 2A and B). The mother was heterozygous for the *POLG1* G517V mutation and the father for the *POLG1* R722H mutation. A c.4934G>A (p.R1645Q) mutation in exon 26 of *SCN1A* was also identified in the proband. Arginine at position 1645 is conserved in different species in genes paralogous to *SCN1A* (Fig. 3). The parents did not carry the mutation and it was absent in 94 controls.

The long PCR showed no mtDNA deletions in the muscle DNA of the patient. The mtDNA content of the muscle was