MUTATIONS IN ATP1A1 CAUSE DOMINANT CHARCOT-MARIE-TOOTH TYPE 2

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Nothing to disclose.

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Abstract

Although mutations in more than 90 genes are known to cause CMT, the underlying genetic cause of CMT remains unknown in greater than 50% of affected individuals. The discovery of additional genes that harbor CMT2-causing mutations increasingly depends on sharing sequence data on a global level. In this way - by combining data from four continents, including seven countries - we were able to define mutations in *ATP1A1*, which encodes the alpha1 subunit of the Na^+,K^+-ATPase, as a cause of autosomal dominant CMT2. Seven missense changes were identified that segregated within individual pedigrees: c.143T>G (p.Leu48Arg), c.1775T>C (p.Ile592Thr), c.1789G>A (p.Ala597Thr), c.1801_1802delinsTT (p.Asp601Phe), c.1798C>G (p.Pro600Ala), c.1798C>A (p.Pro600Thr) and c.2432A>C (p.Asp811Ala). Immunostaining peripheral nerve axons localized ATP1A1 to the axolemma of myelinated sensory and motor axons, and to Schmidt-Lanterman incisures of myelin sheaths. Two-electrode voltage clamp measurements on *Xenopus* oocytes demonstrated significant reduction in Na^+ current activity in some, but not all, ouabain-insensitive ATP1A1 mutants suggesting a loss of function defect of the Na^+,K^+-pump. Five mutants fall into a remarkably narrow motif within the helical linker region that couples the nucleotide-binding and phosphorylation domains. These findings identify a CMT pathway and a potential target for therapy development in degenerative diseases of peripheral nerve axons.

Text

Charcot-Marie-Tooth disease (CMT) is a clinically and genetically heterogeneous group of monogenic causes of peripheral motor and sensory neuropathy, affecting an estimated 1 in 2,500 individuals.[1] Typical clinical features include distal weakness and atrophy, sensory loss, and absence of reflexes; although additional signs may also be present. Mutations in more than 90 genes have been associated with CMT.[2,3] For the dominant demyelinating forms (CMT1 [MIM: 118220]), the identification of the genetic cause is possible for 90% of affected individuals.[4] For the dominant axonal forms (CMT2 [MIM: 609260]), however, even the most comprehensive sequencing studies fail to identify the genetic cause in ~47-75% of families.[5] Most of the ~40 genes carrying CMT and related disease-causing mutations, identified in the past five years, are supported by only a few families and isolated cases, especially for the dominant forms. Thus, the rarity of new disease-gene associations has fostered data sharing and genetic matchmaking efforts.[6,7] This study showcases the success of truly global data sharing by reporting mutations that cause dominant CMT2 in a gene not previously connected to CMT, supported by seven families, including genome wide significant LOD scores in two largest families.

This study started with a CMT2 family from Czech Republic (Family 1) that had been studied for over 14 years. The family steadily expanded to 18 members, 11 of whom were affected. Whole exome sequencing, using Agilent SureSelect All Exon kit (Agilent, USA) and standard Illumina HiSeq2500 sequencing, revealed a single variant in the gene *ATP1A1* [MIM: 182310] co-segregating with the disease in all affected family members as validated by Sanger sequencing (c.143T>G). We then took advantage of a large collection of 753 aggregated exomes and genomes associated with OrphaNet terms for CMT [ORPHA:166], CMT2 [ORPHA:64746], distal hereditary motor neuropathy (dHMN; ORPHA:53739), and hereditary

motor and sensory neuropathy (HMSN; ORPHA:140450) from over 20 independent CMT research groups within the GENESIS genome analysis platform.[7] All the participants or their legal representatives had provided informed consent approved by local Research Ethics committees. In this large dataset, we firstly identified a rare missense change in *ATP1A1* in a large family from Southern Italy. Segregation of this variant was demonstrated by Sanger sequencing. After enrollment of additional family members, we were able to obtain a LOD score of 3.1 at the *ATP1A1* locus. We then filtered the 753 exomes and genomes under an autosomal dominant segregation model for minor allele frequency (MAF) in ExAC < 0.00001 and allele count in the GENESIS database < 5 in *ATP1A1*. Two additional missense variants (c.1798C>A and c.1775T>C) were identified and Sanger sequencing and segregation studies were performed in all available family members (Figure 1, Table 1). Independently, exome sequencing of five affected and two unaffected individuals from an Australian pedigree (Family 6) identified a missense variant co-segregating with disease (c.1801_1802delGAinsTT, p.Asp601Phe). Sanger sequencing of the hotspot exon 13 (NM_000701) in 39 genetically-unresolved Australian CMT probands identified one additional family (Family 5) with an *ATP1A1* variant (c.1789G>A). Family 7 was identified through the analysis of 513 whole exomes from 399 unrelated Korean CMT families. Complete segregation analysis was not possible as the proband's father is deceased; he died in his sixth decade of life without any CMT clinical symptoms. Finally, 80 Taiwanese families with genetically unresolved CMT2 were screened for *ATP1A1* mutations, but no further cases were identified. In total, we confirmed the following *ATP1A1* variants in families from four continents: Family 1 (Czech), c.143T>G, p.Leu48Arg (chr1:116927424); Family 2 (Italy), c.1798C>G, p.Pro600Ala (chr1:116937869); Family 3 (USA), c.1775T>C, p.Ile592Thr (chr1:116937846); Family 4 (USA), c.1798C>A, p.Pro600Thr (chr1:116937869); Family 5 (Australia), c.1789G>A, p.Ala597Thr

(chr1:116937860); Family 6 (Australia), c.1801_1802delGAinsTT, p.Asp601Phe (chr1:116937872); Family 7 (South Korea), c.2432A>C, p.Asp811Ala (chr1:116941690) (Figure 1, Figure 2, Table 1). All reported mutations were absent from >240,000 chromosomes in the gnomAD database, highly conserved across species, predicted to be protein damaging, and were conserved across all four different *ATP1A* paralogs (in the literature also described as ATP1A isoforms) (Figure 2B, Table S2).

Neurological examination and electromyography were performed in 30 individuals across five of the studied families. (Table 2, Table S1). We observed distal weakness in the legs and arms with normal proximal strength in virtually all subjects. Vibratory sensation was typically reduced in the legs and also in the hands in some individuals. The age of symptom onset was variable, ranging from childhood to adult, even within the same family. Several mutation-carrying subjects had normal neurological examinations into the 5th decade of life, but clear abnormalities in their nerve conduction studies (NCS). NCS, when performed, demonstrated reduced compound muscle action potential (CMAP) and sensory nerve actions potential (SNAP) amplitudes with preserved conduction velocity diagnostic of axonal sensorimotor neuropathies (Table 2). CMT Neuropathy Scores version 2 (CMTNSv2) ranged from mild 6 to severely affected 25.[8,9] Many subjects presented with *pes cavus* foot structure (see Supplemental Note).

A sural nerve biopsy was available from a 33-year-old affected individual (Family 7), who carried the functionally most severe change p.Asp811Ala. As shown in Figure 2D, there was a pronounced loss of large myelinated fibers, groups of regenerating axons, and a number of thinly-myelinated large axons were present. No signs of inflammation were observed. These findings are indicative of an axonal peripheral neuropathy and in agreement with the electrophysiological findings in this individual.

ATP1A1 encodes for the α1 subunit of the Na^{+},K^{+}-ATPase, a protein ion pump responsible for the active transport of Na^{+} and K^{+} across the plasma membrane, powered by the hydrolysis of ATP, to maintain the Na+ and K+ chemical gradients in the cell [10]. There are four α paralogs encoded by different genes (*ATP1A1-4*), and each has distinct tissue-specific expression. Mutations in *ATP1A2* [MIM: 182340] and *ATP1A3* [MIM: 182350] are known to cause distinct neurological disorders of the central nervous system, likely through a haploinsufficiency mechanism of action [11,12]. Mutations in *ATP1A1* have not been associated with any human monogenic disease to date. Four of the identified substitutions (p.Ile592Thr, p.Pro600Thr, p.Pro600Ala, and p. Asp601Phe) are clustered in the intracellular loop between transmembrane domains M4 and M5 (Figure 2A), a region where ATP binding and ATP hydrolysis occur. The substitution p.Leu48Arg is located adjacent to a phosphorylation site in the N-terminus region, and p.Asp811Ala is located in the fifth transmembrane domain where Na^{+} binding occurs. The corresponding substitution in ATP1A2 (p.Asp801Asn) has been reported in several individuals with alternating hemiplegia of childhood and rapid-onset dystonia parkinsonism diseases.[12,13]

ATP1A1 is highly constrained (intolerant) for missense and loss-of-function (LoF) variations as calculated by the correlation between observed and expected number of variants.[14] Derived from ExAC, the expected number of missense variants for *ATP1A1* is 357.8, but the observed number of variants is only 91 (Z-score=6.9) (Figure 3A, Table S3). The number of loss-of-function variants is also low, leading to the maximum constraint metric for probability of LoF intolerance (pLI=1.00) (Figure 3B).[14] Other dominant CMT-associated genes are also significantly more constrained than recessive CMT-associated genes (Figure 3C). Interestingly, *ATP1A1* has the second highest constraint value of genes associated with CMT, behind *DYNC1H1* [MIM: 600112] (Z-score=13.88). In fact, *ATP1A1* is ranked number 31 of all

genes in the human genome.[14] Two other disease-associated *ATP1A* genes (*ATP1A2* and *ATP1A3*) also show high intolerance against rare missense variation, but *ATP1A4* does not. This further supports the potential for variations in *ATP1A1* to cause disease.

ATP1A1 is the catalytic α subunit of a P-type Na^+,K^+-ATPase ion pump that establishes Na^+ and K^+ gradients across cell membranes. As with other members of the P-type ATPase superfamily, ion pumping by ATP1A1 is achieved by alternating between two major conformational states that are controlled by ATP hydrolysis and intermediate phosphorylation to regulate the binding, occlusion, and transport of cations into (K^+) and out (Na^+) of the cell.[15] Crystallographic studies have established that ATP1A1 consists of ten transmembrane helices, and three cytoplasmic domains, individually known as the N (nucleotide binding), P (phosphorylation), and A (actuator) domains (Figure 2C).[15,16]

We examined these crystal structures to assess the potential structure-function consequences of the seven unique segregating missense changes that we identified in ATP1A1 (Figure 2C). Remarkably, five substitutions (p.Ile592Thr, p.Ala597Thr, p.Pro600Ala, p.Pro600Thr, and p.Asp601Phe) map to a hot spot within the helical linker region (residues 592 to 608) that couples the N- and P-domains (red linker in Figure 2C). These changes likely de-couple ATP-hydrolysis (N-domain) from intermediate phosphorylation (P-domain) that is essential for ion selectivity and driving the two major alternating conformations of the pump. Only one substitution (p.Leu48Arg) maps to the actuator domain, where it could disrupt the concerted movement of the N-, P-, and A-domains during phosphorylation and pumping. The remaining change, p.Asp811Ala, maps to the transmembrane channel core, where it likely disrupts Na^+ binding and transport through the membrane. From this analysis, we conclude that the helical linker region between the N- and P-domains is a hot spot for mutational defects in the ATP1A1 pump. However, our observation of additional mutations elsewhere in

ATP1A1 indicate that pathogenic mutations are not necessarily localized to a specific region of the pump. This latter observation makes intuitive sense given the long-distance allosteric changes that regulate this complex molecular machine.

To test the functional effects of the different CMT-associated *ATP1A1* mutations, we examined the electrophysiological characteristics of human wild type (WT) *ATP1A1* and the different *ATP1A1* variants (co-expressed with the human □1 subunit) in *Xenopus* oocytes. Two studied CMT substitutions, p.Pro600Ala and p.Asp811Ala, demonstrated significantly less Na^+-dependent currents than WT-ATP1A (Figure 3D). This was especially pronounced for the p.Asp811Ala substitution, in agreement with previous experiments that found mutations of the homologous residue leads to defective Na^+ binding in other ATP1A proteins.[17] D811 is predicted to coordinate Na^+ ions in the crystal structures of Na^+,K^+-ATPases, thus missense variants are potentially resulting in the loss of sodium ion coordination. Substitution p.Pro600Ala also showed significantly reduced Na^+-dependent currents (Figure 3D). This residue falls into a proposed molecular hinge between the N and P domains.[18] Substitutions of p.Pro600 might therefore affect the ability of ATP1A1 to undergo conformational changes during Na^+/K^+ transport. The p.Leu48Arg substitution showed similar Na^+-dependent currents as WT-ATP1 (Figure 3D), suggesting that this substitution causes functional defects that are not detected in this assay (i.e. protein stability) or defects that are masked in the *Xenopus* oocyte expression system, such as cellular trafficking. Mutations that cause trafficking defects in mammalian expression systems are often still trafficked to the plasma membrane in *Xenopus* oocytes, possibly due to the lower culture temperatures for *Xenopus* oocytes.[19]

To further evaluate the functional consequences of the CMT-associated *ATP1A1* variants in mammalian cells, we performed an ouabain survival assay in U2OS cells. Cells were transfected with plasmids encoding full-length human *ATP1A1* (WT-ATP1A1), and a

ATP1A1 construct that had been mutated to be ouabain-insensitive (Oua-WT). Cells transfected to express Oua-WT and selected mutants (Oua-L48R, Oua-I592T, Oua-P600T, and Oua-D811A) were treated with 0.5 uM ouabain for 48 hr. Cell death was observed in WT-ATP1A1 cells, whereas cells transfected with the Oua-WT survived. Cell viability assays were quantified using the CellTiter-Glo assay. We observed a significant decrease in cell viability in cells transfected with the ouabain-insensitive mutants Oua-L48R, Oua-I592T, Oua-P600T, and Oua-D811A (Figure 3E). The mutant Oua-D811A seems to be the most severely affected, in the least number of viable cells. Likewise, it has been demonstrated that cells transfected with pathogenic *ATP1A3* mutants show a higher percentage of cell death compared to *ATP1A3* WT.[20] Thus, the ouabain survival assay further supports a detrimental functional effect of the identified substitutions.

To investigate why *ATP1A1* mutations cause an axonal neuropathy, we evaluated the protein levels of ATP1A1 in the peripheral nervous system using mouse monoclonal antibodies against ATP1A1 and ATP1A3 (Figure 3F and Figure S1).[21,22] Immunostaining of teased fibers from adult rat sciatic nerve (Figure 3F) shows that individual axons are variably ATP1A1- and ATP1A3-positive: some axons are mainly positive for one or the other, but most axons show detectable levels of both. ATP1A1 is also localized to Schwann cells, particularly paranodes and Schmidt-Lanterman incisures, which are regions of non-compact myelin that are thought to facilitate the diffusion of small molecules and ions.[23] Immuno-stained cryosections of adult rat spinal cord (Figure S1) show that ATP1A3 is the main ATP1A paralog localized in the central nervous system, a subset of large sensory axons that are known to be proprioceptors, and small myelinated axons of γ-motor neurons.[24,25,26] ATP1A1 was the predominant ATP1A paralog present in most the motor and sensory axons of the ventral and dorsal roots. In addition, the large motor neurons of

the ventral horn are also ATP1A1-positive. Thus, most peripheral nerve axons and all Schwann cells contain ATP1A1 protein, so that *ATP1A1* mutations could cause an axonal neuropathy by affecting the function of ATP1A1 in one or both cell types.

The membrane bound ATP1A1 is present at high levels in α-motoneurons and most sensory neurons.[27] In peripheral nerve, ATP1A1 localizes to axons (internodal axolemma) and non-compact myelin (Schmidt-Lanterman incisures and paranodes). The demonstrated reduction in Na^+,K^+-ATPase activity in ATP1A1 mutants will potentially reduce the Na^+ gradient across the axonal membrane, as has been shown in other Na^+,K^+-ATPase mutations.[28] This reduced Na^+ gradient would lead to a reduced efflux of Ca^{2+} from the axon by Na^+/Ca^{2+} exchangers and potentially even a Ca^{2+} influx through Na^+/Ca^{2+} exchangers. As has been shown at nodes during repetitive firing, this leads to influx of Ca^{2+} through Na^+/Ca^{2+} exchangers at the nodes of WT mouse peripheral motor axons.[29] Thus, the reduction in Na+,K+-ATPase activity would lead to a build-up of toxic levels of intracellular Ca^{2+}.[13] This offers a pathomechanism for CMT, which will likely yield additional disease-causing genetic factors.

Two other paralogs of *ATP1A* have been shown to cause neurological disease. We hypothesize that the distinctive phenotypes associated with ATP1A proteins results from differential tissue localization patterns and redundancy of local function. For instance, *ATP1A2* and *ATP1A3* are expressed predominantly in the central nervous system and mutations have been associated with familial hemiplegic migraine and dystonia and parkinsonism.[20,30] Reduced activity of Na^+,K^+-ATPase activity in ATP1A2 and ATP1A3 mutants has been shown, indicating haploinsufficiency and loss-of-function mechanism of action.[12,31] *ATP1A1* substitutions may act in a similar fashion as supported by our voltage clamp experiments. However, as not all tested substitutions showed reduced Na^+,K^+-ATPase activity, additional

mechanisms, such as trafficking defects may be revealed in future studies. The identified missense mutations in our study span the gene, with a prominent cluster between residue 592 – 601. Of interest, the large gnomAD database does not record any missense variant alleles between residues 590 and 638 in over ~240,000 chromosomes.

In our study, *ATP1A1* substitutions were associated with a classic axonal to intermediate length-dependent degeneration of motor and sensory peripheral nerves. We predict that as more affected individuals are screened for variants in *ATP1A1*, the phenotypic spectrum may expand. Given that ATP1A proteins are ubiquitously present at low levels, a combination of central and peripheral phenotypes is conceivable in some families. We fully expect that extra-PNS features will appear in affected individuals, as we have already noted anecdotally in Family 3 with migraine headaches.

Taken together, we show that *ATP1A1* is a cause for dominant axonal to intermediate CMT. This conclusion is supported by strong genetic evidence in seven families, structural protein analysis, comparative molecular genetics evidence, *in vitro* and *in vivo* functional experiments, and histological studies. These mutations likely act though haploinsufficiency, similar to *ATP1A2* and *ATP1A3* changes. This CMT pathway offers therapeutic target considerations. The international collaborative nature of this study was enabled by data aggregation and genetic matchmaking capabilities of the GENESIS platform, spanning four continents, and five countries.[7]

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All authors do not report any conflicts of interest.

Supplemental Material

The supplemental information contains detailed case reports and motor and sensory nerve conduction velocity scores for select families; additional confocal images displaying the reciprocal expression of α1 and α3 in cryosections of adult rat spinal cord; *ATP1A1* variant pathogenicity prediction scores; and ATP1 gene family mutational constraint scores.

Figure Legends

Figure 1. Pedigrees of families carrying mutations in *ATP1A1*. Autosomal dominant CMT families show segregation of *ATP1A1* variants: Family 1 (Czech Republic), Family 2 (Italy), Family 3 (USA1), Family 4 (USA2), Family 5 (Australia1), Family 6 (Australia2), Family 7 (Korea).

Figure 2. ATP1A1 mutations identified in CMT families. (A) Schematic depicting the position of pathogenic substitutions in the ATP1A1 protein and their associated locations around the globe. (**B)** ATP1A protein paralogs alignment showing that the ATP1A1 substitutions are located at highly conserved residues across the four different paralogs. **(C)** 3D structural model of ATP1A1. **(D)** Electron micrograph of a sural nerve biopsy of affected individual from family 7 (Korea) showing typical signs of chronic axonal degeneration.

Figure 3. Genetic and functional studies of ATP1A1. (A-B) Mutational constraint analysis of ATP1A genes and known CMT-associated genes. *ATP1A1*, *ATP1A2* and *ATP1A3* have high missense constraint scores **(A)** and pLI (probability of LoF intolerance) **(B);** obtained from the ExAC browser. **(C)** Dominant CMT-associated genes have significantly higher constraint (more intolerant) scores for missense variants than recessive CMT-associated genes. **(D)** Two electrodes voltage clamp (TEVC) recordings at −50 mV from different CMT-associated mutations in the α1 subunit. For comparison, currents through wild type α1 are shown in black. Dashed lines represent zero current. Summary of currents from the CMT-associated mutations shown are normalized to wt currents (mean ± s.e.m. ; n=5-7; P<0.05). **(E)** Ouabain survival assay of U2OS cells treated with 0.5 uM ouabain. Cell viability represents the luminescence values obtained from the CellTiter-Glo assay (n=8; Ttest p<0.05 compared to oua-WT-ATP1A1). **(F)** Distinct localization of α1 and α3 in myelinated axons. These are confocal images of teased fibers from an adult rat, immunostained with a mouse monoclonal antibody against α3 (XVIF9-G10; red) and a rabbit antiserum against α1 (NASE; green), as indicated. Two myelinated axons (1,3) have strong α3 staining (and weak α1 staining), a myelinated axon (4) has strong α1 staining (and weak α3 staining).

The incisures of myelin sheaths are α1-positive (inset). A Remak bundle (2) is α1-positive and α3-negative. Apposed arrowheads mark nodes of Ranvier. Scale bar: 10 μm.

Tables

Table 1. ATP1A1 mutations detected in in CMT2 and CMT dominant-intermediate families

Families (Origin)	gDNA level: Chr1(GRCh37):	cDNA level: NM_001160233.1:	Protein level:	Exon
Family 1 (Czech Republic)	g.116927424T>G	c.143T>G	p.Leu48Arg	5
Family 2 (Italy)	g.116937869C>G	c.1798C>G	p.Pro600Ala	13
Family 3 (USA 1)	g.116937846T>C	c.1775T>C	p.Ile592Thr	13
Family 4 (USA 2)	g.116937869C>A	c.1798C>A	p.Pro600Thr	13
Family 5 (Australia 1)	g.116937860G>A	c.1789G>A	p.Ala597Thr	13
Family 6 (Australia 2)	g.116937872_116937873delinsTT	c.1801_1802delinsTT	p.Asp601Phe	13
Family 7 (Korea)	g.116941690A>C	c.2432A>C	p.Asp811Ala	19

Table 2. Clinical features observed in ATP1A1 individuals.

Site	Individual	Age (y)	AOO	CMTNSv2 (RASCH)	Distal weakness UL	Proximal Weakness UL	Distal Weakness LL	Proximal Weakness LL	Vibration UL	Vibration LL	Foot deformity	Reflexes UL (Biceps/Triceps)	Reflexes LL (Patellar/Achilles)	Positive Sensory Symptoms	Other clinical features
Family 1	II:5	66	20	12 (CMTES)	+ 2,3,2	- 5,5	+ 0,0,0	- 5,5	6/8 (fingers)	2/8 (ankle)	Pes cavus	Normal/ normal	Decreased/decreased	-	
	III:6	33	13	7 (CMTES)	+ 2 ,3,2	- 5,5	+ 0,0,0	- 5,5	Normal (8/8)	Normal (8/8)	Pes cavus	Decreased/ decreased	Decreased/absent	-	
	III:7	28	18	7 (CMTES)	+ 3,3,3	- 5,5	+ 1,1,1	- 5,5	Normal (8/8)	Normal (8/8)	-	Normal/ normal	Decreased/absent	-	
	II:2	71	12	14 (CMTES)	+ 2,2,2	- 5,5	+ 0,0,0	- 5,5	Normal (8/8)	0/8	-	Absent/ Absent	Absent/ Absent	-	
	III:1	42	25	8 (CMTES)	+ 5,4,5	- 5,5	+ 0,0,0	- 5,5	0/8 (fingers)	5/8 (ankle)	-	Decreased/ absent	Absent/ absent	-	
		55		13 (CMTES)	+ 4,4-,4-	- 5,5	+ 0,0,0	- 5,5	6/8 (fingers)	2/8 (ankle	-	Absent/ Absent	Absent/ Absent	-	Uses crutches intermittently
	II:4	68	50	10 (CMTES)	+ 4,4,4	- 5,5	+ 3,3,3+	- 5,5	Normal (8/8)	5/8 (ankle)	Pes cavus	Decreased/ Decreased	Absent/ Absent	-	
	III:3	32	No	0 (CMTES)	- 5,5,5	- 5,5	- 5,5	- 5,5	Normal (8/8)	Normal (8/8)	-	Normal/ Normal	Decreased/ Decreased	-	
		45		0 (CMTES)	- 5,5,5	- 5,5	- 5,5	- 5,5	7/8 (fingers)	7/8 (ankle)	shortened Achilles	Decreased/ Absent	Absent/ Absent	-	
	III:4	47	35	5 (CMTES)	+ 5,4+,4	- 5,5	+ 4,5,4	- 5,5	NA	NA	Pes cavus	Absent/ Absent	Absent/ absent	+ toes	
		59		8 (CMTES)	+ 4,4,4	- 5,5	+ 2,3	- 5,5	7/8 (fingers)	4/8 (ankle)	Pes cavus	Absent/ Absent	Absent/ Absent		
	II:7	63	No	3 (CMTES)	+ 4+,4, 4+	- 5,5	+ 5,5,4	- 5,5	7/8 (fingers)	6/8 (ankle)	-	Decreased/ absent	Absent/ Absent	-	
	III:8	45	No	0 (CMTES)	- 5,5,5	- 5,5	- 5,5	- 5,5	7/8 (fingers)	6/8 (ankle)	shortened Achilles	Normal/ Normal	Normal/ absent	-	
	IV:1	25	No	0 (CMTES)	- 5,5,5	- 5,5	- 5,5	- 5,5		7/7	Pes planus	Normal/ Normal	Normal/ decreased	-	
Family 2	III.2	69	30	19 (23)	+ 4, 4, 4	-	+ 1, 1	+ 4, 4	Normal	RED Toe		Normal/ Normal	Absent/ Absent	-	
	III.7	59	50	14 (17)	+ 4, 4, 4	-	+ 4, 5	-	Normal	RED knee	Pes cavus	Normal/ Normal	Normal/ Normal	-	
	IV.2	44	30	15 (21)	+ 4, 4, 4	-	+ 1, 4	-	Normal	RED knee	Pes cavus	Normal/ Normal	Absent/ Absent	-	Nasal voice
	IV.3	41	30	12 (17)	+ 4, 4, 4	-	+ 3, 4	-	Normal	RED ankle		Normal/ Decreased	Normal/ Absent	-	
	IV.9	20	13	16 (18)	-	-	+ 3, 4	-	Normal	RED knee	Pes cavus	Normal/ Absent	Absent/ Absent	-	
Family 4	76014-1000	33	12	19 (26)	+ 4-,3,3	- 5,5	+ 0,4	- 5,5	Reduced L side, Normal R side	Absent ankle	Pes cavus	Normal/ Normal	Absent/ absent	+	Severe headaches, dizziness, swallow/GI issues, Pulmonary issues, can't sleep on back
	76014-1002	45	30s	15 (20)	+ 4+,4+,3	+ 5,4+, 4+	+ 4,4+	- - 5,5	Normal	Absent ankle, reduced knee	Pes planus	Absent/ Absent	Reduced/ absent	-	Dizziness, swallow/GI issues
	76014-1006	36	31	6 (6)	- 5,5,5	- 5,5,5	- 5,5,5	- 5,5	Normal	Normal	-	Normal/ Normal	Normal/ absent	+	Tripping, foot drop, no hand issues

Family 6	542_II:2	61	9	25	+ 0/5	-	+ 0/5	-	Absent in fingers	Absent to knee	Pes cavus	Reduced	Absent	-	
	542_III:3	55	10						Absent in fingers	Absent to mid shin	Pes cavus	Reduced	Absent	-	
	542_III:6	30	12		+ 2/5	-	+ 0/5	-	Absent in fingers	Absent to mid shin	Pes cavus	Reduced	Absent	-	
	542_III:9	26	11						Absent in fingers	Absent to mid shin	Pes cavus	Reduced	Absent	-	
	542_IV:1	10	8								Pes cavus	Normal	Reduced	-	
	3074_I:2	88	36						Absent in fingers	Absent to mid shin	Pes cavus	Reduced	Absent		
	3074_II:1	66	25	21	+ 3/5	-	+ 0/5	-	Absent in fingers	Absent to mid shin	Pes cavus	Reduced	Absent		
	3074_II:3	64	32		+ 4/5	-	+ 2/5	-	Normal	Absent to ankle	Pes cavus	Normal	Absent		
	3074_II:4	59	21		+ 4/5	-	+ 4/5	-	Normal	Absent to ankle	Pes cavus	Reduced			
	3074_II:5	59	25		+ 4/5	-	+ 4/5	-	Normal	Absent to ankle	Pes cavus	Reduced	Absent		
Family 7	II:2	33	18	22	+ 0	-	+ 0	-	Absent in fingers	Absent to ankle	Pes cavus	Absent/ Absent	Absent/ Absent	+	Gait ataxia, hand muscle atrophy, uses AFOs

Motor weakness based on MRC scale (0-5): "+" = weakness present, "-" = no weakness detected/no problems. LL Distal weakness assessed by Anterior Tibialis and Gastrocnemius, LL Proximal weakness assessed by Ilio Psoas and Quadriceps; UL Distal weakness assessed by First Dorsal Interosseous, Abductor Pollicis Brevis, and Adductor Digiti Minimi, UL Proximal weakness assessed by Deltoids, Biceps Brachii, and Triceps. Vibration based on Rydell tuning fork with "5" on scale of "8" being considered normal and Cutaneous based on Pinprick sensation: Normal is no decrease compared to the examiner, 'Red' is reduced, and 'abs' is absent up to level indicated. Both motor and sensory evaluations were based on worst score observed of the two limbs. CMTNSv2 scores are separable into <10 (mild), 11-20 (moderate) or >20 (severe) impairment. [8]

Online resources

HGMD	(https://portal.biobase-international.com/hgmd
ClinVar	(https://www.ncbi.nlm.nih.gov/clinvar/
OMIM	(https://omim.org
Annovar	(http://annovar.openbioinformatics.org)
ExAC	(http://exac.broadinstitute.org)
gnomAD	(http://gnomad.broadinstitute.org)
SIFT	(http://sift.jcvi.org/)
Provean	(http://provean.jcvi.org/index.php)
LRT	(http://www.genetics.wustl.edu/jflab/lrt_query.html)
MutationTaster	(http://www.mutationtaster.org)

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