



Novel *POLG* mutation in a patient with sensory ataxia, neuropathy, ophthalmoparesis and stroke

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ABSTRACT

Background: Clinical diagnosis of *POLG*-related disorders can be challenging because the phenotypic spectrums are heterogeneous which can mimic different types of mitochondrial disorders.

Case: We report a case of *POLG*-related disorder in an 18 y Chinese girl who had been diagnosed as MELAS syndrome (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) for the past 8 y. She first presented at 10 y with sudden onset of headache, repeated focal seizures and visual loss, complicated with residual sensory and motor neuropathy, ophthalmoparesis and cortical blindness. MRI brain showed extensive cytotoxic edema and ischemia in bilateral parietal–occipital lobes. Mutation analysis for common point mutations in the mitochondrial DNA and muscle biopsy was negative. She was referred to us for mitochondrial whole genome analysis. However, no pathogenic variants can be determined. We initiated further genetic analysis for *POLG* which confirmed compound heterozygous mutations NM_002693.2:c.925C>T (p.Arg309Cys) and a novel mutation c.2244G>T (p.Trp748Cys). Both were determined to be pathogenic using in silico analysis. **Conclusions:** The novel mutation contributes to the expanding spectrum of disease-causing mutations. A definitive diagnosis can benefit our patient and also the relatives by avoiding sodium valproate induced liver toxicity in *POLG* patients and also the heterozygotes.

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1. Introduction

Human mitochondrial DNA (mtDNA) is a double-stranded circular DNA located in the mitochondria inherited in a uniparental maternal manner. The mtDNA genome is composed of 16,569 base pairs, encoding for 37 genes: 22 transfer RNA (tRNA), 13 core proteins in mitochondrial respiratory complexes and 2 mitochondrial ribosome-coding RNA (rRNA) [1]. Mutations of mtDNA would result in various clinical disorders, for example, Alpers-Huttenlocher syndrome, ataxia neuropathy syndromes, chronic progressive external ophthalmoplegia (CPEO), Kearns–Sayre syndrome, Leber hereditary optic neuropathy (LHON), Leigh syndrome, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy myopathy sensory ataxia (MEMSA), myoclonic epilepsy with ragged-red fibers (MERRF), neurogenic weakness with ataxia and retinitis pigmentosa (NARP) and Pearson syndrome [2].

Both DNA polymerase gamma (encoded by the *POLG*) [3] and Twinkle (encoded by the *C10orf2*) [4] are essential for mtDNA replication. DNA polymerase gamma is a nuclear-encoded protein which has both polymerase activity and a proofreading exonuclease activity for mtDNA [5]. Therefore, patients with *POLG* mutation can have similar phenotypes as those patients with inherited mitochondrial disorders [6]. The first reported case of *POLG* mutation was described in 2001 in which the patient presented with progressive external ophthalmoplegia (PEO) [7]. Since then, more *POLG*-related disorders had been reported, for example male infertility [8], MERRF [9], a syndrome of ophthalmoplegia, sensorimotor polyneuropathy, ataxia, and deafness [10], Alpers' syndrome [11], ataxia without myopathy [12], premature ovarian failure [13], familial parkinsonism [14], axonal and demyelinating sensory-motor neuropathy [15], multiple oxidative phosphorylation deficiencies [16], sensory ataxic neuropathy, dysarthria and ophthalmoparesis (SANDO) [17] and MELAS [18]. The phenotypic spectrums of *POLG*-related disorders are heterogeneous. Therefore, clinical diagnosis of *POLG*-related disorders is challenging. Very often, the diagnosis is relied on molecular genetic test for *POLG* gene.

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2. Case report

The proband was an 18-y Chinese girl born to non-consanguineous parents with unremarkable birth history. She enjoyed good past health. She first presented at 10 y with sudden onset of headache, repeated focal seizures and loss of vision. She was treated as having acute meningo-encephalitis. Seizures were controlled with phenytoin and carbamazepine. Ophthalmological assessment confirmed no structural lesion and the sudden loss of vision was compatible with cerebral visual impairment. Her sensorium gradually improved. However, there was no improvement on her vision. Magnetic resonance imaging (MRI) of the brain showed extensive cytotoxic edema and ischemia in bilateral parietal–occipital lobes (Fig. 1). The overall MRI features were compatible with acute ischaemia. However, subsequent magnetic resonance angiogram (MRA) showed no vascular abnormalities. Electroencephalography (EEG) and microbiology investigations were negative. The patient also had unremarkable blood acid–base profile. Blood lactate was 1.0 mmol/l (reference interval (RI): 0.7–2.1). Laboratory testing for plasma amino acid and organic acid profile and very long chain fatty acid levels was unremarkable. Investigation of cerebral spinal fluid (CSF) protein was normal, CSF glucose was 4.7 mmol/l (paired plasma glucose was 8.5 mmol/l), CSF lactate was 1.5 mmol/l (RI: 0.45–2.1), and CSF pyruvate was 0.07 mmol/l (RI: <0.10). There was no CSF oligoclonal band. Screening for autoimmune markers was negative. Muscle biopsy was performed which showed no fiber necrosis or regeneration, no hyaline change, and no peri-fascicular/endomyxial inflammatory infiltrate and fibrosis. There were no ragged red fibers. The muscle fibers showed normal succinic dehydrogenase staining. There was no moth-eaten fiber or sarcoplasmic inclusions. Electron-microscopy of the muscle tissue showed normal mitochondria appearance. MELAS syndrome was suspected for this patient. However, common pathogenic mutations in mtDNA, including m.3243A>G, m.8344A>G and m.8893T>G were negative. She was given arginine supplement, vitamin cocktail and anticonvulsants (initially phenytoin followed by carbamazepine monotherapy).

The patient showed good recovery on sensorium with residual sensory and motor ataxia. Nerve conduction study performed at the first admission confirmed sensory and motor neuropathy. However, her vision was severely impaired with only light perception remained. Visual evoked potential (VEP) test confirmed conduction delay along the optic nerve secondary to ischemic optic neuropathy. She also had

limited abduction of both eyes. Subsequently, she had two episodes of recurrent seizure attacks and hemiparesis about 1 year and 2 years after the first admission. Serial MRI of the brain showed no new changes. The patient was followed up regularly with serial blood lactate monitoring. The blood lactates were all normal except one episode of mild lactate elevation (2.3 mmol/l). All along, her liver function test and liver ultrasonography were normal.

3. Materials and methods

3.1. Mitochondrial whole genome sequencing analysis

Blood samples and/or urine sediment samples were collected from the proband and her unaffected brother and parents after informed consent. Extraction of DNA from urine sediments was performed according to the manufacturer procedures using Qiagen DNA extraction kit (Qiagen). The entire mitochondrial genome was amplified with genomic DNA extracted from the urine sediment sample of the proband in 5 overlapping fragments, m.782 to m.8484, m.4502 to m.11527, m.5197 to m.11524, m.7819 to m.15274 and m.11714 to m.3527 using long range polymerase chain reaction (PCR). Primer sequences are available upon request. The long range PCR products were purified using Agencourt AMPure XP (Beckman Coulter) and quantitated by Quant-iT Picogreen (Invitrogen). A library was built using the GS Junior Titanium Rapid Library Prep Kit (Roche Diagnostics) by shearing of the DNA using nebulization (kit part of the library prep kit) followed by ligation of Rapid Library Adaptor to the DNA according to the manufacturer's protocols (454 Life Sciences). The library was amplified using emulsion PCR with the GS Junior Titanium emPCR Kit (Roche) following the manufacturer's protocol. An input of 2 molecules of library DNA per capture bead was used. After emulsion PCR, the beads capturing the DNA library were enriched and the enriched beads were annealed with sequencing primers. The amplified fragments were sequenced using 500,000 enriched beads and 200 cycles with full processing for shotgun or paired end pipeline in the GS Junior Benchtop System (Roche). Post-sequencing raw data was filtered to eliminate low-quality sequence data. The reads were mapped to mitochondrial genome using GS Reference Mapper. Mutational analysis and pipeline used to rule out variants of unknown significance were based on published American College of Medical Genetics (ACMG) clinical laboratory standards for next-generation sequencing [19].

3.2. Mutational analysis using Sanger sequencing for POLG

Methods for PCR and sequencing are described previously [20]. Primer sequences for *POLG* are available upon request. Sequencing results of all coding exons and the flanking intronic regions of the *POLG* genes were compared with National Center for Biotechnology Information (NCBI) reference sequences NM_002693.2 and NP_002684.1. The pathogenicity of novel mutation was determined using *in silico* analysis, Polymorphism Phenotyping v2 (PolyPhen-2) online, <http://genetics.bwh.harvard.edu/pph2/>.

3.3. NMR-based urinalysis

NMR-based urinalysis was acquired using a 600 MHz Bruker NMR spectrometer using a “noesygppr1d” pulse sequence. Details of the sample preparation, proton NMR acquisition parameters and positive identifications of metabolites had been described previously [21,22].

4. Results

4.1. Mutational analysis

In the mitochondrial whole genome sequencing analysis, 114,779 DNA sequence reads were obtained after processing with quality filters

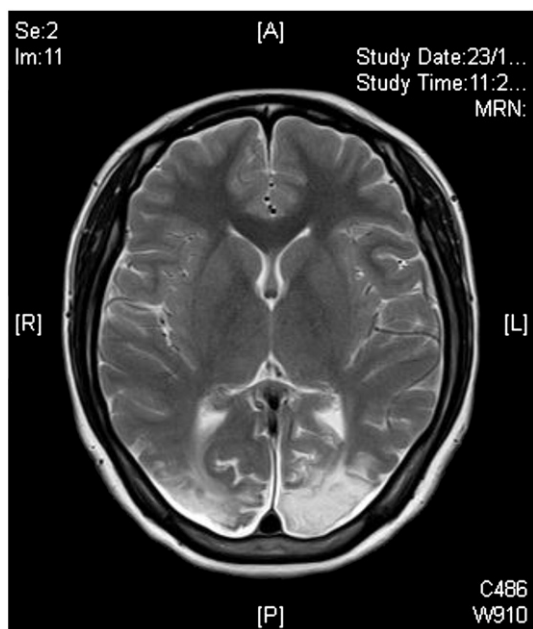


Fig. 1. Extensive T2-hyperintense signal over bilateral parietal–occipital areas.

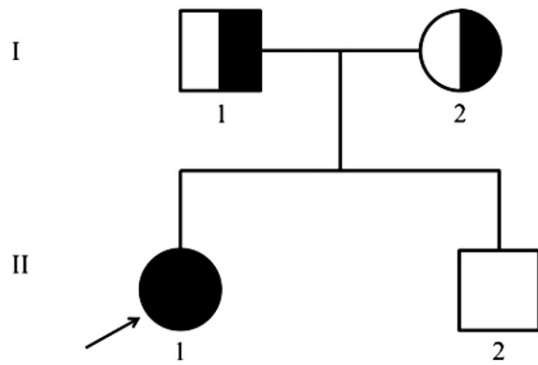


Fig. 2. Pedigree of a *POLG*-affected family.

with average read length of 457 base-pairs. The percentage of reads mapped to the mitochondrial genome was 98.69% and the coverage of the mitochondrial genome was 100%. The average read depth was 400×. The mitochondrial whole genome sequencing analysis in the urine sediment sample of the proband did not detect any point mutations or large mitochondrial deletion.

The pedigree of the family is shown in Fig. 2. Mutation analysis for *POLG* gene of the proband (II-1) showed a compound heterozygous mutation NM_002693.2:c.925C>T (p.Arg309Cys) and NM_002693.2:c.2244G>T (p.Trp748Cys). Homozygous p.Arg309Cys had been previously reported in patient with peripheral neuropathy, progressive external ophthalmoplegia, ataxia and myopathy [23]. The p.Trp748Cys is a novel mutation. Using PolyPhen-2 prediction, both mutations were predicted to be probably damaging. The father (I-1) was heterozygous

for c.2244G>T (p.Trp748Cys) and the mother (I-2) was heterozygous for c.925C>T (p.Arg309Cys). The two disease-causing mutations were not found in the unaffected brother (II-2) (Fig. 3).

4.2. ¹H-NMR-based urinalysis

The ¹H NMR-based urinalysis showed increased urine ascorbic acid and carnitine. The findings were compatible with the drug record of the proband which was put on L-carnitine and vitamin C supplements. Overall, the urine metabolic pattern was unremarkable.

5. Discussion

The phenotype of *POLG*-related disorders is heterogeneous which can mimic different types of mitochondria disorders [6,24]. The phenotypes of *POLG*-related disorders include (i) mitochondrial DNA depletion syndrome 4A (Alpers type), (ii) mitochondrial DNA depletion syndrome 4B (MNGIE type); (iii) Mitochondrial recessive ataxia syndrome (includes SANDO and SCAE); (iv) progressive external ophthalmoplegia, autosomal dominant and (v) progressive external ophthalmoplegia, autosomal recessive [25]. Most of the reported *POLG* mutations were recessive mutations with few of them being dominant mutations [26].

The patient in this case report presented with stroke-like episode, sensory ataxia, neuropathy and ophthalmoplegia, and the mode of inheritance was autosomal recessive, therefore the overall clinical features is compatible with SANDO syndrome (sensory ataxic neuropathy, dysarthria and ophthalmoparesis; OMIM #607459). However, she had no remarkable dysarthria. Indeed, dysarthria can be absent in patients with SANDO caused by *POLG* mutations [27]. In a recent study



Fig. 3. Electropherograms showing the *POLG* mutations in the family.

of SANDO syndrome, dysarthria was found to be absent in some patients who carried compound heterozygous pathogenic *POLG* mutations. Therefore, etiology of dysarthria in SANDO remained inconclusive [28]. Intriguingly, our patient also presented with stroke-like episode which was further evident by extensive cytotoxic edema and ischemia in bilateral parietal–occipital lobes by MRI brain study. The patient also showed hemiparesis in her subsequent admissions. Stroke-like episode is not a usual feature in SANDO. This case implies the heterogeneous clinical features of *POLG*-related disorders. To our knowledge, none of the known *POLG*-related syndrome can fit all the clinical features of our patient.

A definitive diagnosis can benefit our patient and also the relatives by avoiding sodium valproate induced liver toxicity in *POLG* patients and also the heterozygotes [29,30]. Clinician should be aware of *POLG*-related disorder in patients with unexplained MELAS-like presentations, neurological deficit, liver dysfunction and suspected mitochondrial diseases.

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