Familial Parkinsonism and Ophthalmoplegia From a Mutation in the Mitochondrial DNA Helicase Twinkle

Robert H. Baloh, MD, PhD; Ezequiel Salavaggione, PhD; Jeffrey Milbrandt, MD, PhD; Alan Pestronk, MD

Objective: To describe the clinical phenotype and genetic basis of a family with autosomal dominant progressive external ophthalmoplegia and parkinsonism from a Twinkle mutation.

Design: All coding exons of POLG1, Twinkle (aka C10ORF2, PEO1), and ANT1 (SLC25A4) were sequenced in the proband with targeted sequencing of the Twinkle gene in all additional subjects.

Subjects: Members of a 3-generation family followed up in a neuromuscular disease center for dominantly inherited progressive external ophthalmoplegia.

Results: We identified a heterozygous G1121A mutation (R374Q) in exon 1 of Twinkle that segregated with the disease phenotype in all affected family members. No pathogenic mutations were present in POLG1 or ANT1.

Conclusion: This finding broadens the clinical spectrum of Twinkle gene mutations and further implicates loss of mitochondrial DNA integrity in the pathogenesis of Parkinson disease.

Arch Neurol. 2007;64(7):998-1000

A role for mitochondrial dysfunction in the pathogenesis of Parkinson disease (PD) has been suggested since the description of parkinsonism induced by the complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)1 and the finding of decreased complex I activity in the substantia nigra of patients with PD.2 More recently, mutations in the mitochondrial proteins Pink1 and Dj-1 were associated with early-onset recessive PD, further strengthening the tie between mitochondrial function and PD pathogenesis.3 Parkinsonism is also part of the broad clinical spectrum associated with mutations in POLG1, the mitochondrial DNA (mtDNA) polymerase.4,5 Mutated POLG1 leads to accumulation of mtDNA deletions and mitochondrial dysfunction, and recently a POLG1 mutation was identified in 2 sisters with early-onset parkinsonism in the absence of other features of mitochondrial disease.6 Additionally, recent studies demonstrating accumulation of mtDNA deletions neurons of the substantia nigra from patients with PD and with normal aging suggest that mtDNA deletions may play a role in sporadic PD as well.7,8

Mutations in Twinkle, a mitochondrial DNA helicase essential for mtDNA replication and stability, are associated with autosomal dominant progressive external ophthalmoplegia (PEO) but not parkinsonism.9 Here we describe the first family with parkinsonism as a prominent feature associated with a mutation in Twinkle (PEO1). The finding of a mutation in a mitochondrial DNA helicase important for mtDNA stability further strengthens the connection between mitochondrial DNA integrity, mitochondrial dysfunction, and the pathogenesis of PD.

METHODS

CASE DESCRIPTIONS

The proband (subject III-3) first developed progressive eyelid drooping in her early 30s, accompanied by limitation of extraocular eye movements without diplopia. In her early 50s, she began having gait difficulty, including several falls. Members of her family noticed that she was somewhat “stiff” and that she had a resting tremor of her right foot. Neurologic examination at age 56 years showed bilateral ocular ptosis with nearly complete eye closure, near complete limitation of eye movements in all directions of gaze, and mild neck flexor weakness. Additionally she was found to have moderate parkinsonism with decreased facial expression, bradykinesia, and increased tone (lead-pipe rigidity with superimposed cogwheeling) in the left more than the right arm that was further increased with contralateral arm movement. Tone in the legs was likewise increased, and she had a bilateral resting foot
tremor that was also present but less prominent in the hands (the left more than the right). Vibration sense was diminished distally in a gradient fashion. She walked with a normal base and stride, and she had moderate retrophalgeal instability. Nerve conduction studies confirmed a mild axonal sensory-motor polyneuropathy.

Subject III-2 first developed progressive eyelid ptosis and ophthalmoplegia in her 20s. She had her first episode of severe depression requiring inpatient psychiatric care after the birth of her first child at age 22 years and had subsequent psychiatric admissions for major depression and bipolar disorder at ages 40 and 43 years. She was treated transiently with neuroleptics at age 22 years but had no symptoms of parkinsonism at that time. At age 41 years, she noticed a resting tremor of her right leg followed by a similar tremor of the right hand and progressively small handwriting. She was diagnosed with parkinsonism and treated using carbidopa and levodopa, which resulted in improvements in her handwriting and gait. Trihexyphenidyl hydrochloride improved her tremor. Magnetic resonance images of the brain were normal at age 42 years. On examination at age 45 years, she had moderate bilateral ptosis with near complete gaze limitation in all directions. She had decreased facial expression, bradykinesia, a resting tremor of her right arm and leg, and moderate muscle rigidity with superimposed cogwheeling in both the upper and lower extremities on the right side more than the left. Her stride was normal length, but she had decreased arm swing and retrophalgeal instability. Testing of muscle strength, all sensory modalities, reflexes, and coordination was normal.

Subject III-4 developed PEO in her early 30s and developed asymmetric parkinsonism, including resting tremor, muscle rigidity, and bradykinesia, in her 40s that responded well to dopamine replacement therapy. She did not have psychiatric disease or neuropathy. When examined at age 52 years, subject III-1 had PEO but no signs or symptoms of parkinsonism. Subject IV-2 developed PEO at age 25 years and had otherwise normal findings from an examination performed at age 28 years. Subjects I-1 and I-2 had PEO on record in the family, but no records were available. Subject II-1 had PEO, developed "stiffness" and walking difficulties in her 60s, and died from pneumonia after developing pharyngeal weakness at age 70 years. Subjects were recruited and consented according to a study protocol approved by the Washington University human studies committee institutional review board.

MOLECULAR GENETIC ANALYSIS

We isolated DNA from peripheral blood leukocytes, saliva samples using a DNA-self collection kit (Oragene; DNA Genotek, Ottawa, Ontario), or stored muscle biopsy tissue using the QIAamp DNA mini kit (Qiagen Inc, Valencia, California). All coding exons of the POLG1, PEO1 (C10ORF2), and SLC25A4 (ANT1) genes were amplified by polymerase chain reaction and sequenced on both strands in the proband. Subsequently exon 1 of the PEO1 gene was sequenced in all available family members. Muscle histology was performed using standard methods, and long-range polymerase chain reaction for mtDNA deletions was performed as previously described.10

RESULTS

In addition to PEO, 3 members of the family also had mild to moderate parkinsonism, including asymmetric resting tremor, muscle rigidity, and bradykinesia (Figure 1). Parkinsonism developed 10 to 20 years after PEO and was responsive to dopamine replacement therapy. Other features in some family members included psychiatric disease (subject III-2) and mild axonal peripheral neuropathy on electrophysiologic studies (subjects II-1, III-3). Muscle biopsy performed in 2 affected subjects (II-1, III-2) showed mitochondrial proliferation and cytochrome oxidase-negative muscle fibers (Figure 2A and B), and mtDNA deletions were present (Figure 2C).

All coding exons of the genes POLG1, PEO1, and SLC25A4 were sequenced in the proband. This revealed a heterozygous G-to-A substitution at position 1121 in exon 1 of the PEO1 gene corresponding to an R374Q mutation in the Twinkle protein (Figure 1). No mutations were present in POLG1 or SLC25A4/ANT1 in the proband. The R374Q mutation was also present in 4 other affected family members across 3 generations (subjects II-1, III-1, III-2, IV-2), indicating that the mutation segregates with the disease phenotype.

COMMENT

We report the first description of a family with PEO and parkinsonism caused by a mutation in the Twinkle mtDNA helicase. The R374Q mutation in Twinkle was previously described in 2 individuals with PEO in a family of a different ethnic background; however, no additional clinical information was reported.9 The fact that the R374Q mutation arose independently in the family described here, and also manifested with PEO, strongly argues that the mutation is pathogenic. Furthermore, as the previous report did not describe the pedigree, our findings validate that the R374Q allele leads to dominantly inherited disease. Given that 3 of the 4 subjects examined over age 45 years had parkinsonism, the previous report of a sporadic case of PEO and parkinsonism with com-
Our observations broaden the clinical spectrum of disorders associated with Twinkle mutations and the clinical overlap with POLG1 mutations and further implicate disrupted mtDNA integrity in the pathogenesis of parkinsonism. Given the recent report of a POLG1 mutation in individuals with isolated parkinsonism, our findings suggest PEO1/Twinkle should also be considered a candidate gene in dominantly inherited PD.

Accepted for Publication: January 6, 2007.
Correspondence: Robert H. Baloh, Department of Neurology, Washington University School of Medicine, PO Box 8111, 660 S Euclid Ave, St Louis, MO 63110 (balohb@neuro.wustl.edu).

Author Contributions: Study concept and design: Baloh, Milbrandt, and Pestronk. Acquisition of data: Baloh and Salavaggione. Analysis and interpretation of data: Baloh and Salavaggione. Drafting of the manuscript: Baloh, Milbrandt, and Pestronk. Critical revision of the manuscript for important intellectual content: Baloh and Salavaggione. Statistical analysis: Salavaggione. Obtained funding: Baloh and Milbrandt. Administrative, technical, and material support: Baloh, Salavaggione, Milbrandt, and Pestronk. Study supervision: Baloh, Milbrandt, and Pestronk.

Financial Disclosure: None reported.

Funding/Support: Dr Baloh receives support from grant 1K12RR023249-01 from the National Institutes of Health and a grant from the Muscular Dystrophy Association.

Additional Contributions: Amy Overbey and Amanda Knoten provided technical assistance.

REFERENCES