OBSERVATION

POLG1 Mutations Manifesting as Autosomal Recessive Axonal Charcot-Marie-Tooth Disease

Timothy Harrower, MRCP; Joanna D. Stewart, BSc; Gavin Hudson, PhD; Henry Houlden, MRCP(UK); Graham Warner, MRCP; Dominic G. O'Donovan, FRCPath; Leslie J. Findlay, FRCP; Robert W. Taylor, PhD; Rajith De Silva, FRCP; Patrick F. Chinnery, PhD, FRCP

Background: Although a molecular diagnosis is possible in most patients having Charcot-Marie-Tooth disease (CMT), recessively inherited and axonal neuropathies still present a diagnostic challenge.

Objective: To determine the cause of axonal CMT type 2 in 3 siblings.

Design: Case report.

Setting: Academic research.

Participants: Three siblings who subsequently developed profound cerebellar ataxia.

Main Outcome Measures: Muscle biopsy specimen molecular genetic analysis of the *POLG1* (polymerase γ-1) gene, as well as screening of control subjects for *POLG1* sequence variants.

Results: Cytochrome *c* oxidase deficient fibers and multiple deletions of mitochondrial DNA were detected in skeletal muscle. Three compound heterozygous substitutions were detected in *POLG1*.

Conclusion: Even in the absence of classic features of mitochondrial disease, *POLG1* should be considered in patients having axonal CMT that may be associated with tremor or ataxia.

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Author Affiliations:
Departments of Neurology (Drs Harrower, O'Donovan, Findlay, and De Silva) and Neuropathology (Dr O'Donovan), Essex Centre for Neurological Sciences, Queen's Hospital, Romford; Mitochondrial Research Group, The Medical School (Drs Hudson, Taylor, and Chinnery and Ms Stewart), and Institute of Human Genetics (Drs Taylor and Chinnery), Newcastle University, Newcastle upon Tyne; Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London (Dr Houlden); and Department of Neurology, Royal Surrey County Hospital, Guildford (Dr Warner); England.

THE PAST DECADE HAS SEEN major advances in our understanding of the causes of inherited neuropathies. Duplication of the *PMP22* (peripheral myelin protein 22) gene and point mutations in *GJB1* (gap junction protein, beta 1) and *MPZ* (peripheral myelin protein zero) account for approximately 85% of unselected cases, but a growing number of genes have been implicated in the remaining 15% of patients, affecting a diverse group of cellular processes ranging from axonal transport to DNA repair.[1] The central role of mitochondria in maintaining peripheral nerve integrity is an emerging theme, as illustrated by pathogenic mutations in *MFN2* (mitofusin 2) and *DNM2* (dynamin 2) that disrupt organelle fusion and fission.[1] Although axonal neuropathies are well recognized in patients having classic multisystem mitochondrial disease, isolated neuropathy is uncommon.[2] Herein, we describe a family manifesting an autosomal recessive axonal sensorimotor neuropathy that dominated the clinical phenotype for more than 2 decades. This was due to compound heterozygous mutations in the *POLG1* gene, which codes for mitochondrial DNA (mtDNA) polymerase γ.

REPORT OF A CASE

A 35-year-old man had manifested abnormal gait and pes cavus at age 10 years. When initially seen at age 22 years, his main symptoms were reduced dexterity and sensory disturbance affecting his face, hands, and feet. Findings on physical examination disclosed that he had marked bilateral distal weakness and wasting of the small muscles of the forearms, hands, calves, and feet. His proximal power was normal. There was clawing of the toes on the left foot and minimal tremor of the outstretched hands. Joint position and vibration sense were lost in the toes of both feet. Nerve conduction studies revealed an axonal sensorimotor neuropathy consistent with Charcot-Marie-Tooth disease type 2 (CMT2) (**Table**). He subsequently developed progressive, predominantly distal muscle wasting and a postural tremor at age 33 years, followed by dysarthria.

Video available online at www.archneurol.com

Neurological examination at age 35 years (a video is available at http://www.archneurol.com) disclosed that he had a

Table. Peripheral Neurophysiological Findings in the Proband and in His Affected Brother

Variable	II-1 at Age 21 y	II-2 at Age 34 y
Arm		
Right sensory		
Median (F2-wrist)	Absent	Absent
Ulnar (F5-wrist)	Absent	Absent
Radial (forearm-wrist)	NA	Absent
Sural (calf-ankle)	Absent	Absent
Superior peroneal (calf-ankle)	NA	Absent
Motor		
Right median (SE on abductor pollicis brevis) DML, ms	3.2	4.2
CV (wrist-elbow), m/s	58	50
MAP, mV		
Wrist	4.2	4.7
Elbow	NA	4.0
F latency, ms	NA	31
Right ulnar (SE on abductor digiti minimi) DML, ms	2.6	3.5
CV (wrist–above elbow), m/s	61	64
MAP, mV		
Wrist	3.2 Broadened MAP	4.5
Above elbow	2.8 Broadened MAP	4.0
Leg		
Right sensory		
Common peroneal (SE on extensor digitorum brevis) DML	NA	Absent
Right posterior tibial (SE on abductor hallucis) DML, mV	6.5	Absent
Motor		
Right common peroneal (SE on tibialis anterior) DML, ms	N/A	3.4
CV (popliteal fossa–fibular neck), m/s	42 At posterior tibial	56
MAP, mV		
Fibular neck	NA	1.2
Popliteal fossa	NA	1.3

Abbreviations: CV, conduction velocity, DML, distal motor latency; MAP, muscle action potential, NA, not available; SE, stimulating electrode.

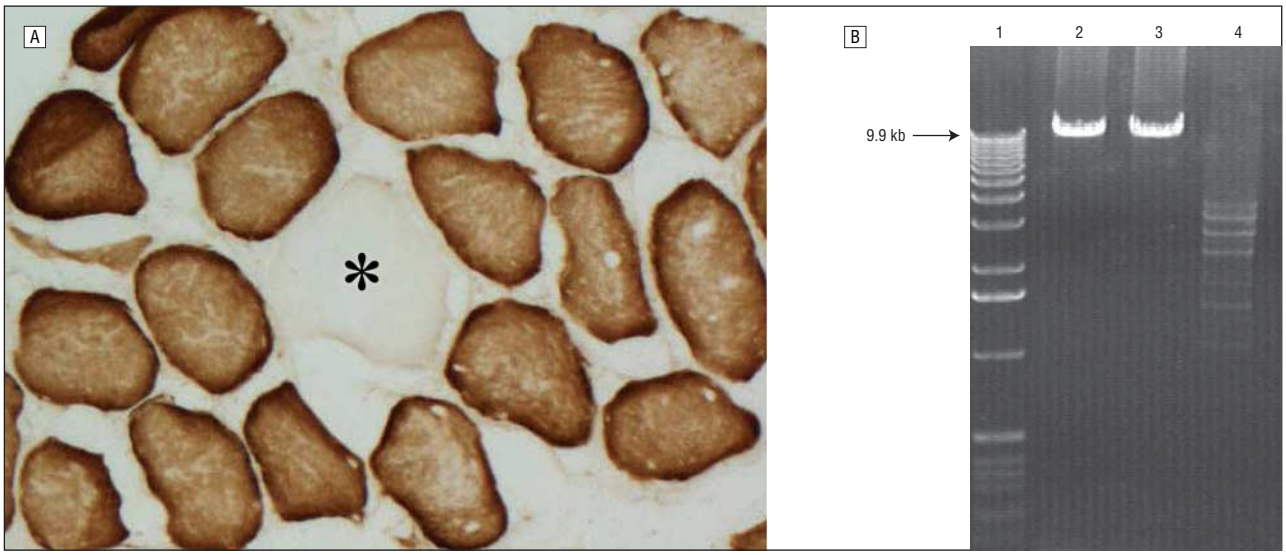

Figure 1. Skeletal muscle histochemistry and mitochondrial DNA (mtDNA) analysis in the proband. A, Histochemical demonstration of cytochrome *c* oxidase activity in muscle highlighting a cytochrome *c* oxidase–deficient muscle fiber (asterisk) among fibers with normal enzyme activity. B, Long-range polymerase chain reaction of skeletal muscle DNA amplifying a 9.9-kilobase (kb) fragment across the major mtDNA arc (nucleotide positions 6249-16215). Lane 1 indicates DNA size marker; lanes 2 and 3, control muscle; and lane 4, muscle from the proband showing multiple mtDNA deletions.

prominent side-to-side (no-no) head tremor, slow saccades with upbeat nystagmus on upgaze, and pendular nystagmus on right lateral gaze, with a full range of external ocular movements. His visual fields, acuity, and fundi were normal, but he had cerebellar dysarthria and dysphagia. Limb examination revealed a postural and action tremor, marked distal wasting, dysmetria, and dysdiadochokinesis. He had predominantly distal symmetric limb weakness, absent tendon reflexes, and diminished sensation to all 4 modalities in all

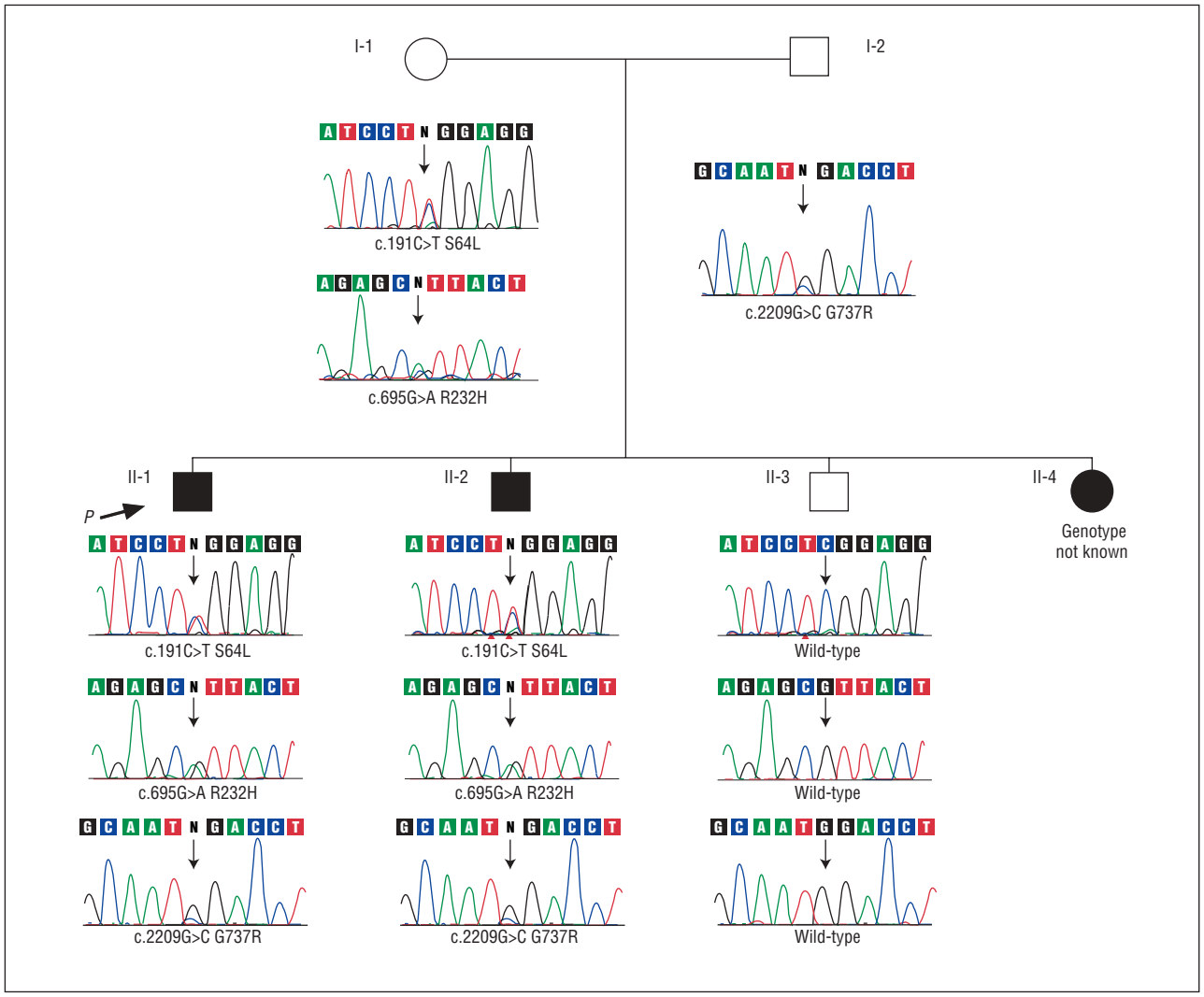

Figure 2. Pedigree showing segregation of *POLG1* (polymerase γ-1) gene mutations. N indicates heterozygous substitution; P, the proband; squares, men; circles, women; shaded symbols, affected; and open symbols, unaffected.

4 limbs. He was unable to walk because of weakness and ataxia.

He was the product of a nonconsanguineous union, with healthy parents aged 63 and 61 years. His 40-year-old brother (II-2) was also initially diagnosed as having CMT2 (Table) but had a less severe clinical course. His 42-year-old sister (II-4) developed similar symptoms at age 9 years, was diagnosed as having CMT2 in her early 20s, and is now unable to walk, with severe ataxia (a video is available at http://www.archneurol.com). A 41-year-old brother (II-3) was clinically unaffected. Three half-siblings of the father (data not shown) remain unaffected.

Results of routine blood tests in the proband were normal. Cerebrospinal fluid examination results were unremarkable, except for an elevated lactate level (21.5 mg/dL [to convert to millimoles per liter, multiply by 0.111]). Brain magnetic resonance imaging, echocardiography, and pure-tone audiometry findings were normal. Neuropsychometry revealed no major deficits. Results of genetic testing for chromosome 17 duplication, *PMP22*, *MPZ*, and spinocerebellar ataxia types 1, 2, 3, 6, and 7 expansions were negative. A nerve biopsy specimen revealed epineural fibrosis but no evidence of inflammation or amyloid.

RESULTS

Muscle histochemistry findings were consistent with ongoing denervation and reinnervation, with fiber-type grouping. There was a mosaic defect of cytochrome *c* oxidase affecting 6% of muscle fibers (**Figure 1**A). Long-range polymerase chain reaction of skeletal muscle DNA revealed multiple deletions of mtDNA (Figure 1B). *POLG1* sequencing revealed the following 3 heterozygous substitutions in the proband: c.191C>T in exon 2, c.695G>A in exon 3, and c.2209G>C in exon 13. Segregation analysis in the family (**Figure 2**) showed that the c.191C>T and c.695G>A substitutions were in *cis* and inherited from the mother and that the c.2209G>C substitution was inherited from the father. The affected brother (II-2) had the same genotype as that of the proband. The unaffected brother (II-3) had a wild-type sequence.

The frequency of the *POLG1* substitutions was determined by primer extension and by matrix-assisted laser

desorption ionization–time-of-flight mass spectrometry (MALDITOF; Sequenom, San Diego, California). The c.191C>T substitution was detected in 90 of 294 control alleles (30.6% [95% confidence interval, 25.4%-36.2%]). The c.695G>A substitution was not detected in 282 control alleles (95% confidence interval, 0%-1.06%). The c.2209G>C substitution was detected in 2 of 666 control alleles (0.3% [95% confidence interval, 0.04%-1.08%]).

COMMENT

Mitochondrial DNA codes for 13 essential components of the respiratory chain that is linked to oxidative phosphorylation, the principal source of adenosine triphosphate within the cell. *POLG1* mutations affect the maintenance of mtDNA, causing deletions, point mutations, and depletion of mtDNA. Secondary mtDNA defects lead to a biochemical defect of the respiratory chain in affected tissues, organ dysfunction, and the clinical phenotype.[3]

The c.695G>A and c.2209G>C substitutions are the likely cause of the autosomal recessive axonal sensorimotor neuropathy in this family. The c.695G>A substitution is predicted to alter the highly conserved arginine 232 to histidine in the exonuclease (proofreading) domain of polymerase γ, and the c.2209G>C substitution is predicted to change the highly conserved glycine 737 to arginine in the linker region of polymerase γ. Both have previously been described in compound heterozygotes having other *POLG1* mutations and having multiple mtDNA deletions[4,5]; they did not cause disease in the heterozygous parents and are uncommon in the general population. The c.191C>T substitution is predicted to cause a serine to leucine substitution at position 64, but this amino acid is located outside the functional domain of the protein, is not as conserved across species, is not positioned close to any reported pathogenic mutations, and was present in approximately 30% of control alleles.[3] Therefore, this substitution is unlikely to be directly responsible for the disorder in this family, although we cannot exclude the possibility that the serine to leucine substitution at position 64 contributes to the phenotype, as has been described for other polymerase γ substitutions present at high frequencies in control subjects.[4]

Axonal neuropathies have been described in patients having dominant and recessive *POLG1* mutations,[4,6-10] often causing profound sensory ataxia; however, the neuropathy in each case was associated with additional neurological features, pointing toward a multisystem mitochondrial disorder. By contrast, in all 3 siblings in the present family, peripheral neuropathy dominated the clinical picture for 2 decades. Only later did they develop tremor and ataxia, but without the ophthalmoplegia or dysphagia that characterizes the well-described mitochondrial phenotype of SANDO (sensory ataxic neuropathy with dysphagia and ophthalmoplegia).[11] Therefore, our observations add to the broad phenotypic spectrum of *POLG1*-related disease and suggest that *POLG1* should be sequenced in patients having unexplained axonal hereditary neuropathy. Although muscle biopsy may reveal the presence of cytochrome *c* oxidase–negative fibers, this is not universally the case,[12] and *POLG1* should be sequenced if there is a strong clinical suspicion.

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Correspondence: Patrick F. Chinnery, PhD, FRCP, Mitochondrial Research Group, The Medical School, Newcastle University, Room M41014, Framlington Place, Newcastle upon Tyne NE2 4HH, England (p.f.chinnery@ncl.ac.uk).
Author Contributions: *Study concept and design:* Stewart, Findlay, De Silva, and Chinnery. *Acquisition of data:* Harrower, Stewart, Hudson, Houlden, Warner, O'Donovan, Findlay, Taylor, and De Silva. *Analysis and interpretation of data:* Stewart, Hudson, Taylor, and Chinnery. *Drafting of the manuscript:* Harrower, Stewart, Houlden, and Chinnery. *Critical revision of the manuscript for important intellectual content:* Harrower, Stewart, Hudson, Warner, O'Donovan, Findlay, Taylor, and De Silva. *Obtained funding:* Taylor and Chinnery. *Administrative, technical, and material support:* Harrower, Stewart, Hudson, Houlden, O'Donovan, Findlay, and De Silva. *Study supervision:* De Silva and Chinnery.
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Additional Information: Videos are available online at http://www.archneurol.com.

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