Published in final edited form as:

J Exp Integr Med. 2012 ; 2(2): . doi:10.5455/jeim.200312.cr.001.

A novel *POLG* gene mutation in a patient with SANDO

Bulent Kurt¹, Ali B. Naini¹, William C. Copeland², Jiesheng Lu¹, Salvatore DiMauro¹, and Michio Hirano¹

¹Department of Neurology, Columbia University Medical Center, New York, NY

²Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, Durham, NC; USA

Abstract

The human mitochondrial genome is replicated by DNA polymerase γ , which is encoded by polymerase γ gene (*POLG1*) on chromosome 15q25. Patients with *POLG1* mutations usually present as Alpers' syndrome or progressive external ophthalmoplegia. Our patient was a 48-year old woman with sensory ataxic neuropathy, dysarthria, ophthalmoplegia, and dysphagia. Sequence analysis revealed that she has two heterozygous missense mutations in the *POLG1*, a c.1774C>T substitution in exon 10, which results in a p.L591F amino acid change; and a c.3286C>T substitution in exon 21, which results in a p.R1096C amino acid change. The 1774C>T substitution is a novel mutation.

Previously described adult patients with one mutation in exon 10 and the other in exon 21 of *POLG1* had presented with progressive external ophthalmoplegia. We now describe a patient with mutations in the same exons but suffering from the more complex clinical syndrome of sensory ataxic neuropathy, dysarthria, ophthalmoplegia.

Keywords

Novel mutation; PEO; *POLG1*; SANDO

Introduction

The human mitochondrial genome is replicated by the nuclear-encoded DNA polymerase (pol γ) [1]. The catalytic subunit of DNA pol γ , encoded by the polymerase γ gene (*POLG1*) on chromosome 15q25 [2], is a 140-kDa protein with three functional domains: an exonuclease domain located at the N-terminus, a polymerase domain encompassing the C-terminus, and a linker region connecting the two [3].

The first pathogenic mutations in *POLG1* were identified in families with autosomal dominant chronic progressive external ophthalmoplegia (adPEO) and multiple mitochondrial DNA (mtDNA) deletions in muscle [4]. Subsequent reports have shown that *POLG1* mutations are associated with at least six different neurodegenerative phenotypes [3] and, to date, about 150 pathogenic mutations have been identified [5].

In this article, we report a patient with two heterozygous missense mutations (one of which is novel) in *POLG1* and discuss the relationship between these mutations and the patient's clinical findings.

Materials and methods

Patient history

This 48-year-old woman was born after a normal pregnancy and delivery. She had normal childhood and young adult life. At the age of 38, she developed difficulty in walking and her work performance deteriorated so rapidly that she had to be retired. At the age of 41, progressive diplopia and ptosis added to the symptoms. Five years later, she gradually had dysarthria and restless leg syndrome. Her current clinical findings include sensory ataxic neuropathy, dysarthria, ophthalmoplegia (SANDO), and dysphagia.

Most of the routine laboratory tests were normal except for a low creatinine (0.34 mg/dL; normal range 0.5–1.2 mg/dL) and increased BUN/creatinine ratio (38; normal range 6–22). At age of 40, a muscle biopsy was performed. It showed many ragged red fibers, which stained intensely with the succinate dehydrogenase and were negative for the cytochrome oxidase reaction. In addition, there was mild to moderate angulated fiber atrophy, predominantly in type 2 fibers, and some fiber-type grouping.

The patient's 76-year-old father and 52-year-old sister had also present ptosis. Droopy eyelids in the sister were noted when she was 40 and she also had difficulty in walking.

DNA sequencing

Nine (forward and backward) oligonucleotide primers were used to amplify the 22 coding exons of the *POLG1*. Sequencing was performed using the BigDye Terminator Cycle Sequencing Kit. Amplified products were purified using Performa DTR Gel Filtration Cartridges (Edge Biosystem, Gaithersburg, MD, USA) and analyzed on an ABI 3130XL Genetic Analyzer with Sequencing Analyzer Software v5.2 (Applied Biosystems, Foster City, CA, USA). The cDNA sequence corresponding to GenBank accession number NM_002693.2 was used as reference.

Results

Sequence analysis revealed two heterozygous missense mutations. The first, a novel mutation, was a c.1774C>T substitution in exon 10 (Fig.1A), which resulted in a p.L591F amino acid change (Fig.1B). The second was a c.3286C>T substitution in exon 21 (Fig.1C), resulting in p.R1096C amino acid change (Fig.1D).

Discussion

Patients with *POLG1* mutations have a wide range of phenotypes, but the genotype-phenotype relationship is not well understood. In 2006, Horvath *et al* reviewed 38 patients with one or more *POLG1* mutations [6], the most common of which was c.1399G>A in exon 7, resulting in a p.A467T substitution in the linker domain. They grouped the patients in four clinical variants characterized by: (1) liver dysfunction and encephalopathy; (2) encephalopathy; (3) PEO; and (4) other features, including ataxia, dysphagia, neuropathy, myopathy, cardiomyopathy, cortical blindness, seizures, myoclonus, diabetes, dementia, thyroid dysfunction, hearing loss, and heart block. In this series, most children presented with hepatoencephalopathy while most adults had PEO and limb myopathy, often in association with ataxia and axonal sensorimotor peripheral neuropathy (similar to sensory ataxic neuropathy, dysarthria, and ophthalmoplegia; SANDO).

One of the two mutations in our patient was in exon 10 (p.591L>F) and the other one was in exon 21 (p.1096R>C). To our knowledge, only three previously reported adult patients with mutations in both exon 10 (linker domain) and exon 21 (polymerase domain) were reported

and all three presented with PEO (Table 1). Our patient had adult-onset SANDO, but also gastrointestinal dysmotility and dysphagia. In addition, she had exercise intolerance, proximal myopathy, diplopia, and restless leg syndrome. Mutation in exons 10 and 21 were previously reported in two children [6, 7]. The first one had infantile hepatocerebral syndrome with mtDNA depletion while the second one had encephalopathy, liver failure, stroke-like episodes, and seizures (Table 1).

The p.1096R>C mutation has been described in Alpers' syndrome, in arPEO, and in sporadic cases of PEO [8] or PEO-myopathy (in trans with p.648P>R) [6]. In Alpers' syndrome, the mutation could be either homozygous, associated with the p.1236Q>H polymorphism [3], or compound heterozygous with p.914T>P mutation [9]. Arg1096 is part of the active site of pol γ in a position that can interact with the template DNA and may be involved in DNA binding.

The novel p.591L>F mutation is likely to be pathogenic for the following reasons: first of all, Leu591 is in an area of the protein that is known to interact with the p55 accessory subunit, although the precise role of the Leu residue in this interaction is not clear; secondly, the Leu residue at this position is conserved in animal DNA pol γ sequences and the conservation is consistent with its role in accessory subunit binding (lower eukaryotic DNA pol γ 's do not have an accessory subunit); lastly, because Leu591 makes a close contact with Gln456, its substitution with the larger Phe side chain would predictably clash with the Gln456, pushing away the alpha-helix that makes up the thumb domain and consequently disturbing the interaction of Leu591 with Gln456. In conclusion, all three previously reported adult patients with mutations in exons 10 and 21 of *POLG1* had presented with PEO. We now report an additional adult patient, who is also compound heterozygous for mutations in these exons but presented with a more complex clinical syndrome, namely SANDO.

Acknowledgments

This work was supported by NIH grant HD-032062 and by the Marriott Mitochondrial Disorder Clinical Research Fund (MMDCRF).

References

1. Clayton DA. Replication of animal mitochondrial DNA. *Cell*. 1982; 28:693–705. [PubMed: 6178513]
2. Kaguni LS. DNA polymerase gamma, the mitochondrial replicase. *Annu Rev Biochem*. 2004; 73:293–320. [PubMed: 15189144]
3. Wong LJ, Naviaux RK, Brunetti-Pierri N, Zhang Q, Schmitt ES, Truong C, Milone M, Cohen BH, Wical B, Ganesh J, Basinger AA, Burton BK, Swoboda K, Gilbert DL, Vanderver A, Saneto RP, Maranda B, Arnold G, Abdenur JE, Waters PJ, Copeland WC. Molecular and clinical genetics of mitochondrial diseases due to *POLG* mutations. *Hum Mutat*. 2008; 29:E150–E172. [PubMed: 18546365]
4. Van Goethem G, Dermaut B, Lofgren A, Martin JJ, Van Broeckhoven C. Mutation of *POLG* is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nat Genet*. 2001; 28:211–212. [PubMed: 11431686]
5. Mitochondrial DNA Replication Group. [Accessed 14 March 2012] Human DNA Polymerase Gamma Mutation Database. National Institute of Environmental Health Science (NIEHS). Available via <http://tools.niehs.nih.gov/polg>
6. Horvath R, Hudson G, Ferrari G, Futterer N, Ahola S, Lamantea E, Prokisch H, Lochmuller H, McFarland R, Ramesh V, Klopstock T, Freisinger P, Salvi F, Mayr JA, Santer R, Tesarova M, Zeman J, Udd B, Taylor RW, Turnbull D, Hanna M, Fialho D, Suomalainen A, Zeviani M,

Chinnery PF. Phenotypic spectrum associated with mutations of the mitochondrial polymerase gamma gene. *Brain*. 2006; 129:1674–1684. [PubMed: 16621917]

7. Taanman JW, Rahman S, Pagnamenta AT, Morris AA, Bitner-Glindzicz M, Wolf NI, Leonard JV, Clayton PT, Schapira AH. Analysis of mutant DNA polymerase gamma in patients with mitochondrial DNA depletion. *Hum Mutat*. 2009; 30:248–254. [PubMed: 18828154]
8. Agostino A, Valletta L, Chinnery PF, Ferrari G, Carrara F, Taylor RW, Schaefer AM, Turnbull DM, Tiranti V, Zeviani M. Mutations of *ANTI*, *Twinkle*, and *POLG1* in sporadic progressive external ophthalmoplegia (PEO). *Neurology*. 2003; 60:1354–1356. [PubMed: 12707443]
9. Ashley N, O'Rourke A, Smith C, Adams S, Gowda V, Zeviani M, Brown GK, Fratter C, Poulton J. Depletion of mtDNA in fibroblast cultures from patients with *POLG1* mutations is a consequence of catalytic mutations. *Hum Mol Genet*. 2008; 17:2496–2506. [PubMed: 18487244]

Figure 1.

(A) c.1774C>T substitution in exon 10 was seen in the sequence of *POLG1* of our patient; (B) this substitution caused a p.L591F amino acid change; (C) the second substitution was a c.3286C>T transition in exon 21; and (D) This mutation caused a p.R1096C amino acid change.

Table 1

Reported patients with mutations in both exons 10 and 21 of *POLG1*.

Patient number	Age-Sex	Exon 10		Exon 21		Additional mutation/s		Clinical presentation
		N	AA	N	AA	N	AA	
1	35-F	C1760T	P587L	G3616A	V1106I	C752T (in exon 13)	T2511	PEO, myopathy
2	45-F	G1880A	R627Q	A3428G	E1143G	G3708T (in exon 23), T2894G (in exon 18)	Q1236H, L965X	PEO, ataxia, neuropathy, myoclonus epilepsy
3	55-F	C1943G	P648R	C3286T	R1096C	-	-	PEO, myopathy
4*	48-F	C1774T	L591F	C3286T	R1096C	-	-	SANDO, dysphagia, diplopia
5	5 months	C1760T	P587L	G3406A	E1136K	C752T (in exon 13)	T2511	Infantile hepatocerebral mtDNA depletion syndrome
6	7-M	G1880A	R627Q	G3287A	R1096H	-	-	Encephalopathy Liver failure Stroke-like episodes Seizures

* Current case; N, nucleotide substitution; AA, amino acid substitution.