Novel POLG Splice Site Mutation and Optic Atrophy

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**Objective:** To investigate the molecular etiology of 2 unrelated patients with a multisystem mitochondrial disorder accompanied by optic atrophy in one of them.

**Design:** Clinical examination and neurophysiological, radiological, morphological, and molecular analyses.

**Setting:** Tertiary care neuromuscular clinic and molecular genetics laboratory.

**Patients:** A 65-year-old man (patient 1) with dyschromatopsia and vision loss since childhood developed progressive external ophthalmoplegia, ptosis, and myopathy in the seventh decade of life and was found to have optic atrophy. A 63-year-old man (patient 2) with a similar phenotype, without visual symptoms, experienced also hearing loss and parkinsonism.

**Main Outcome Measures:** Description of the clinical and molecular findings.

**Results:** A muscle biopsy specimen showed ragged-red, ragged-blue, and cytochrome c oxidase–negative fibers in both patients. Because optic atrophy in patient 1 suggested an autosomal dominant OPA1-related disorder, the OPA1 gene was first sequenced, the results of which did not detect any mutations. Southern blot and polymerase chain reaction analyses of muscle mitochondrial DNA revealed multiple deletions. Sequencing of POLG detected a novel variant, c.3104 + 3A>T, in both patients. Patient 1 was compound heterozygous for a known p.F749S mutation; patient 2 had p.G848S as the second mutation.

**Conclusion:** Early-onset dyschromatopsia and optic atrophy can occur not only in OPA1-related but also in POLG-related disorders with significant impact on genetic counseling.

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The nuclear gene POLG encodes for the catalytic subunit of the sole mitochondrial DNA (mtDNA) polymerase gamma (Polγ). Mutations in POLG result in a broad phenotypic spectrum ranging from fatal infantile hepatoencephalopathy to late-onset progressive external ophthalmoplegia. Recently, POLG mutations were reported in 2 patients who manifested with optic neuritis and unmatched cerebrospinal fluid oligoclonal bands years before the development of a classic POLG phenotype.

Mutations in OPA1, the leading gene for autosomal dominant optic atrophy, can result in a multisystem neurological disease in which optic atrophy may be absent. While OPA1 mutations are inherited with autosomal dominant modality, although with variable penetrance, POLG mutations can demonstrate autosomal recessive or dominant inheritance. We investigated the molecular etiology of 2 unrelated patients with a multisystem mitochondrial disorder, accompanied by optic atrophy in one of them.

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**REPORT OF CASES**

**Case 1**

A 65-year-old man with dyschromatopsia and poor vision since childhood presented with a 3-year history of progressive ptosis, dysphagia, exercise-induced myalgia, proximal limb weakness, and unintentional weight loss. His medical history was significant for type 2 diabetes mellitus. A cataract was removed when the patient was 50 years old. Clinical examination revealed bilateral optic atrophy (Figure 1), reduced visual acuity of 20/150 OD and 20/200 OS, moderate symmetric bilateral ophthalmoplegia, and severe ophthalmoparesis, mild generalized weakness, and mild distal superficial sensory loss in his feet with hyperactive tendon reflexes. Ishihara test results confirmed dyschromatopsia; optical coherence tomography showed retinal nerve fiber layer thickness loss. The patient's creatine kinase level was normal. Electromyographic studies showed mixed myopathic and neurogenic changes with normal nerve conduction. Tibial somatosensory evoked potentials were normal. Brain magnetic
resonance imaging showed diffuse volume loss of the optic chiasm and optic nerves, a left-sided chronic occipital infarct, multiple lacunar infarcts, and mild diffuse cerebral atrophy (Figure 2A and B).

Case 2

A 63-year-old man with long-standing exercise intolerance and sensorineural hearing loss developed progressive ptosis, limited eye movements, dysphagia, and generalized weakness in his mid-40s, and, more recently, parkinsonism and cataracts. Clinical examination showed muscle weakness similar to that observed in patient 1 with no optic atrophy. In addition, patient 2 had asymmetric parkinsonism that was levodopa responsive. The patient's creatine kinase level was 596 U/L (reference range, 52-336 U/L) (to convert to microkatal std per liter, multiply by 0.0167). Electromyographic studies showed diffuse myopathic changes. Brain magnetic resonance imaging revealed mild diffuse cerebral atrophy and moderate periventricular and subcortical white matter signal changes (Figure 2C). Magnetic resonance spectroscopy disclosed no increased lactate peaks.

In both patients, a muscle biopsy specimen showed scattered ragged-red and ragged-blue fibers and numerous cytochrome c oxidase–negative fibers (Figure 3). The biochemical measurement of the muscle respiratory enzymes did not reveal deficiencies in complex activities in patient 1. Electrocardiogram, 24-hour Holter monitoring, and echocardiogram were normal in both patients. Neither patient had similarly affected family members.

BIOCHEMICAL AND MOLECULAR ANALYSES

Total DNA was extracted from the muscle tissue of patient 1 and from a blood sample of patient 2 using DNA isolation kits (Gentra Systems, Inc, Minneapolis, Minnesota) according to the manufacturer protocols. Mito-
chondrial DNA common point mutations, including 3243A>G, 3271T>C, 3460G>A, 8344A>G, 8356T>C, 8363G>A, 8993T>G, 8993T>C, 11778G>A, 14459G>A, and 14484T>C, were screened by the polymerase chain reaction (PCR)/allele-specific oligonucleotide hybridization method. Mitochondrial DNA deletions were analyzed by Southern blot and PCR.

RESULTS

Both patients harbor a novel splice-site mutation in intron 18 of POLG (OMIM 174763), c.3104 + 3A>T. This mutation occurs in compound heterozygosity with previously reported pathogenic mutations, p.F749S in patient 1 and p.G848S in patient 2 (http://tools.niehs.nih.gov/polg/). Using exonic primers spanning from exon 17 to 19, analysis of POLG cDNA showed 2 bands. The 447-base pair (bp) band represented the wild-type transcript, while the 324-bp band represented a transcript lacking exon 18 (Figure 4A and B). Sequencing of the PCR product confirmed exon 18 skipping (Figure 4C). The novel mutation was detected in the asymptomatic mother of patient 2, suggesting that the mutation is recessive. The asymptomatic family member’s blood of patient 1 was unavailable for analysis. However, Southern blot and PCR analyses of patient 1’s muscle DNA showed multiple mtDNA deletions (Figure 5), a pattern consistent with the pathogenicity of the POLG mutations. Mitochondrial DNA common point mutations were not detected in the muscle mtDNA of patient 1. In particular, mtDNA mutations associated with Leber optic neuropathy were not found. Sequencing of genes causing late-onset progressive external ophthalmoplegia and/or optic atrophy, OPA1, ANT1, PEO1, POLG2, and OPA3 in patient 1 did not detect any pathogenic mutations. Large deletions in OPA1 or other mitochondrial-related nuclear genes were not detected by oligonucleotide array comparative genomic hybridization.

COMMENTS

We identified a novel POLG mutation, c.3104 + 3A>T, in 2 patients, occurring in compound heterozygosity with previously reported pathogenic mutations. The c.3104 + 3A>T is predicted to abolish the splicing donor site. Indeed, analysis of c.3104 + 3A>T POLG transcripts revealed skipping of exon 18, resulting in in-frame deletion of 41 amino acids in the polymerase catalytic domain. Previously reported point mutations in exon 18 have resulted in reduced enzyme catalytic ac-
tivity or reduced DNA-binding affinity in light of their location in the putative DNA-binding channel. Therefore, skipping of exon 18 is expected to reduce both Pol catalytic activity and DNA-binding affinity. The novel mutation was detected in the blood sample of the asymptomatic mother of patient 2, suggesting that the mutation is likely recessive. In addition, the novel mutation was not detected in 2600 individuals analyzed for POLG, including 200 normal control subjects.

Although the optic atrophy and long history of dyschromatopsia and vision loss in patient 1 were suggestive of an autosomal dominant OPA1 gene mutation, patient 1 had an autosomal recessive POLG-related disorder. This finding has a significant impact on genetic counseling, underscoring the importance of molecular analysis for a definite diagnosis. Patient 2 had no visual symptoms and no optic atrophy. Because optical coherence tomography was not performed in patient 2, sub-

Figure 4. Analysis of POLG complementary DNA. A, Location of c.3104 + 3A>T, polymerase chain reaction (PCR) and sequencing primers. B, Polymerase chain reaction: P, shows a 447–base pair (bp) band similar to that observed in the control, C, and a 324–base pair band; DNA marker, M. C, Sequencing of PCR product-confirmed exon 18 skipping.
clinical optic atrophy, eventually segregating with c.3104 + 3A>T, cannot entirely be excluded. We have identified another patient with POLG mutations, homozygous for p.A467T, manifesting optic atrophy in the setting of Alpers syndrome (L.-J.W., unpublished observation, September 1, 2010). In this case, sequencing analysis of OPA1 and OPA3 did not detect any point mutations. Large deletions in mitochondrial-related nuclear genes were also not detected by oligonucleotide array. Thus, we attribute with confidence the optic atrophy to the POLG mutations. Of interest, 2 patients with optic neuritis and oligoclonal bands in cerebrospinal fluid, previously diagnosed as having multiple sclerosis, developed ataxia, myopathy, progressive external ophthalmoplegia, and cognitive impairment later in life and were found to carry recessive POLG mutations. Conversely, patients with pathogenic dominant OPA1 mutations can lack optic atrophy. Therefore, POLG- and OPA1-related disorders may have clinical overlap.

The other 2 detected POLG mutations were previously reported in compound heterozygosity and expected to be pathogenic. p.F749S was described in association with p.A467T in Alpers syndrome. p.G848S has been reported in compound heterozygosity with several other mutations and in association with a broad phenotypic spectrum but not with levodopa-responsive parkinsonism, as observed in patient 2 (http://tools.niehs.nih.gov/polg/).

In conclusion, the present study demonstrates that optic atrophy may occur in POLG-related disorders and that POLG-related disorders can be differentiated from OPA1-related disorders only by molecular analysis.

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