# Early-Onset Familial

## BRIEF COMMUNICATIONS

directly related to mitochondrial function, parkin and DJ-1, have been associated with autosomal recessive

# Parkinsonism Due to *POLG*

Mutations

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**Objective:** To define the molecular etiology of early-onset parkinsonism and peripheral neuropathy.

**Methods:** Two sisters had early-onset parkinsonism (dys- tonic toe curling, action tremor, masked face, bradykinesia, stooped posture, and rigidity), together with clinical and electrophysiological signs of sensorimotor axonal peripheral neuropathy.

**Results:** No mutations were found in the genes for parkin or PINK1. Muscle biopsies showed ragged-red and cytochrome *c* oxidase–negative fibers, and biochemistry showed decreased activities of respiratory chain complexes containing mito- chondrial DNA–encoded subunits. Multiple mitochondrial DNA deletions were seen by long polymerase chain reaction, and sequencing of the *POLG* gene showed that the patients were compound heterozygous for two patogenic mutations. **Interpretation:** *POLG* mutations can cause early-onset par- kinsonism in the absence of progressive external ophthalmo- plegia.

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Mitochondrial involvement in the pathogenesis of Par- kinson’s disease (PD) was suspected since the late 1980s, when respiratory chain complex I activity was found to be reduced in tissues from patients (reviewed in Mizuno and colleagues1) and in animals intoxicated with the PD-inducing molecule 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP).2 It was also sug- gested that PD may result from excessive age-related accumulation of mitochondrial DNA (mtDNA) muta- tions in the substantia nigra, but this mechanism was never convincingly substantiated.3 However, in the past few years, mutations in three nuclear genes encod- ing the mitochondrial protein PINK1 and two proteins

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and early-onset forms of PD.4–6 Mutations in the cat- alytic subunit of mtDNA polymerase gamma (POLG), a nuclear-encoded protein involved in the synthesis, replication, and repair of mtDNA, have been associ- ated with depletion or multiple deletions of mtDNA in a striking variety of neurological phenotypes, including progressive external ophthalmoplegia (PEO), ataxia, pe- ripheral neuropathy, and an infantile hepatocerebral disorder known as Alpers’ syndrome.7,8 Several families harboring mutations in *POLG* suffered from parkin- sonism, usually associated with other clinical manifes- tations, most commonly PEO.7–9

Here, we describe two sisters with early-onset par-

kinsonism and neuropathy but without PEO, who were compound heterozygotes for two autosomal reces- sive *POLG* mutations, one in the polymerase domain and the other in the linker region of the enzyme.

## Patients and Methods

*Patients*

Two sisters, ages 27 (Patient 1) and 33 (Patient 2), the only children of nonconsanguineous parents, were normal at birth and through childhood except for migraine headaches and anxiety.

When first seen at age 26, Patient 1 had dystonic toe curl- ing, postural-action tremor of all limbs, reduced arm swing, hyporeflexia, “foot numbness,” and mild distal weakness of toes and fingers. An electromyogram showed length- dependent axonal predominantly sensory neuropathy. Within 1 year, she developed facial masking, hypophonia, cogwheeling of the wrists, stooping, shuffling gait, bradyki- nesia, and positive pull test. Resting venous lactate was ele- vated (2.5 and 3.1mmol/L; reference range, 0.93–1.65mmol/ L). Serum creatine kinase was normal. Because of her sister’s intolerance to carbidopa/L-dopa (Sinemet), she was started on pramipexole (0.5mg three times daily), with moderate improvement of her action tremor.

Patient 2 presented at age 20 with dystonic toe curling, intermittent action tremor of all limbs, and facial masking and stiffness, leading to the diagnosis of parkinsonism. At age 27, she developed numbness in her feet, and a year later, when seen at the Mayo Clinic, she had signs of both parkinsonism (bradykinesia, stooped posture, and rigidity) and peripheral neuropathy (bilateral stocking and glove sen- sory deficits). An electromyogram showed axonal sensori- motor, predominantly sensory neuropathy. Magnetic reso- nance imaging of the brain showed moderate generalized cerebral and cerebellar atrophy. Serum lactate and creatine kinase values were normal. Her tremor improved on a low dose of carbidopa/levodopa (25/100 immediate-release tab- let three times daily); higher doses caused severe oral dys- kinesia.

MUSCLE MORPHOLOGY AND HISTOCHEMISTRY. Routine

histological and histochemical studies were performed as de- scribed previously.10

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BIOCHEMICAL ANALYSIS. Respiratory chain enzyme and citrate synthase activities in muscle were measured by previ- ously described methods.11

DNA ANALYSIS. Total DNA from each patient’s muscle was extracted using standard protocols.12 Southern blot anal- ysis and long polymerase chain reaction (PCR) were per- formed as described previously.13 The gene for PINK1 was screened with primers designed according to GenBank acces- sion number NP\_004610, and the gene for parkin was

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16 (cDNA C2839T) (see the Human DNA Polymer- ase Gamma Mutation Database for more information: [http://dir-apps.niehs.nih.gov/polg/index.cfm?edit=](http://dir-apps.niehs.nih.gov/polg/index.cfm?edit) allPolg) in the polymerase domain and a novel G737R in exon 13 (cDNA G2491C) mutation in the linker region (Fig). The father carried the G737R mutation, and the mother carried the R853W mutation.

## Discussion

These sisters were compound heterozygous for two

screened as described previously. The entire coding region

of the *POLG* gene was amplified by PCR,14 and mutations were detected by direct sequencing of all exons utilizing in- tronic primers. Apparently pathogenic changes were con- firmed by reverse transcriptase PCR and by complementary DNA (cDNA) sequencing analysis as described previously.15

## Results

The muscle biopsy from Patient 1 showed 2 to 3% ragged-red fibers (RRFs) and cytochrome *c* oxidase– deficient fibers. Focal fiber–type grouping and scarce targetoid structures were also seen. Common mtDNA mutations for MELAS (mitochondrial encephalopathy, lactic acidosis, and strokelike episodes), MERRF (myo- clonic epilepsy and ragged-red fiber disease), and neu- ropathy, ataxia, retinitis pigmentosa (NARP) were ab- sent. Biochemical studies of mitochondrial enzyme showed increased citrate synthase activity. When nor- malized to the nucleus-encoded citrate synthase, the ac- tivities of respiratory chain complexes containing mtDNA-encoded subunits were decreased, varying from 10 to 18% of normal (data not shown). Both histological and biochemical abnormalities were consis- tent with mitochondrial proliferation and a defect of mitochondrial protein synthesis.

The muscle biopsy specimen from Patient 2 showed

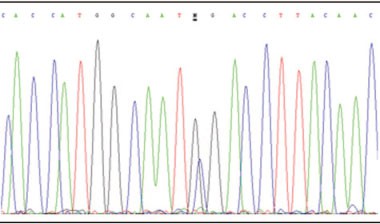
1 to 2% RRFs, which were cytochrome *c* oxidase de- ficient, mild focal fiber–type grouping, and sparse tar- getoid fibers consistent with neuropathy. The activity of citrate synthase was not increased, but when respi- ratory chain enzyme activities were referred to the ac- tivity of succinate dehydrogenase, another nucleus- encoded enzyme, all complexes containing mtDNA- encoded subunits were decreased, with residual activities varying from 40 to 60% of normal (data not shown). Real-time PCR showed only 37% mtDNA de- pletion, and Southern blot analysis for multiple dele- tions was negative on two occasions. However, long PCR assays showed multiple deletions. Direct sequenc- ing of the mtDNA genes encoding the seven subunits of complex I, cytochrome *b,* and 12S RNA demon- strated only some known nonpathogenic polymor- phisms. The nuclear genes encoding parkin and PINK1 were also normal. However, DNA and cDNA sequencing of the catalytic subunit of *POLG* (GenBank accession numbers NP\_002684 and NM\_002693) showed the already reported R853W mutation in exon

missense mutations in the *POLG* gene, a novel

G737R mutation and the R853W mutation, which had been associated with PEO (see the Human DNA Polymerase Gamma Mutation Database for more infor- mation: http://

dir-apps.niehs.nih.gov/polg/index.cfm?edit=allPolg). We consider these mutations to be pathogenic for several rea- sons. First, although the clinical phenotype is atypical, there is a good correlation between genotype and bio- chemical and molecular phenotype. Second, the R853W mutation, already documented as pathogenic, is in the polymerase domain, where the catalytic function resides, whereas the novel mutation affects the linker region, and changes in this domain have been associated with severe manifestations.16 Third, this latter mutation alters a highly conserved site (Table 1). Finally, both mutations were absent in more than 100 controls.

The clinical presentation in these sisters was domi- nated by parkinsonism and peripheral neuropathy. This disorder differed from idiopathic PD because of early onset, symmetrical involvement, postural-action tremor (but no resting tremor) in the four limbs, and incomplete L-dopa response in both patients. The clin- ical and electrophysiological evidence of a predomi- nantly sensory polyneuropathy was also distinctive and atypical for PD. Anxiety was notable in both sisters and dated back to childhood in Patient 1. This is in- teresting because anxiety is commonly experienced by patients with idiopathic PD and may precede motor symptoms by two decades or more.17 The presenting



*Fig. Electropherogram of the* POLG1 *sequence showing the novel G2491C substitution* (underlined) *resulting in a G737R amino acid change.*

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*Table. Regions of Polg1 Containing Glycine (Wild-Type) or Arginine (Mutated) at Position 737 Are Aligned with Sequences of Polg1 from Other Species*

ARGGPKDTQPSYHHGN**R**PYNDVDIPGCWFFKL [Patients] ARGGPKDTQPSYHHGN**G**PYNDVDIPGCWFFKL [*Homo sapiens*] AAGGPKASQPAYHHGN**G**PYNDVDIPGCWFFKL [*Bos taurus*] AACAPKSSQPTYHHGN**G**PYNDVNIPGCWFFKL [*Mus musculus*] VSRASALGQPAYHHGN**G**PYNDVDIPGCWFFKL [Canis *familiaris*] LELVEESSQPSFHHGN**G**PYNDVNIPGCWFFKL [*Gallus gallus*] SVFKSLNGECPYHHGN**G**PYNDVNIPGCWFFKL [*Danio rerio*]

DGTLPEQSQCHYHHGN**G**PYSDVDVPGCWFFKL [*Tetraodon nigroviridis*]

symptom of dystonic toe curling in both sisters is also common in idiopathic PD. Patient 2 also suffered from depression, which has been associated with PD and with parkinsonism due to *POLG* mutations.9

In contrast, in the absence of PEO, no clinical clues pointed to a mitochondrial disorder, which was sug- gested only by the increased blood lactate in Patient 1 and by the cytochrome *c* oxidase–negative RRFs in the muscle biopsies of both patients. Parkinsonism has been described in a few families with mtDNA muta- tions, including a microdeletion in the cytochrome *b* gene18 and a mutation in the 12S ribosomal RNA gene,19 but is not a common presentation of primary mtDNA-related syndromes.

In contrast, parkinsonism has been reported in sev- eral patients with PEO and multiple mtDNA deletions in muscle due to mutations in *POLG1.* The largest se- ries included seven families from Finland, Sweden, and the United Kingdom, and the clinical picture was char- acterized by PEO, parkinsonism, and premature meno- pause.9 Transmission was autosomal recessive in one family and autosomal dominant in all others. We have also described a US family with autosomal dominant PEO, neuropathy, hypogonadism, and parkinsonism.20 There are several interesting differences between the patients in these families and the two sisters described here. First, all other patients had PEO, which had in- variably preceded the appearance of parkinsonism by several years. Second, the age at onset of parkinsonism ranged from 36 to 75 years in the European families and was 46 years in the US patient, whereas both sis- ters described here started showing parkinsonian symp- toms in their 20’s. Interestingly, the age at onset was younger (36 – 46 years) in the one European family with autosomal recessive inheritance, and those pa- tients were, similar to ours, compound heterozygotes for *POLG* mutations. Third, parkinsonism was part of complex syndromes in previous reports, whereas the only major additional clinical feature in our patients was peripheral neuropathy. As a consequence, these two sisters presented as juvenile PD, leading us to sus- pect *PINK1* mutations before sequencing *POLG,* a step that was motivated by the coexistence of peripheral neuropathy and by presence of RRFs and multiple

mtDNA deletions (albeit detected only by long PCR) in muscle.

We conclude that mutations in *POLG* have to be included in the differential diagnosis of familial early- onset parkinsonism, even in the absence of PEO.

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# Rapid Diagnosis of Glycine Encephalopathy by 13C- Glycine Breath Test

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**Objective:** It is currently problematic to confirm the clinical diagnosis of glycine encephalopathy, requiring either invasive liver biopsy for enzymatic analysis of the glycine cleavage sys- tem or exhaustive mutation analysis. Because the glycine cleav- age system breaks down glycine generating carbon dioxide, we suppose that the glycine cleavage system activity could be eval- uated *in vivo* by measuring exhaled 13CO2 after administration of [1-13C]glycine.

**Methods:** The [1-13C]glycine breath test was performed in 10 control subjects and 5 glycine encephalopathy patients with *GLDC* mutation, including 1 patient with mild glycine encephalopathy.

**Results:** All the patients showed lower 13CO2 excretion than any control subject.

**Interpretation:** Not only typical GE but also atypical GE can be reliably diagnosed by the 13C-glycine breath test. Be- cause it is rapid, non-invasive, and requires little expertise, the breath test could be useful as a standard test for diagnos- ing GE.

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Glycine encephalopathy (GE; MIM 605899), also termed nonketotic hyperglycinemia (NKH), is an in- born error of glycine metabolism caused by deficiency

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