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POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement

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Abstract—Objective: To identify *POLG* mutations in patients with sensory ataxia and CNS features. Methods: The authors characterized clinical, laboratory, and molecular genetic features in eight patients from five European families. The authors conducted sequencing of coding exons of *POLG*, *C10orf2* (Twinkle), and *ANT1* and analyzed muscle mitochondrial DNA (mtDNA), including Southern blot analysis and long-range PCR. Results: Ataxia occurred in combination with various CNS features, including myoclonus, epilepsy, cognitive decline, nystagmus, dysarthria, thalamic and cerebellar white matter lesions on MRI, and neuronal loss in discrete gray nuclei on autopsy. Gastrointestinal dysmotility, weight loss, cardiomyopathy, and valproate-induced hepatotoxicity occurred less frequently. Two patients died without preceding signs of progressive external ophthalmoplegia. In muscle, typical findings of mitochondrial disease, such as ragged red fibers and Southern blot mtDNA abnormalities, were absent. *POLG* mutations were present in eight patients, including two isolated cases, and one Finnish and two unrelated Belgian families contained in total six patients. All *POLG* mutations were recessive, occurring in a homozygous state in seven patients and in a compound heterozygous state in one patient. The novel W748S mutation was identified in five patients from three unrelated families. Conclusions: The clinical spectrum of recessive *POLG* mutations is expanded by sensory ataxic neuropathy, combined with variable features of involvement of CNS and other organs. Progressive external ophthalmoplegia, myopathy, ragged red fibers, and Southern blot abnormalities of muscle mitochondrial DNA also are not mandatory features associated with *POLG* mutations.

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Mitochondrial DNA (mtDNA) maintenance disorders are characterized by somatic depletion¹ or multiple deletions of mtDNA in different, mainly postmitotic tissues.² The majority of disorders characterized by multiple mtDNA deletions present with progressive external ophthalmoplegia (PEO),² but several other phenotypes have been reported.^{3–9} In PEO, four nuclear genes associated with multiple mtDNA deletions have been identified. In autosomal dominant PEO (adPEO; MIM 157640), mutations were demonstrated in the genes *ANT1*,¹⁰ *C10orf2*,¹¹ and *POLG*.¹² In autosomal recessive PEO (arPEO), mutations were identified in *ECGF1*¹³ and *POLG*.¹² *ANT1* (MIM

103220) encodes adenine nucleotide translocator 1. *C10orf2* (MIM 606075) encodes the mitochondrial helicase Twinkle. *POLG* (MIM 174763) encodes DNA polymerase gamma subunit 1 (synonyms, mitochondrial DNA polymerase catalytic subunit or PolG- α [Swiss-Prot ID, P54098; EC 2.7.7.7]). *ECGF1* (MIM 131222) encodes thymidine phosphorylase, and mutations are associated with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE; MIM 603041), an arPEO variant characterized by gastrointestinal dysmotility¹⁴ and leukodystrophy.¹⁵

POLG mutations are the most frequent cause of multiple mtDNA deletions in PEO, both in families with dominant or recessive inheritance¹⁶ and in isolated patients. Isolated patients are most frequently compound heterozygous for two distinct recessive *POLG* mutations.^{17,18}

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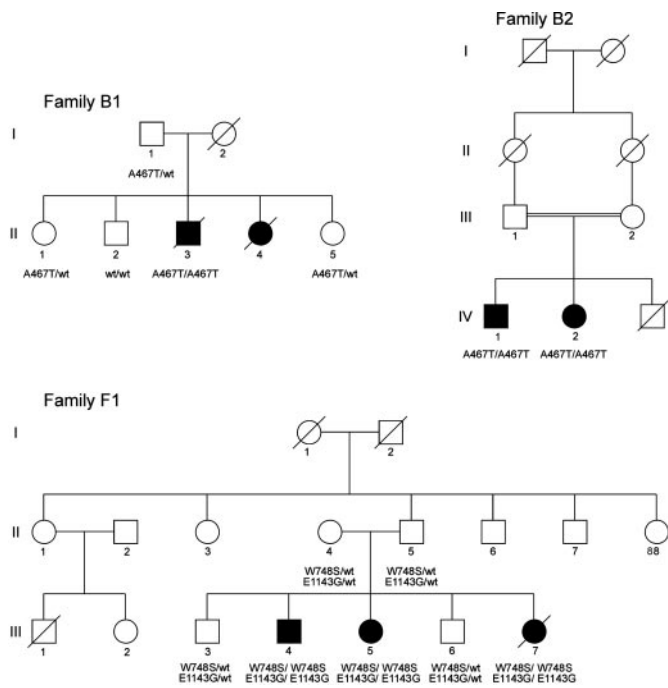


Figure 1. Segregation analysis of *POLG* mutations in Belgian Families B1 and B2 and in Finnish Family F1. Filled symbols represent affected individuals. All available family members were clinically examined, including the deceased affected individual, F1.III.7.

We previously reported that sensory ataxia or other features of sensory neuropathy can precede PEO associated with recessive *POLG* mutations.¹⁹ Here we report *POLG* mutations in patients initially seeking treatment for sensory ataxia or CNS abnormalities and who subsequently do not develop PEO or only discrete and asymptomatic ophthalmoparesis.

Methods. *Clinical investigations.* In seven of eight genotyped patients, clinical examination was recently performed, including testing of strength, tone, muscle bulk, eyelid elevation, and extraocular movements. If possible, video-oculography was performed. Unaffected family members, included in the segregation analyses (figure 1), were also examined. In seven of eight patients, electrophysiologic examination was performed, and the eighth patient had a nerve and muscle biopsy. Muscle biopsy was also performed in four of five probands. Laboratory investigations are presented in the patient reports and listed in table E-2 (available on the *Neurology* Web site at www.neurology.org). Clinical data from previous examinations and findings of deceased Patient B1.II.4 were extracted from hospital records or were collected from a previous study (Family F1).²⁰

DNA analysis. Genomic DNA was isolated from total blood using standard DNA extraction procedures. Mutation analyses were performed using direct sequencing of PCR amplicons of coding exons of *POLG* on an ABI 3100 or ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA).¹² In index patients carrying *POLG* mutations, mutations in *ANT1* and *C10orf2* were subsequently excluded.²¹ One hundred sixty-eight Belgian and 70 Finnish control individuals were examined for the presence of the c.2243G→C transition (W748S) and the c.3428A→G transition (E1143G). We used pyrosequencing²² to examine Belgian control subjects and direct PCR sequencing for Finnish control subjects, including 46 individuals from the genetic isolate of North Karelia in Finland, from which family F1 originated.

Mitochondrial DNA analysis. DNA extraction from muscle biopsy specimens and Southern blot analysis of mtDNA were performed as described previously²³ with linearization of mtDNA by

*Bam*HI or *Pvu*II restriction enzymes and with a fragment of mtDNA (nucleotides 1 to 740) as a hybridization probe. The radioactive signals were detected with the Typhoon 9400 Imager (Amersham Biosciences, Piscataway, NJ). Quantification of mtDNA amounts compared with a nuclear 18S ribosomal RNA gene was done with ImageQuant 5.1 software (Amersham Biosciences) to detect possible mtDNA depletion. The same Southern blots were hybridized with a nuclear 18S ribosomal RNA gene probe. The amount of mtDNA signal on each lane was compared with that of the nuclear gene in two different experiments.

Long-range PCR analysis was performed using a pair of oligonucleotides that primed on 8.3-kb separated regions of mtDNA, namely, at nucleotide positions 8,238 to 8,262 (forward) and 16,496 to 16,465 (reverse). PCR amplification was done using Expand Long PCR long template PCR system (Roche, Basel, Switzerland) according to the manufacturer's instructions, but with 10 ng of total DNA template, with buffer system 1, and either 8 minutes or 3 minutes of extension time. The short extension time selectively amplifies smaller-sized deletion-containing mtDNA fragments, whereas long time also allows amplification of the wild-type template.

As controls, we used diagnostic muscle DNA samples from healthy siblings of patients with *POLG* mutations or *POLG* patients' leukocyte DNA. As controls for PCR reactions, we also used a muscle sample of a patient with a dominant *POLG* mutation (Y955C), known to harbor multiple mtDNA deletions, and a fibroblast DNA sample from a patient with Kearns-Sayre syndrome carrying a single mtDNA deletion in muscle.

Brain autopsy studies. Fixation was performed with formalin according to standard procedures. On frozen sections, cresyl violet staining was used to assess neuronal loss, and the Spielmeyer method was used to observe myelin pallor. On paraffin-embedded sections, H-E staining and cresyl violet staining were performed to study neuronal populations. The Bodian method was used to evaluate axons, and the Klüver-Barrera method was used to observe myelin. Immunohistochemistry was performed using markers of hyperphosphorylated tau (AT8), A β amyloid (4G8), glial fibrillary acidic protein (GFAP), ubiquitin, and the macrophages/microglia marker CD68.

Results. The clinical findings are summarized in table E-1 (available on the *Neurology* Web site at www.neurology.org). Laboratory features are summarized in table E-2. Patient reports are presented in detail below.

Patient reports. Patients B1.II.3 and B1.II.4. At age 18 years, B1.II.3 was evaluated for status epilepticus lasting 8 days, followed by a "Todd paralysis" of the left arm and face. Five years later, he had an acute psychiatric illness, hyperventilation, gastrointestinal symptoms, gait unsteadiness, and disturbed limb coordination. Between 32 and 35 years of age, he lost 13 kg of weight. On examination, he had sensory gait ataxia, limb ataxia, areflexia, generalized dystrophy, and loss of vibration and static joint position sense at the distal lower limbs, severe dysarthria, and a left-sided Babinski sign. Romberg test was positive. On funduscopy, optic disks were pale without pigmentary retinopathy. At age 38 years, he had intestinal pseudo-obstruction, anorexia, and further weight loss. A few weeks later, he developed stupor (Glasgow coma scale, 5/15), hyperventilation, myoclonic jerks, and seizures necessitating intensive care and artificial ventilation. Because of lactic acidosis, sodium valproate was changed to phenytoin and levetiracetam, but seizures and myoclonus persisted. Postpyloric enteric feeding failed because of severe gastroparesis and intestinal pseudo-obstruction, and weight further decreased to 35 kg. Numerous medical complications preceded death at age 39 years. Blepharoptosis and ophthalmoparesis were never noted.

From onset, EEGs repeatedly showed slowed background activity. Nerve conduction studies and EMG, first performed at age 34 years, were consistent with a predom-

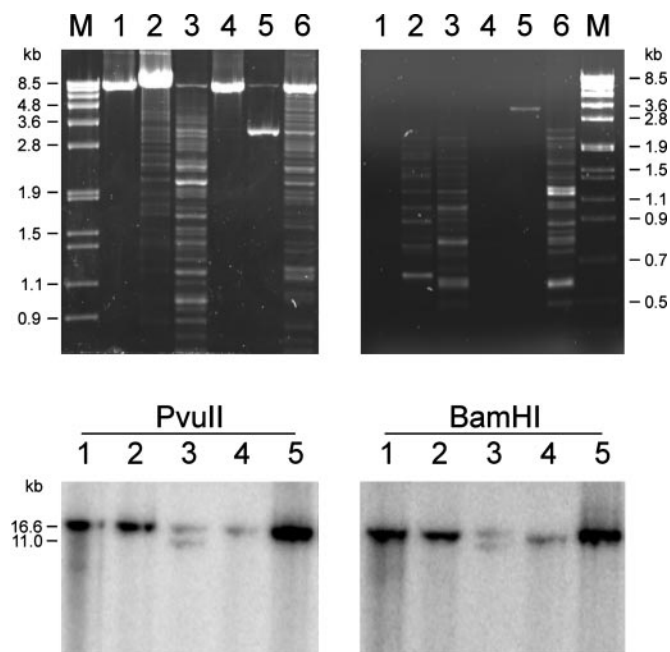


Figure 2. Mitochondrial DNA analysis. Top, PCR analysis. Mitochondrial DNA (mtDNA) fragment between the mtDNA nucleotides 8.2 kb and 16.5 kb was amplified from total muscle DNA. Left, 8-minute PCR extension time, allowing amplification of full-length fragments. Single product of 8.3 kb, from wild-type mtDNA is present in lane 1, leukocyte DNA of Patient F2.II.1, and in lane 4, loaded with DNA from 58-year-old control muscle. Additional products corresponding to multiple deleted mtDNA molecules can be seen in lanes 2 and 3 of two ataxia patients (lane 2, F1.III.7; lane 3, B1.II.3), and in lane 6, muscle DNA of a multiple-deletion control progressive external ophthalmoplegia (PEO) patient with a dominant Y955C mutation in POLG. Lane 5, as a second disease control, shows a single mutant product from an isolated patient harboring a single 5-kb mtDNA deletion. M denotes marker VII (Roche Applied Sciences, Penzberg, Germany); kb denotes the kilobase sizes of marker fragments. Right, Same PCR as on the left, but with 3-minute extension time, favoring the amplification of short mutant mtDNA. Multiple products corresponding to mtDNA deletions are present in samples of F1.III.7 and B1.II.3 (lanes 2 and 3), as well as the multiple mtDNA deletion control Y955C POLG mutant (lane 6). Only the mutant molecule containing a single deletion can be seen in lane 5. No amplification product was obtained from leukocytes of ataxia Patient F2.II.1 or from the healthy control muscle DNA. Bottom, Southern hybridization analysis of mtDNA. Samples: lane 1, muscle DNA of Patient B1.II.3; lane 2, muscle DNA of Patient F1.III.7; lane 3, fibroblast DNA of a disease control individual with a single mtDNA deletion; lane 4, control lymphoblast DNA sample; lane 5, control myoblast DNA sample; kb, denoting kilobases, is the molecular size marker. As indicated, total DNA was digested with either PvuII or BamHI restriction enzymes, linearizing mtDNA. The blot was hybridized with a short cloned mtDNA fragment (mtDNA nucleotides 1 to 740). In lane 1, a faint "smear" signal is present below the wild-type mtDNA signal. In lanes 2, 4, and 5, just wild-type mtDNA signal is present. Lane 3 shows two signals: one from wild-type mtDNA and one from mtDNA molecule containing a 5-kb single deletion.

inant sensory axonal peripheral neuropathy. On several occasions, CSF analysis revealed elevated protein at 129 to 183 mg/dL but normal lactate. Total and unconjugated serum bilirubin levels were slightly elevated, but liver enzymes were always normal. Serum lactate was initially normal but was elevated at 8.1 mmol/L (normal values < 2.0) with sodium valproate treatment. Creatine kinase (CK) was mildly elevated at 342 U/L (normal values < 170). Brain MRI remained normal, even at age 39 years, and SPECT was normal at age 34 years. Echocardiography demonstrated dilated cardiomyopathy. There was no hearing loss on audiometry. At age 34 years, a muscle biopsy was performed, and succinate dehydrogenase (SDH) stain showed no abnormalities. However, electron microscopy demonstrated some fibers with subsarcolemmal accumulation of mitochondria containing paracrystalline inclusions. Diagnostic mtDNA analysis revealed no pathogenic mtDNA point mutations.²⁴ There was a faint smear but no visible deletions on Southern analysis of muscle mtDNA (figure 2).

The sister (Patient B1.II.4) was examined at age 17 years for an acute encephalopathy, myoclonus, partial epileptic seizures, and left hemianopsia, followed by cortical blindness, stupor, Cheyne–Stokes respiration pattern, and sudden death. DNA of this patient was not available for molecular genetic studies. Three siblings appeared unaffected on clinical examination at ages 43, 41, and 33 years, as well as the father (age 67 years). The mother, who died before this study was performed, was reportedly unaffected. The pedigree is presented in figure 1.

Patients B2.IV.1 and B2.IV.2. The patients are a 49-year-old Belgian man and his 47-year-old sister. Their unaffected younger brother died at age 38 years from leishmaniasis. Paternal and maternal grandmothers were sisters (see figure 1). Clinical features were almost identical in both siblings (see tables E-1 and E-2 on the *Neurology* Web site), but the sister (B2.IV.2) was more severely affected. From adolescence, both patients experienced gradually increasing gait unsteadiness, which was worse in the dark. In her fourth decade, the sister had frequent falls, and she became wheelchair dependent in the fifth decade. The brother had mild cognitive decline in the fifth decade. He underwent cataract surgery. Both siblings had an ataxic gait. Heel-to-shin testing was more ataxic than finger-to-nose testing, which markedly deteriorated with eye closure. Deep tendon reflexes were absent. Vibration and position sense were absent at the lower limbs early on, whereas light touch sensation was decreased at a later stage. Both had pes cavus. Dysarthria was only present in the sister. There was no blepharoptosis. Mild and asymptomatic upward gaze paresis appeared at age 48 years in Patient B2.IV.1, whereas in Patient B.IV.2, from age 40 years, smooth pursuit ocular movements became saccadic, and there was a horizontal and vertical gaze-evoked nystagmus. Muscle strength was always preserved. Electrophysiologic examinations in both patients showed signs of axonal generalized peripheral neuropathy, predominantly involving sensory nerves. Needle EMG was normal (B2.IV.2) or showed minor neurogenic abnormalities in distal lower-extremity muscles (B2.IV.1). Visual evoked potential (VEP) and electroretinography (ERG) were normal (B2.IV.2). Oculography confirmed gaze-evoked nystagmus and saccadic pursuit movements and was suggestive of

brainstem dysfunction (B2.IV.2; age 40 years). Brain MRI showed symmetric cerebellar white matter lesions similar to those observed in Family F1²⁰ (B2.IV.2; age 49 years).

Patient UK1.II.1. This patient of British origin noticed minor tremor of the hands from age 12 years. In his late 20s, he experienced tingling in both hands, followed by tingling in the legs. In the early fourth decade, he became unsteady at walking, especially in the dark. From his early 40s, his speech became slurred. When relaxed, he noticed abnormal movements of the toes. His son died suddenly at age 30 years, without obvious cause.

On examination at age 52 years, he had an ataxic gait, and Romberg test was positive. There was dysarthria and bilateral ophthalmoparesis. In the limbs, muscle tone and strength were normal. There was generalized areflexia. Pinprick sensation was reduced in a stocking distribution; proprioception was reduced bilaterally to the ankle; and vibration sensation was reduced bilaterally to the iliac crests.

Brain MRI showed minor atrophy. CSF protein was 75 mg/dL. Sural nerve biopsy showed marked decrease of myelinated fibers of all diameters but no active demyelination. Pathologic findings were consistent with axonal neuropathy. Muscle biopsy, performed at age 52 years, showed no abnormalities on histochemical staining for oxidative enzymes.

Patients F1.III.4, 5, and 7. The pedigree is presented in figure 1. The detailed clinical and laboratory features of these patients of Finnish (Karelian) origin have been reported previously.²⁰ Briefly, they had a combination of adult-onset progressive ataxia with severe gait ataxia, dysarthria, mild cognitive impairment, and, in the case of Patients F1.III.4 and F1.III.7, epilepsy. Patient F1.III.7 unexpectedly died at age 36 years. She had no ophthalmoparesis, but her two affected siblings developed mild upward gaze paresis in the fifth decade. Blepharoptosis was absent on the original study in all three patients.²⁰ Electrophysiology was consistent with a predominantly sensory axonal neuropathy. Brain MRI showed symmetric thalamic lesions and white matter changes in the cerebellum. Southern analysis on muscle DNA had not revealed large mtDNA deletions (see figure 2), and pathogenic mtDNA point mutations were not observed.²⁰ After we detected *POLG* mutations in this pedigree, the authors re-examined the two surviving patients, F1.III.4 and F1.III.5, with particular attention to external eye muscle function and other muscular features. In both patients, mild blepharoptosis and ophthalmoparesis had now appeared between ages 46 and 48 years. Patient F1.III.4 also had mild diffuse muscle weakness and atrophy, particularly at distal lower limbs. In both patients, ataxia of gait and stance had progressed, necessitating help or a walker. At age 47 years, needle EMG and nerve conduction velocity studies were consistent with moderate to severe axonal sensorimotor neuropathy, whereas CK was normal (Patient F1.III.4).

Patient F2.II.1. This 33-year-old Finnish man is one of three children from unrelated parents originating from North Karelia and Central Lapland. He was not closely related to Family F1. He reported balance problems in two maternal second cousins. He had plurimetabolic syndrome. At age 30 years, he noted disturbed balance, and ataxia and severe axonal neuropathy were diagnosed. On examination at age 32 years, he had slowed ocular pursuit movements but with full range of motion. There were

spontaneous slow lateral eye movements but no nystagmus. He had no blepharoptosis. He had severe gait ataxia, imbalance, and trunk ataxia, as well as clumsiness of the hands. Achilles tendon reflexes were absent. Vibration sensation was decreased at the lower limbs, and pain and touch sensations were decreased in a stocking-like distribution to knee level at the lower extremities. His speech was dysarthric. He was obese. Brain MRI showed bilateral symmetric intense signals in the cerebellar white matter on T2-weighted images. Electrophysiology revealed severe sensory and moderate chronic motor axonopathy at the lower limbs and moderate sensory neuropathy at the upper limbs. Muscle biopsy showed mild neurogenic atrophy, no ragged red fibers, and 1:100s cytochrome c oxidase-negative fibers. Analysis of muscle mtDNA revealed no pathogenic mutations.²⁴ Biochemical analysis of respiratory chain function was normal.

Morphologic studies and autopsy findings. In muscle biopsy, no findings suggestive of mitochondrial disease were detected on light microscopy, including histochemical SDH and cytochrome c oxidase stains (Patients B.II.3, UK1.II.1, F1.III.7, and F.II.2.1).

On autopsy of Patient B1.II.3, cerebellum and cerebral hemispheres were preserved. All gray nuclei appeared paler than normal, particularly in the substantia nigra and locus ceruleus. Unfortunately, because of incomplete autopsy, the peripheral nerves, dorsal root ganglia, spinal cord, medulla oblongata, and lower part of the pons were unavailable for this study. Frozen material was not available for histochemical studies and DNA extraction.²⁵

On light microscopy, optic nerves and cerebral and cerebellar cortex were normal. Caudate nucleus, putamen, and pallidum were normal. In the thalamus, there was mild neuronal loss in some nuclei belonging to the lateral formation and in the magnocellular part of the centromedian nucleus, whereas the large association nuclei (pulvinar and dorsomedial nucleus) were normal. Neuronal loss was present, most prominent in the subthalamic nucleus and in the cerebellar dentate nucleus (figure 3). The locus ceruleus was also affected, whereas the compact zone of the substantia nigra was barely affected. Gliosis, strongly reactive to GFAP antibodies, was present in the white matter, particularly at the level of the subcortical U fibers and in subependymal and subpial regions. Myelin was normally stained. Moderate amounts of CD68⁺ cells were present in the perivascular spaces in the white matter.

Genetic findings. *POLG* analysis. In all eight patients, we sequenced *POLG* because of the presence of a sensory ataxic neuropathy. Mutations in *POLG* are presented in the table.

Four Finnish ataxia patients (F1.III.4, 5, and 7 and F2.II.1) carried a single base change in *POLG*, namely, c.2243G→C, predicting a W748S substitution. This mutation occurred in the homozygous state in all patients and in the heterozygous state in unaffected siblings and parents of Family F1 (see figure 1). The British ataxia patient was compound heterozygous for W748S and for the previously reported A467T mutation.¹² Interestingly, in addition to the W748S mutation, all four Finnish patients and the British patient also carried the previously reported c.3428A→G mutation, predicting an E1143G substitution (<http://www.ncbi.nlm.nih.gov/SNP/>). E1143G occurred in the homozygous state in the Finnish patients and in the

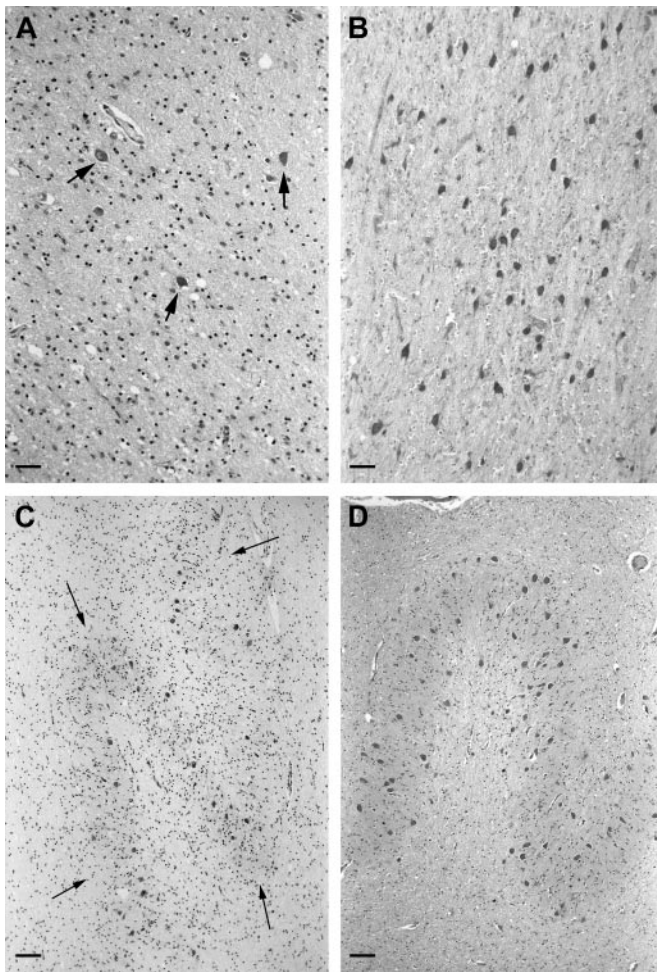


Figure 3. Neuropathology. (A) Subthalamic nucleus of Patient B1.II.3: severe neuronal losses and astrocytic gliosis. A few neurons are remaining, some of which are arrowed. (B) Subthalamic nucleus of control individual. (C) Cerebellar nucleus dentatus of Patient B1.II.3: neuronal losses and astrocytic gliosis. The thin arrows point to the curves of the dentate nucleus, and similar curves can easily be seen in the control nucleus (D). (D) Cerebellar nucleus dentatus of control individual (formalin fixation, paraffin slides, cresyl violet staining; scale bar = 100 μ m).

heterozygous state in the British patient. W748S was absent in 168 Belgian and 70 Finnish control subjects, of which 46 were of North Karelian origin. Eleven of 168 Belgian and 3 of 70 Finnish control subjects were heterozygous for E1143G, and control subjects homozygous for E1143G were not encountered. This implies allele G frequencies of 3.3% (11/336) in the Belgian population and of 2.1% (3/140) in the Finnish population. One North Karelian control subject carried E1143G, corresponding with a 1.0% (1/92) allele G frequency in the genetic isolate.

Three Belgian patients were homozygous for the previously reported c.1399G→A mutation, predicting an A467T substitution.¹² The isolated British patient UK1.II.1 was heterozygous for A467T.

Mutations in *ANT1* and *C10orf2* were absent in four index patients (not tested on isolated Patient F2.II.1).

Mitochondrial DNA analysis. Southern blot hybridization of total muscle DNA showed the 16.5-kb signal de-

Table *POLG* mutations causing ataxia

Patients	Origin	Occurrence	<i>POLG</i> mutations	Amino acid changes
B1.II.3	Belgian	Familial	Homozygous c.1399G→A	A467T/A467T
B2.IV.1 and 2	Belgian	Familial	Homozygous c.1399G→A	A467T/A467T
UK1.II.1	British	Isolated	Heterozygous c.1399G→A c.2243G→C c.3428A→G	wt/A467T W748S/wt E1143G/wt
F1.III.4, 5, and 7	Finnish	Familial	Homozygous c.2243G→C c.3428A→G	W748S/W748S E1143G/E1143G
F2.II.1	Finnish	Isolated	Homozygous c.2243G→C c.3428A→G	W748S/W748S E1143G/E1143G

rived from normal mtDNA in control samples, in muscle of Patient F1.III.7, and in leukocytes of Patient F2.II.1. Long-range PCR, which selectively amplified short deleted molecules, revealed clearly different-sized products in muscle biopsy samples of Patients B1.II.3 and F1.III.7, whereas control subjects only showed the 16.5-kb mtDNA fragment or no PCR product (see figure 2). mtDNA depletion was not present in any of the patients.

Discussion. We report a neurodegenerative phenotype of recessive *POLG* mutations. We present eight patients, from five unrelated families, with adult- or juvenile-onset sensory ataxic neuropathy in combination with various CNS abnormalities. Notably, there was no apparent skeletal muscle involvement, and on muscle biopsy samples, there were no signs of mitochondrial disease on morphologic, biochemical, or routine mtDNA analyses.

The presenting and major feature in all patients was ataxia caused by axonal sensory neuropathy. Ataxia occurred in combination with CNS abnormalities, including myoclonus, seizures, transient hemiparesis, cognitive decline, dysarthria, and nystagmus. In some patients, MRI showed lesions in the thalamus and/or cerebellar white matter. Brain autopsy findings, unfortunately incomplete for Patient B1.II.3, were not typical of primary mtDNA disorders but showed prominent neuronal loss in gray nuclei, including the subthalamic and cerebellar dentate nucleus.²⁶ For Patient F1.III.7, a more complete autopsy had also shown atrophy in dorsal root ganglia, posterior columns, and, to a lesser extent, in posterior spinocerebellar and corticospinal tracts. There was neuronal loss in the dorsal nucleus (Clarke column), and, contrary to Patient B1.II.3, in the pulvinar and the dorsomedial thalamic nuclei. There was also degeneration of inferior olives.²⁰ The involvement pattern differs from other spinocerebellar ataxias but resembles findings in system atrophies.²⁷

Reports on patients with *POLG* mutations have mentioned associated CNS features and neuropathy in some instances.^{16,19,28-30} However, PEO was a con-

sistent feature, except for one teenager with myoclonus.²⁹ In our present study, two of eight patients died at ages 36 and 39 years without extraocular muscle involvement. In the other six patients, PEO was absent at clinical presentation, although minor signs of incipient PEO appeared in a late disease stage in five. Hence, PEO represents merely one variant of the clinical phenotypic expression of *POLG* mutations; therefore, *POLG* could be designated as an “ataxia gene” and a “PEO gene.”

One isolated patient had the clinical triad of sensory ataxic neuropathy, dysarthria, and ophthalmoplegia (SANDO), previously claimed as a unique mitochondrial disorder.³¹ Further studies are necessary to test the hypothesis that SANDO could be a specific phenotype of mutations in *POLG*.¹⁹

The large differences in phenotypic expression of recessive *POLG* mutations are puzzling. Although PEO and sensory ataxia are common, no single clinical feature is shared by all patients.³² Differences between our own series and those reported by others^{16-18,33} could be attributed to clinical selection bias. Several speculations could be made about molecular causes of the observed clinical heterogeneity. Data on familial patients suggest that clinical variability is larger between families than within families (e.g., Families B1 and B2 in this article and the family in Van Goethem et al.,²⁹ which all contain individuals homozygous for A467T). In our view, the contribution of the genetic background would explain best the observed clinical similarities in siblings.

In view of this considerable heterogeneity, what are the clinical clues to mutated *POLG*? Clinical features are confined to energy-demanding postmitotic tissues, with the exception of the liver. Hepatocytes have a low mitotic index, and they may become affected with sodium valproate treatment (e.g., Patient B1.II.3 and Van Goethem et al.²⁹). Because substantial tissue accumulation of mtDNA deletions putatively causes pathology,³⁴ the absence of muscle mtDNA deletions on diagnostic Southern analysis correlates with the absence of clinical and morphologic abnormalities in the biopsied muscle. However, long-range PCR did show low levels of multiple mtDNA deletions in muscle, suggesting that the proportion of deleted mtDNA in muscle can be estimated as <5%.

In all patients (two isolated patients and six patients belonging to three unrelated families), we demonstrated recessive missense mutations in *POLG*. Seven patients (six familial, one isolated) carried homozygous mutations. One isolated patient was compound heterozygous. A467T is a known mutation,¹² whereas W748S is a novel pathogenic mutation, and E1143G is most likely a low frequency polymorphism. W748S and E1143 always occurred together (five patients from three unrelated families). In four Finnish patients, the novel W748S mutation occurred in the homozygous state, and in a British patient, it occurred in the compound heterozygous state with A467T. In Family F1, segrega-

tion analysis indicated recessive inheritance. Interestingly, all five W748S patients carried a second missense mutation, namely, E1143G. E1143G was previously designated as a polymorphism because of its high frequency (2.9%) in a North American control population (<http://www.ncbi.nlm.nih.gov/SNP/>). We measured a comparable frequency in the Belgian and Finnish control population. Control individuals homozygous for E1143G were not encountered. In Families F1 and F2, haplotype analysis using five informative polymorphic DNA markers surrounding *POLG*²⁹ revealed a common haplotype suggestive of a common ancestral origin of the W748S/E1143G alleles (data not shown). W748S and E1143G alter evolutionary conserved amino acid residues. Secondary structure prediction (program PHDsec, EMBL, Heidelberg, Germany) suggests that W748 is one residue out of a highly conserved block of six amino acids forming a β -sheet in the spacer region of the enzyme. This so-called “palm” region is presumably involved in primer-template interaction of the DNA polymerase.^{35,36} The contribution of E1143G to the phenotype remains unclear. Because E1143G accompanied W748S in three of three unrelated families, we cannot conclude that W748S alone is sufficient to cause disease in homozygous or compound heterozygous patients. This situation parallels the finding of *POLG* mutations T251I and P587L in cis in arPEO patients of different ethnic origins.^{18,28} Such data may suggest the concept of a recessive haplotype.³⁷ Therefore, segregation analysis of novel low-frequency mutations remains crucial for the determination of their pathogenic nature because in vivo functional studies on DNA polymerase gamma are still unavailable, and phenotypic expression is absent in patients’ mitotic cells, including cultured fibroblasts, lymphocytes, or myoblasts.

The current study presents three novel patients homozygous for A467T, which is common to Belgian arPEO patients³² and originates from a common ancestral chromosome in the Belgian population.²⁹ This mutation is also retrieved in the compound heterozygous state in a patient of British origin, and previously it was reported in an Italian PEO patient.¹⁷ We had predicted a homozygous appearance of A467T in 36×10^{-6} Belgian individuals.¹² The present findings support our suggestion that a subgroup of patients homozygous for A467T could have clinical features different from PEO.²⁹

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References

1. Moraes CT, Shanske S, Tritschler HJ, et al. mtDNA depletion with variable tissue expression: a novel genetic abnormality in mitochondrial diseases. *Am J Hum Genet* 1991;48:492-501.
2. Zeviani M, Servidei S, Gellera C, Bertini E, DiMauro S, DiDonato S. An autosomal dominant disorder with multiple deletions of mitochondrial DNA starting at the D-loop region. *Nature* 1989;339:309-311.
3. Yuzaki M, Ohkoshi N, Kanazawa I, Kagawa Y, Ohta S. Multiple deletions in mitochondrial DNA at direct repeats of non-D-loop regions in cases of familial mitochondrial myopathy. *Biochem Biophys Res Commun* 1989;164:1352-1357.
4. Cormier V, Rotig A, Tardieu M, Colonna M, Saudubray JM, Munnich A. Autosomal dominant deletions of the mitochondrial genome in a case of progressive encephalomyopathy. *Am J Hum Genet* 1991;48:643-648.
5. Ohno K, Tanaka M, Sahashi K, et al. Mitochondrial DNA deletions in inherited recurrent myoglobinuria. *Ann Neurol* 1991;29:364-369.
6. Suomalainen A, Paetau A, Leinonen H, Majander A, Peltonen L, Somer H. Inherited idiopathic dilated cardiomyopathy with multiple deletions of mitochondrial DNA. *Lancet* 1992;340:1319-1320.
7. Bohlega S, Tanji K, Santorelli FM, Hirano M, al Jishi A, DiMauro S. Multiple mitochondrial DNA deletions associated with autosomal recessive ophthalmoplegia and severe cardiomyopathy. *Neurology* 1996;46:1329-1334.
8. Blumenthal DT, Shanske S, Schochet SS, et al. Myoclonus epilepsy with ragged red fibers and multiple mtDNA deletions. *Neurology* 1998;50:524-525.
9. Paul R, Desnuelle C, Pouget J, et al. Importance of searching for associated mitochondrial DNA alterations in patients with multiple deletions. *Eur J Hum Genet* 2000;8:331-338.
10. Kaukonen J, Juselius JK, Tiranti V, et al. Role of adenine nucleotide translocator 1 in mtDNA maintenance. *Science* 2000;289:782-785.
11. Spelbrink JN, Li FY, Tiranti V, et al. Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat Genet* 2001;28:223-231.
12. 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.
13. Nishino I, Spinazzola A, Hirano M. Thymidine phosphorylase gene mutations in MNGIE, a human mitochondrial disorder. *Science* 1999;283:689-692.
14. Hirano M, Silvestri G, Blake DM, et al. Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): clinical, biochemical, and genetic features of an autosomal recessive mitochondrial disorder. *Neurology* 1994;44:721-727.
15. Nishino I, Spinazzola A, Papadimitriou A, et al. Mitochondrial neurogastrointestinal encephalomyopathy: an autosomal recessive disorder due to thymidine phosphorylase mutations. *Ann Neurol* 2000;47:792-800.
16. Lamantea E, Tiranti V, Bordoni A, et al. Mutations of mitochondrial DNA polymerase gammaA are a frequent cause of autosomal dominant or recessive progressive external ophthalmoplegia. *Ann Neurol* 2002;52:211-219.
17. Agostino A, Valletta L, Chinnery PF, et al. Mutations of ANT1, Twinkle, and POLG1 in sporadic progressive external ophthalmoplegia (PEO). *Neurology* 2003;60:1354-1356.
18. Di Fonzo A, Bordoni A, Crimi M, et al. POLG mutations in sporadic mitochondrial disorders with multiple mtDNA deletions. *Hum Mutat* 2003;22:498-499.
19. Van Goethem G, Martin JJ, Dermaut B, et al. Recessive POLG mutations presenting with sensory and ataxic neuropathy in compound heterozygote patients with progressive external ophthalmoplegia. *Neuromuscul Disord* 2003;13:133-142.
20. Rantamaki M, Krahe R, Paetau A, Cormand B, Mononen I, Udd B. Adult-onset autosomal recessive ataxia with thalamic lesions in a Finnish family. *Neurology* 2001;57:1043-1049.
21. Van Goethem G, Lofgren A, Dermaut B, Ceuterick C, Martin JJ, Van Broeckhoven C. Digenic progressive external ophthalmoplegia in a sporadic patient: recessive mutations in POLG and C10orf2/Twinkle. *Hum Mutat* 2003;22:175-176.
22. Alderborn A, Kristofferson A, Hammerling U. Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing. *Genome Res* 2000;10:1249-1258.
23. Suomalainen A, Majander A, Haltia M, et al. Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. *J Clin Invest* 1992;90:61-66.
24. Kogelnik AM, Lott MT, Brown MD, Navathe SB, Wallace DC. MITO-MAP: a human mitochondrial genome database. *Nucleic Acids Res* 1996;24:177-179.
25. Cottrell DA, Ince PG, Blakely EL, et al. Neuropathological and histochemical changes in a multiple mitochondrial DNA deletion disorder. *J Neuropathol Exp Neurol* 2000;59:621-627.
26. Sparaco M, Bonilla E, DiMauro S, Powers JM. Neuropathology of mitochondrial encephalomyopathies due to mitochondrial DNA defects. *J Neuropathol Exp Neurol* 1993;52:1-10.
27. Martin JJ. Thalamic degenerations. In: de Jong JMBV, ed. *Handbook of Clinical Neurology*, vol. 16/60, Hereditary Neuropathies and Spinocerebellar Atrophies. Amsterdam: Elsevier Science Publishers, 1991:701-715.
28. Van Goethem G, Schwartz M, Lofgren A, Dermaut B, Van Broeckhoven C, Vissing J. Novel POLG mutations in progressive external ophthalmoplegia mimicking mitochondrial neurogastrointestinal encephalomyopathy. *Eur J Hum Genet* 2003;11:547-549.
29. Van Goethem G, Mercelis R, Lofgren A, et al. Patient homozygous for a recessive POLG mutation presents with features of MERRF. *Neurology* 2003;61:1811-1813.
30. Van Goethem G, Martin JJ, Lofgren A, et al. Unusual presentation and clinical variability in Belgian pedigrees with progressive external ophthalmoplegia and multiple deletions of mitochondrial DNA. *Eur J Neurol* 1997;4:476-484.
31. Fadic R, Russell JA, Vedanarayanan VV, Lehar M, Kuncl RW, Johns DR. Sensory ataxic neuropathy as the presenting feature of a novel mitochondrial disease. *Neurology* 1997;49:239-245.
32. Van Goethem G, Martin JJ, Van Broeckhoven C. Progressive external ophthalmoplegia characterized by multiple deletions of mitochondrial DNA: unraveling the pathogenesis of human mitochondrial DNA instability and the initiation of a genetic classification. *Neuromolecular Med* 2003;3:129-146.
33. Filosto M, Mancuso M, Nishigaki Y, et al. Clinical and genetic heterogeneity in progressive external ophthalmoplegia due to mutations in polymerase gamma. *Arch Neurol* 2003;60:1279-1284.
34. Moraes CT, Ricci E, Petruzzella V, et al. Molecular analysis of the muscle pathology associated with mitochondrial DNA deletions. *Nat Genet* 1992;1:359-367.
35. Ye F, Carrodegua JA, Bogenhagen DF. The gamma subfamily of DNA polymerases: cloning of a developmentally regulated cDNA encoding *Xenopus laevis* mitochondrial DNA polymerase gamma. *Nucleic Acids Res* 1996;24:1481-1488.
36. Kaguni LS, Clayton DA. Template-directed pausing in in vitro DNA synthesis by DNA polymerase α from *Drosophila melanogaster* embryos. *Proc Natl Acad Sci USA* 1982;79:983-987.
37. Parton MJ, Broom W, Andersen PM, et al. D90A-SOD1 mediated amyotrophic lateral sclerosis: a single founder for all cases with evidence for a Cis-acting disease modifier in the recessive haplotype. *Hum Mutat* 2002;20:473.

***POLG* mutations in neurodegenerative disorders with ataxia but no muscle involvement**

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