Mutation of the Linker Region of the Polymerase γ-1 (POLG1) Gene Associated With Progressive External Ophthalmoplegia and Parkinsonism

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Objective: To define the molecular basis of the autosomal dominant progressive external ophthalmoplegia and parkinsonism in a large family with a dominantly transmitted multiple mitochondrial DNA deletion disorder.

Design: Microsatellite analysis and screening of the progressive external ophthalmoplegia 1 (PEO1), adenine nucleotide translocator 1 (ANT1), and polymerase γ-1 (POLG1) genes.

Results: We identified 3 novel heterozygous POLG1 substitutions in the same family. Autosomal dominant progressive external ophthalmoplegia segregated with 1532G>A in exon 8 and an intronic variant c.2070+158G>A in cis. The one patient with parkinsonism had an additional heterozygous substitution in exon 7 in trans (1389G>T). Both coding region mutations were predicted to alter conserved amino acids in the linker region of polymerase γ. None of the substitutions were found in 192 ethnically matched control chromosomes. 108 patients with progressive external ophthalmoplegia, nor 140 cases of sporadic idiopathic Parkinson disease.

Conclusion: Both autosomal dominant progressive external ophthalmoplegia and parkinsonism can be caused by mutations that directly affect the polymerase domain of polymerase γ.

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Original Contribution

METHODS

PATIENTS

Our index case, II:8, was a 61-year-old woman who developed ptosis in her early 40s, followed by limb ataxia and progressive hearing loss. On examination, she had prominent ptosis, mild bilateral facial weakness, symmetric hip flexion weakness, and absent lower limb tendon reflexes with a mild gait ataxia. Biochemical investigations included an elevated serum creatine kinase level (2568 U/L, normal <140 U/L) and normal fasting plasma glucose (88.45 mg/dL [4.91 mmol/L]), fasting plasma lactate (12.16 mg/dL [1.35 mmol/L]), and fasting cerebrospinal fluid constituents (protein, 0.26 g/L; glucose, 57.65 mg/dL [3.2 mmol/L]; lactate, 19.19 mg/dL [2.13 mmol/L]). An electroencephalogram and electrocardiogram showed normal findings. Peripheral
neuropsychiatric examination revealed an axonal sensory neuropathy with additional myopathic features. Muscle biopsy revealed an increased range of muscle fiber diameters, 20% being cytochrome c oxidase–deficient with evidence of mitochondrial proliferation (Figure 1A). Respiratory chain complex analysis identified a combined defect of complex I, III, and IV. Southern blotting identified multiple deletions of skeletal muscle mtDNA (Figure 1B).

Her 52-year-old son (III:2) presented with fatigue and was noted to have bilateral ptosis since the age of 46 years. He subsequently developed an unsteady gait, progressive deafness, and dysphagia. Extrapyramidal features were first noted 2 years later, at age 48 years, and they progressed until his symptoms improved after receiving ropinirole. He had an expressionless face and hypophonia with bilateral ptosis but were otherwise asymptomatic. Neurological examination was otherwise normal. Two individuals (II:5, 68 y; II:6, 69 y) had no relevant symptoms and normal neurologic examination findings.

A 30-year-old woman (III:1) developed progressive bilateral ptosis in her early 20s followed by external ophthalmoplegia. She came to medical attention through an infertility clinic investigating her primary ovarian failure. A 50-year-old woman (III:3) had no relevant symptoms and normal neurological examination findings.

**NUCLEAR DNA ANALYSIS**

**POLG1** was initially screened using denaturing high-performance liquid chromatography (Transgenicom, Omaha, Neb) as described. The progressive external ophthalmoplegia 1 (PEO1) and adenine nucleotide transporter 1 (ANT1) genes were sequenced using a fluorescent chain terminating sequencing kit (Beckman Coulter Quickstart, Beckman Coulter CEQ 8000; Beckman Coulter, Fullerton, Calif). Microsatellite analysis using markers flanking **POLG1**, **PEO1**, and **ANT1** was performed using part of the 9cm Cooperative Human Linkage Centre Weber Human Screening set (version 10aRC; Invitrogen, Carlsbad, Calif) and sized using a Beckman Coulter CEQ 8000 fluorescent DNA analyzer. **POLG1** was then sequenced directly as described. The frequency of the sequence variants was determined in 96 control subjects and 140 cases fulfilling UK Parkinson Disease Society Brain Bank criteria for the diagnosis of PD by direct sequencing of exons 7 and 8 of **POLG1**.

No heteroduplexes were identified by denaturing high-performance liquid chromatography analysis of **POLG1** in III:2, who also had a wild-type sequence for **PEO1** and **ANT1**. Haplotype analysis using microsatellite markers showed that affected individuals were discordant for the haplotypes flanking **PEO1** and **ANT1**, but all affected individuals shared the same haplotype flanking **POLG1** (Figure 2). We therefore sequenced **POLG1** in III:2 and identified 2 novel heterozygous coding region substitutions in exons 7 (1389G>T) and 8 (1532G>A) and a novel intronic variant (c.2070+158G>A) (Figure 3). The entire coding region of **POLG1** was sequenced in II:1, II:3, II:8, III:2, and III:11 were sequenced in the other living family members shown in Figure 2. All individuals with PEO harbored 1532G>A and c.2070+158G>A. Only III:2 and his unaffected sister (III:3) were found to have 1389G>T. None of the novel sequence variants were found in 192 ethnically matched control chromosomes nor in 108 patients with PEO and 140 cases of sporadic PD.

**OTHER FAMILY MEMBERS**

The other family members were independently examined by 2 neurologists (A.M.S. and P.F.C.). The family described one man (I:1) who had an obvious bilateral ptosis that developed in later years. He died many years ago, preventing formal assessment. There was nothing to suggest he had parkinsonism.

Three individuals (II:1, 60 y; II:3, 65 y; II:4, 58 y) had bilateral ptosis but were otherwise asymptomatic. Neurological examination was otherwise normal. Two individuals (II:5, 68 y; II:6, 69 y) had no relevant symptoms and normal neurologic examination findings.

A 30-year-old woman (III:1) developed progressive bilateral ptosis in her early 20s followed by external ophthalmoplegia. She came to medical attention through an infertility clinic investigating her primary ovarian failure. A 50-year-old woman (III:3) had no relevant symptoms and normal neurological examination findings.

We have identified 3 **POLG1** substitutions in the same family. c.2070+158G>A in intron 11 is unlikely to have functional consequences because it is not predicted to alter splice sites for the flanking exons. 1532G>A is pre-

**Figure 1.** Muscle histochemistry, mitochondrial DNA (mtDNA) analysis, and DaT (dopamine transporter) scan from the family with autosomal dominant progressive external ophthalmoplegia. A, Cytochrome c oxidase (COX)–succinate dehydrogenase histochemistry on II:8 showing COX-deficient fibers (blue). B, Southern blot of skeletal muscle DNA probed with an mtDNA-specific probe. Lanes 1 and 2 show healthy controls; lane 3, known single heteroplasmic mtDNA deletion; and lane 4, II:8, multiple mtDNA deletions. C, DaT scan from the index case (III:2) showing reduced uptake in the left caudate nucleus and both putamen, consistent with a nigrostriatal dopaminergic deficiency. kb indicates kilobase.
dicted to alter a highly conserved serine to asparagine (S511N) in the linker region of polγ (Figure 3B). This mutation was found in all affected individuals in phase with c.2070 + 158G>A and is thus likely to be the pathogenic mutation causing autosomal dominant PEO in this family. This substitution is close to a previously described POLG1 mutation (Q497H)4 and was not found in controls. The c.1389G>A substitution also affects the linker domain of the polγ and has been described in an Austrian family with dominant PEO.15 Although A467T is also found in healthy control subjects (0.69% of the British population, 95% confidence interval, 0.14-2.02),12 functional studies have shown that it reduces polymerase activity to 5% of normal values.15,16 Our observations support previous observations of dominantly inherited ptosis cosegregating with a single heterozygous linker domain substitution.15 Although we cannot completely rule out the possibility that 1532G>A (S511N) is a rare polymorphism, this is unlikely because it was not seen in 192 control chromosomes, nor in 248 disease controls, including 140 patients with idiopathic Parkinson disease who fulfilled UK Parkinson Disease Society brain bank criteria. Intriguingly, the subject II:6 was also heterozygous for 1532G>A (S511N) and remained unaffected at 69 years of age (examined by A.M.S. and P.F.C.). This demonstrates that dominant POLG1 mutations can remain clinically nonpenetrant, potentially explaining why some sporadic heterozygotes have no family history.

Figure 2. Pedigree showing individuals affected by ptosis with and without external ophthalmoplegia (black symbols). Squares indicate men; circles, women; slashes, deceased subjects. III:2 also had parkinsonism as indicated on the pedigree by P. Chromosome 15 microsatellite markers are shown below each symbol. The polymerase γ-1 (POLG1) gene substitutions identified in each subject are shown between D15S655 and D15S652, which flank the gene. Black indicates wild-type nucleotide; red, variant allele. The region of the green chromosome containing POLG1 is shared by all affected individuals. The exon 8 substitution (1532G>A) and the exon 11 substitution (c.2070 + 158G>A) are on the same chromosome. The exon 7 substitution (1389G>T) was only found in III:2 and III:3 and was probably inherited from II:7, but no samples were available to confirm this.
1389G>T is predicted to alter a highly conserved leucine to phenylalanine (L463F), also in the linker domain of polγ close to A467T (Figure 3). The unaffected sister of the proband (III:3) also harbored 1389G>T (L463F), indicating that the substitution was transmitted from their father (II:7). Although we were unable to examine II:7, nor obtain DNA samples, he was not thought to have ptosis. Based on current evidence, it is therefore unlikely that 1389G>T (L463F) can cause disease on its own.

Even within this small family, there is striking clinical variability. This raises the question: could 1389G>T (L463F) be contributing to the phenotype in III:2 and specifically the parkinsonism? It would be premature to draw firm conclusions at this stage, and we hope that further clinical and functional studies will address this question. Although his symptoms improved on a dopamine agonist, he had no tremor and the extrapyramidal rigidity was symmetric—features that mitigate against a diagnosis of idiopathic Parkinson disease. Abnormal DaT scan imaging results (Figure 1C) have been described in parkinsonian patients with PEO.8

Our observations provide further evidence that more than one mutation can be segregating in the same family.12 This is remarkable given the apparent rarity of these substitutions in the general population. Mutations in mitochondrial DNA polymerase 1 (MPI), the yeast homologue of POLG1, not only cause secondary mutations of mitochondrial DNA, but also induce higher rates of nuclear gene mutations.17 Although the reasons for this are not clear, it may be related to oxidative stress related to the mitochondrial damage.17 If correct, then it is conceivable that POLG1 mutations themselves lead to further mutation in this and other genes, which may also be transmitted down the germ line. This provides a potential mechanism for epistatic genetic interactions contributing to the variable phenotype of the disorder.12

Neither 1389G>T (L463F) nor 1532G>A (S511N) directly affect the polymerase domain of polγ. Like other linker region mutations, they are, however, likely to affect polymerase activity of the enzyme.13,16 Progressive external ophthalmoplegia with parkinsonism can therefore be caused by mutations in both the linker and polymerase domain of polγ.

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Correction

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