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## **POLG mutations presenting as CMT**

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### **Abstract**

We report on two patients, with different *POLG* mutations, in whom axonal neuropathy dominated the clinical picture. One patient presented with late onset sensory axonal neuropathy caused by a homozygous c.2243G>C (p.Trp748Ser) mutation that resulted from uniparental disomy of the long arm of chromosome 15. The other patient had a complex phenotype that included early onset axonal CMT caused by compound heterozygous c.926G>A (p.Arg309His) and c.2209G>C (p.Gly737Arg) mutations.

### **Keywords**

neuropathy; uniparental disomy; mitochondria

### **Introduction:**

Charcot-Marie-Tooth disease (CMT) is the eponym for inherited neuropathies that are not part of a larger syndrome (Fridman, et al., 2015). CMT affects ~1 in 2500 individuals and is caused by mutations in more than 100 different genes. In addition to the conventionally recognized forms of CMT, there are hundreds of genetic syndromes that include neuropathy,

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Supplemental Data: Primer sequences.

PCR and Sanger Sequencing Primers:

POLG-ex13-F: 5' – CATTTAATTCCACCACCATGC – 3'

POLG-ex13-R: 5' – TGAGACCTCATGGCCATCTT – 3'

POLG-ex4-F: 5' – AGGATGAGATCTGGGGAACC – 3'

POLG-ex4-R: 5' – CTTACAGGTGATGGGGCAAG – 3'

Microsatellite markers for RFLP analysis.

D6S1552-F: 5' – AGCCTGAACGACAGAACAAG – 3'

D6S1552-R: 5' – CTGCTTAACCTAGATCTTTGGTAT – 3'

D6S1624-F: 5' – AAGTCTTCAGTGGAGAGAGT – 3'

D6S1624-R: 5' – ACTCCAGGTGTTTGTGGTTT – 3'

D61517-F: 5' – GGACCTACGCATCTGGTG – 3'

D61517-R: 5' – TGGCTCTAATGGTTACTTTTACA – 3'

MOG-CA-F: 5' – TCACCTCGAGTGAGTCTCTTT – 3'

MOG-CA-R: 5' – ACCATGGGTAACCTGAAGCAT – 3'

LIZ600 (orange) was used as the size standard.

and for a few of these, neuropathy can be the initial clinical manifestation (Rossor, et al., 2017; Scherer, et al., 2008). Here we describe two patients, both of whom presented with an axonal neuropathy, and were found to harbor mutations in *POLG*.

## Methods:

### Whole Exome Sequencing (WES)

WES was performed on genomic DNA samples taken from the two index patients. The SureSelect Human All Exon Kit (Agilent) was applied for in-solution enrichment, and the HiSeq 2500 instrument (Illumina) was used to produce 120 base pair paired-end sequence reads. The Burrows-Wheeler aligner and Freebayes were utilized for sequence alignment and variant calling. Exome data was uploaded into the GENESIS software for subsequent analysis (Gonzalez, et al., 2015).

### Amplifying and Sanger Sequencing of *POLG* exons 4 and 13

The *POLG* sequences for exons 4 and 13 from transcript NM\_002693 were used to design amplification primers using Primer3 software. The primer design achieved amplification of ~500 base pair regions of *POLG*, which included the mutations of interest (c.2243C>G/ p.Trp748Ser; c.2209G>C/ p.Gly737Arg; c.926G>A/p.Arg309His) flanked on both sides by approximately 250 base pairs. *POLG* exon 13 was amplified from the genomic DNA of the proband and her parents (family 1). *POLG* exons 4 and 13 were amplified from the genomic DNA of the proband and her parents (family 2). Amplification was achieved through PCR with Platinum Taq Polymerase (Thermo Fisher) on a thermal cycler (Applied Biosystems). PCR products were purified using the Qiagen PCR purification kit and submitted to Eurofins Genomics (Louisville, KY) for Sanger sequencing with their corresponding sequencing primers. The trace sequences were then analyzed using Sequencher software (Gene Codes). The primer sequences for PCR and Sanger sequencing can be found in Supplemental Figure 1.

### Paternity Testing

Paternity was established by comparative analysis of restriction fragment length polymorphisms (RFLPs) between the proband and her parents (family 1) using fluorescently tagged microsatellite markers as probes. Microsatellites were amplified from the proband's and the parent's genomic DNA by PCR using Platinum Taq Polymerase (Thermo Fisher Scientific). PCR reactions were carried out in a thermal cycler (Applied Biosystems). Fragment analysis was performed in the 3130xl Genetic Analyzer (Applied Biosystems) whereupon the raw data was analyzed using GeneMapper software (Thermo Fisher Scientific). The microsatellite markers are found in Supplemental Figure 1.

### Copy-Number Variant Analysis

The patient in family 1 had separate genomic testing performed through GeneDx. Whole-Genome Oligonucleotide Array CGH+SNP Microarray analysis was performed. This microarray is based on human genome build GRCh37/hg19 and it contains approximately 118,000 probes that provide copy-number data and 66,000 probes that generate genotype data through the analysis of single nucleotide polymorphisms (SNPs).

## Isodisomy Mapping from Whole-Exome Sequencing

The WES data from the proband of family 1 was screened for uniparental isodisomy. Using a previously published protocol (Bis, et al., 2017), isodisomy mapping was performed by detection of long regions of homozygosity ( $> 10$  Mb) isolated to a single chromosome. Regions of homozygosity were detected using H3M2 (Magi, et al., 2014) with recommended parameters (DNorm=100000, P1=0.1, P2=0.1).

## Results:

All patients were seen by one of the authors (SSS) at the University of Pennsylvania. They had a CMT Neuropathy Score version 2, which includes electrophysiology of the upper extremities (Murphy, et al., 2011). Blood samples were collected from participants after obtaining informed consent, DNA was extracted at the University of Miami, where WES was performed on the proband of each family. Standard software packages produced annotated next-generation sequencing results, which were then further analyzed within the GENESIS software platform. All results were confirmed in a CLIA-approved commercial laboratory

The proband of family 1 (Figure 1a) was a 54-year-old woman of German ancestry. She reported a progressive balance disturbance since age 49 on the background of having had trouble walking heel-to-toe and hopping for many years. Her neurologic exam showed reduced vibration sensation (Rydell-Seiffer tuning fork 0–1 at the toes, 3–4 at the ankles and knees) and pinprick sensation (reduced to the mid-calves). Deep tendon reflexes, as well as strength, bulk and tone were normal. Smooth pursuit and saccadic eye movements and rapid alternating movements were normal. A MRI of the brain at age 52 was normal. At age 57, neuro-ophthalmic evaluation revealed a mild supranuclear vertical gaze paresis and a mild right internuclear ophthalmoparesis (slowed right adducting saccades with dissociated abducting nystagmus) localizing to the rostral midbrain (*Liu, et al.*). There was no gaze-evoked nystagmus, saccadic dysmetria, or abnormal lack of suppression of the vestibulo-ocular reflex, and her optic nerves were normal in appearance. Nerve conductions of the left arm (Table 1) revealed mildly reduced sensory amplitudes and normal to reduced motor responses in the feet; electromyography of the left leg showed moderate, chronic denervation in distal muscles. When last seen at age 58, her balance had worsened. At age 79 and 85, her mother and father were evaluated, respectively. Both parents had had surgery for lumbar spinal stenosis. The mother had normal strength in the arms and legs; electrophysiology of the left arm showed no evidence of a neuropathy. The father had no movement in ankle dorsi- or plantar-flexion; strength in the arms was normal, and EMG of his left arm showed only mild, chronic denervation in his intrinsic hand muscles. For this reason, neuropathy alone is unlikely to be cause of the severe denervation in the legs. The nerve conduction of the father's arms also showed evidence of a sensory neuropathy (antidromic left and right radial amplitudes were 10.5 and 9.3  $\mu$ V;  $>15$   $\mu$ V is normal).

WES of the proband in family 1 identified a homozygous variant (c.2243G>C/p.Trp748Ser) in exon 13 of *POLG*, previously described as pathogenic (Hakonen, et al., 2005). Sanger sequencing and segregation analysis revealed that the mother, but not the father, carried the allele in heterozygous state (Fig. 1A). After confirming the paternity of the father with RFLP

analysis using microsatellite markers as probes (Fig. 1B), further analysis of the proband's whole-exome data with the GENESIS software and revealed a ~60Mb region of homozygosity on chromosome 15 (15q15.1–15q26.3) that included the *POLG* gene. To confirm that this was the only long region of homozygosity, we performed global isodisomy mapping utilizing the H3M2 algorithm (Magi, et al., 2014). Filtering for regions greater than 10 Mb (Bis, et al., 2017) revealed an extended region of homozygosity (chr15:43804173–102359499), covering 72% of chromosome 15 (Fig. 1C). The isolation of the homozygous region to a single chromosome supports the occurrence of a uniparental isodisomic event rather than homozygosity due to consanguinity. Microarray analysis ruled out deletions on chromosome 15 in the proband and confirmed the presence of the ~60 Mb region of homozygosity. Deletion/duplication analysis for the *POLG* gene confirmed the copy-number neutral state of the *POLG* gene.

The proband of family 2 (Figure 1) was evaluated at age 6 years for slowed psychomotor development, including delayed ambulation (age 3) and delayed speech, and subsequently developed progressive balance difficulties and motor issues. She had Achilles tendon lengthening, plantar flexor release, and transfer of the tibialis posterior tendon at age 9, followed by a Jones procedure at age 15, and has required a walker to ambulate ever since. She had achalasia with vomiting beginning at age 15, and two dilations within one month to mitigate symptoms. When first examined at age 16 (by SSS), she had severe weakness in ankle dorsiflexion, ankle plantar flexion, and intrinsic hand muscles, and moderate weakness of proximal leg muscles (hamstring 4/5 and quadriceps 4+/5). Vibration was absent at the toes and ankles and 2 at the knees. Pinprick was reduced to the knees. Neurophysiology of the right arm (Table 1) showed absent sensory responses and a reduced ulnar motor amplitude, and EMG showed severe, chronic denervation in first dorsal interosseous and extensor indices proprius. At age 19, she had started experiencing bolts of pain in her left leg, about once a day. She had no ankle dorsiflexion or plantar flexion and was areflexic in her arms and legs, and later that year, weakness had progressed to 2/5 in the right and 4-/5 left quadriceps and 4-/5 in the hamstrings. An MRI of the brain at age 19 was normal. At age 20, she was falling 1–2 times a day. Strength was unchanged, and pinprick was reduced to above the kneecap; vibration was absent at toes, ankles, and knees.

WES of the proband in family 2 showed compound heterozygous mutations in *POLG* exon 4 (c.926G>A/p.Arg309His) and exon 13 (c.2209G>C/p.Gly737Arg). Sanger sequencing and subsequent segregation analysis showed that she had inherited one mutant allele from each of her parents (Figure 1). The patient also had a nonsynonymous mutation (p.Gly931Ser) in *AARS*, a gene that also causes CMT, but this mutation is too common (7/1,000 individuals in gnomAD) to be the cause of the proband's disease. It is possible, however, that the *AARS* variant, or another, unidentified mutation, contributed to the proband's neuropathy, as the father had electrophysiology evidence of an axonal neuropathy. It is possible that a heterozygous, p.Arg309His *POLG* mutation could cause a mild, late-onset, sensory neuropathy, but that has not been reported.

## Discussion

These two cases add to the literature on *POLG* mutations presenting as CMT (Harrower, et al., 2008; Hoyer, et al., 2014). In family 1, uniparental disomy (UPD) of the well documented p.Trp748Ser mutation caused a sensory neuropathy and subtle eye movement abnormalities. In family 2, compound heterozygous *POLG* mutations (p.Arg309His and p.Gly737Arg) caused a phenotype that is similar to that described in some cases in which the p.Arg309His or the p.Gly737Arg mutations are found in trans with other pathogenic *POLG* mutations (Da Pozzo et al. 2017, Harrower, et al. 2008). Thus, *POLG* should be considered for inclusion in genetic testing panels for CMT in order to effectively utilize NGS panels for CMT patients.

*POLG* encodes the only DNA polymerase for mitochondrial DNA (mtDNA) and mutations are associated with severe mtDNA depletion and accumulation of mutations in mtDNA over time as well as a bewildering spectrum of clinical syndromes (Rahman and Copeland, 2019). These include Alper-Huttenlocher spectrum (OMIM 203700; encephalopathy, intractable epilepsy, and hepatic failure), mitochondrial DNA depletion syndrome 4B (MNGIE; OMIM 613662; gastrointestinal dysmotility and pseudo-obstruction, cachexia), progressive external ophthalmoparesis (PEO) which can be further divided into autosomal dominant PEO (OMIM 157640; generalized myopathy and a combination of axonal neuropathy, ataxia, and sensorineural hearing loss), autosomal recessive PEO (OMIM 258450; weakness of extraocular muscles and exercise intolerance), and mitochondrial recessive ataxia syndromes (OMIM 607459), which include sensory ataxic neuropathy with dysarthria and progressive external ophthalmoparesis (SANDO) and spinocerebellar ataxia with epilepsy and parkinsonism (SCAE). Mitochondrial recessive ataxia syndrome (MIRAS; not in OMIM) can also refer to a more narrowly defined syndrome caused by Ala467Thr and Trp748Ser mutations (Mignarri, et al., 2015). SANDO is more commonly categorized within the Ataxia Neuropathy Spectrum (ANS; not in OMIM) and SCAE within Myoclonic Epilepsy Myopathy Sensory Ataxia (MEMSA) (Rahman and Copeland, 2019). There is a loose correlation between the degree of mitochondrial DNA depletion and the severity of the neurological manifestations.

We report here the first case of UPD as the cause of a homozygous *POLG* mutation. UPD has been described in individual patients in a handful of inherited neuropathies - recessive *NDRG1* (Safka Brozkova, et al., 2017), *ARSA* (Gonorazky, et al., 2017), *MPZ* (Benko, et al., 2008), *GAN* (Miyatake, et al., 2015), and *SACS* (Anesi, et al., 2011) mutations. UPD refers to the genetic phenomenon whereby an individual inherits both copies of a chromosomal fragment from one parent, instead of one chromosome from each parent. UPD arises through mitotic and/or meiotic error, as well as through trisomic rescue. Larger regions of homozygosity, such as the 60 Mb region in our case, are the hallmark of UPD, in contrast to smaller regions of homozygosity that are subject to copy number variation. In the absence of parental genotype information in typical clinical testing scenarios, any long stretches of homozygosity should be followed up with additional tests as UPD has potentially consequences for genetic counseling of patients. UPD is a rare cause of recessive disease (King, et al., 2014). For example, out of 96 families with unresolved causes of motoneuron disease or ataxia, one person (with spastic paraplegia) was found to have a

homozygous mutation in *FA2H* resulting from UPD (Bis, et al., 2017). However, a recent screening of 214,915 trios revealed a UPD prevalence of 1 in 2000 births in the general population; this is twice the current clinical estimate (Nakka, et al., 2019).

The p.Trp748Ser *POLG* variant that was affected by UPD in family 1 is common in European populations, especially Finnish (Hakonen, et al., 2005), and several homozygous patients have been described (Tzoulis, et al., 2006). They are phenotypically variable, although they usually develop ataxia, with a highly variable age of onset, from age 2 and into the 60s. Juvenile-onset cases can present with Alper-Huttenlocher spectrum, with liver failure provoked by valproic acid (Uusimaa, et al., 2008), but a SCAE/MEMSA presentation of migraine-like headache or seizures, and subsequent ataxia, with other features such as myoclonus, PEO, and dysarthria are more typical (Hakonen, et al., 2005; Tang, et al., 2011; Tzoulis, et al., 2006; Van Goethem and al., 2004). Adult-onset patients present with a SANDO/ANS phenotype. Initial clinical symptoms include balance disturbances, neuropathy, and/or ataxia, often accompanied by eye movement abnormalities (including PEO), dysarthria, parkinsonism, exercise intolerance, and cognitive decline (Hakonen, et al., 2005; Paucar, et al., 2016; Remes, et al., 2008). Hearing loss is common across all ages.

The proband in family 2 is the first person to be described with the combination of p.Arg309His and p.Gly737Arg mutations. Both mutations cause neurological disease in trans with other *POLG* mutations. In trans with a p.Arg627Gln mutation, the p.Arg309His mutation caused Alper-Huttenlocher spectrum (Horvath, et al., 2006); in trans with p.Gly1051Arg mutation, it caused neuropathy, achalasia, developmental delay, and epilepsy (Da Pozzo, et al., 2017); except for the seizures, these are similar features of our patient. In trans with a p.Ala767Asp mutation, the p.Gly737Arg mutation caused Alper-Huttenlocher spectrum (Horvath, et al., 2006); in trans with a p.Arg853Trp mutation, it caused axonal sensorimotor neuropathy, mild distal myopathy, and parkinsonism (Davidzon, et al., 2005); in trans with a p.Trp748Ser mutation, it caused a late onset axonal sensory neuropathy, bilateral hearing loss, complete external ophthalmoplegia, and ptosis (Tzoulis, et al., 2009), in trans with p.Ala467Thr, it caused SANDO (Wong, et al., 2008); in trans with p.Arg232His, it caused a phenotype similar to our patient - abnormal gait and pes cavus at age 10, distal myopathy and reduced dexterity and sensory disturbances, progressing to cerebellar dysarthria and dysphagia, absent tendon reflexes, diminished sensation to all limbs, and inability to walk by the age 35 (Harrower, et al., 2008).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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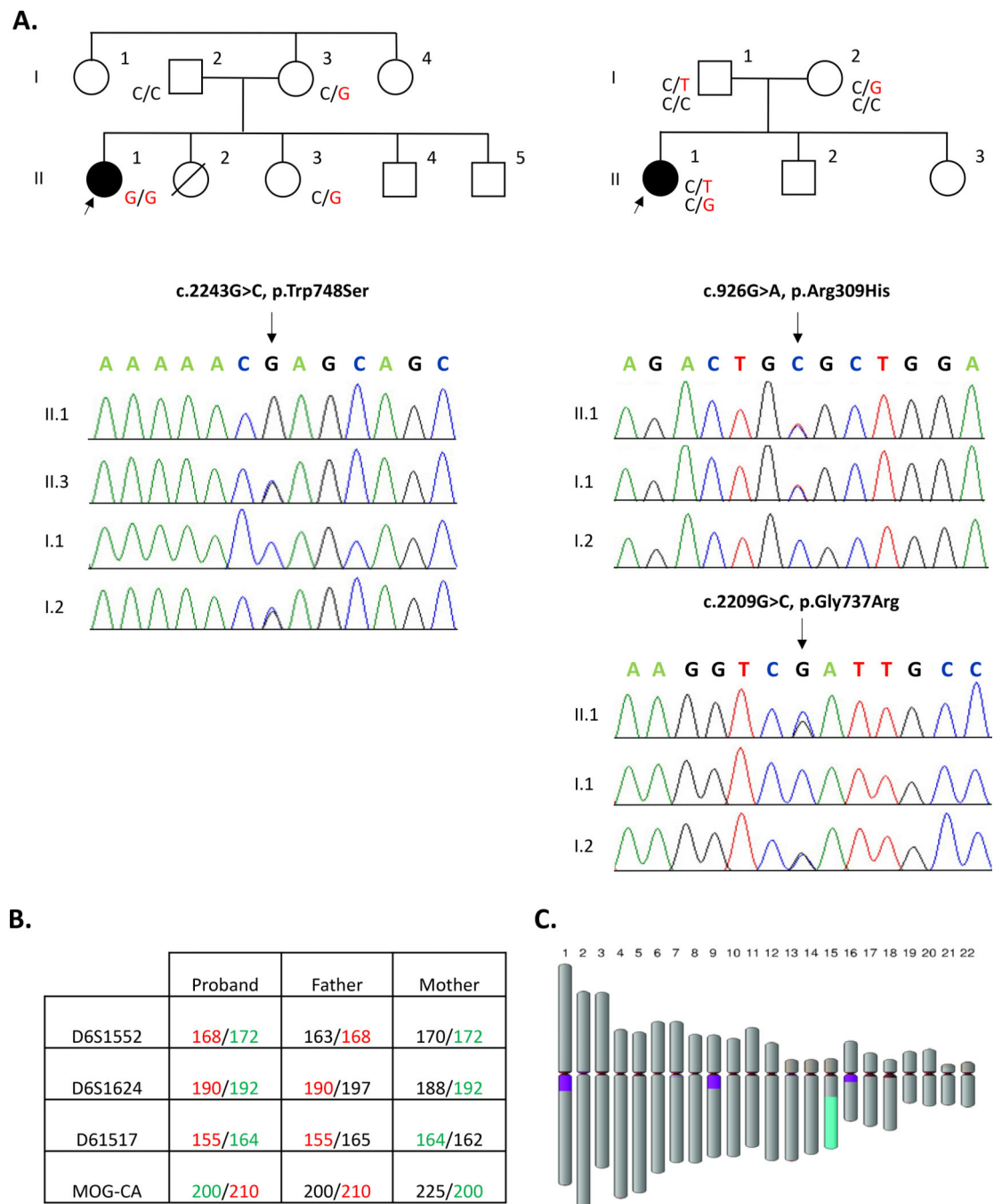


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**Figure 1: Genetic studies of families with *POLG* mutations.**  
**A.** Family pedigree and Sanger traces of Family 1 (left) show inheritance of homozygous c.2243G>C variant in the proband. The mother is heterozygous for the mutation and the father is wild-type. Sanger traces of Family 2 (right) confirm the compound heterozygous segregation of the two *POLG* variants. **B.** RFLP analysis of the proband and her parents (Family 1) confirm the paternity of the father. **C.** Graphical representation of proband's

(Family 1) exome with the 60 Mb region of homozygosity on chromosome 15 represented in blue.

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**Table 1.**

Summary of nerve conduction for affected family members

	ulnar MNCV 49 m/s	ulnar CMAP 6 mV	median MNCV 49 m/s	median CMAP 4 mV	peroneal MNCV 41 m/s	peroneal CMAP EDB 2 mV	tibial MNCV 41 m/s	tibial CMAP 4 mV	ulnar SNAP 7 $\mu$ V (O)	median SNAP 10 $\mu$ V (O)	radial SNAP 15 $\mu$ V (A)	sural SNAP 6 $\mu$ V (A)	CMTNS
Family 1 II-1 (57 y)	55	8.8	52	7.0	39	2.6	36 m/s	2.0	4.1	2.3	11.2	NR	7
Family 2 II-1 (16 y)	54	2.1	51	6.0	ND	ND	ND	ND	NR	NR	NR	ND	27

MNCV = motor nerve conduction velocity; CMAP = compound muscle action potential; SNAP = sensory nerve action potential; O = orthodromic; A = antidromic;

CMTNS = CMT Neuropathy Score version 2; (Murphy, et al., 2011)