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A FAMILY WITH NEUROPATHIES AND AN MFN2 VARIANT

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Background

- Charcot-Marie-Tooth (CMT) is a phenotypically and genetically heterogenous group of inherited peripheral neuropathies. With a prevalence of 1:2500, it is the most common hereditary neurologic condition.[1-4]
- CMT typically manifests as chronic, length-dependent motor and sensory loss resulting from demyelination (CMT1) or axonal loss (CMT2). Onset and severity are widely variable, even amongst individuals with identical mutations.[1-4,13-15,19-22]
- Next-generation sequencing (NGS) has facilitated rapid CMT gene discovery in recent years. To date, >100 CMT-causative genes are reported, with substantial allelic and locus heterogeneity observed.[5-10]
- Mutations in the mitofusin-2 (MFN2) gene cause axonal CMT (CMT2A), typically dominantly-inherited missense mutations.[1,2,11-16] Additional MFN2-associated symptoms are common,[10] including: optic atrophy,[17-20] autonomic dysfunction,[20-21] diaphragm/respiratory dysfunction,[20] neuropathic pain.[10,25]
- MFN2, a mitochondrial membrane GTPase, is essential for mitochondrial dynamics and trafficking. It is critically involved in axonal transport. [11-16, 22-25]
- Today, NGS is employed clinically through targeted multigene panels. Panels are a sensitive, efficient tool for establishing definitive molecular CMT diagnosis, with diagnostic yields approaching 70% in some cohorts.[5-10]

Case Description & Results

We present a case of a 55-year-old man with chronic length-dependent sensory changes, autonomic symptoms, and a positive family history.

Initial evaluation (age 52). Presented with painful parasthesias in bilateral feet and occasional orthostatic lightheadedness. Examination revealed decreased sensation below the ankles bilaterally with preserved strength and reflexes. NCS/EMG demonstrated mild, symmetric axonal sensorimotor polyneuropathy consistent with CMT2 (Table 1). Autonomic function testing was abnormal, suggesting axonal small fiber neuropathy and dysautonomia. Laboratory evaluation was unremarkable.

Re-evaluation (age 55). Presented with worsening neuropathic and orthostatic symptoms. He reported severe orthostatic hypotension and exertional syncope. He endorsed subacute visual changes. Examination and NCS/EMG confirmed progression of axonal polyneuropathy. Further family history revealed similar symptoms in his mother (IB) and sister (IIB). Inherited neuropathy was suspected.

SENSORY NCS

Nerve	LEFT LE Lat. ms	LEFT LE Amp. µV	LEFT LE SCV m/s	RIGHT LE Lat. ms	RIGHT LE Amp. µV	RIGHT LE SCV m/s
Superficial Peroneal N.	2.55	3.7 ↓	47	*NR*	*NR*	*NR*
Sural N.	2.97	2.7 ↓	47	2.81	1.1 ↓	50

MOTOR NCS

Nerve		LEFT LE Lat. ms	LEFT LE Amp. mV	LEFT LE MCV m/s	RIGHT LE Lat. ms	RIGHT LE Amp mV	RIGHT LE MCV m/s
Peroneal N. (EDB)	A	5.73	4.5 *	-	4.79	3.3	-
	FH	14.01	3.4	38	13.65	2.5	38
	PF	16.3	3.1	43	15.52	2.5	43
Tibial N. (AH)	A	4.79	4.6	-	4.69	4.7 *	-
	PF	15.83	2.8	38	14.53	3	45

Table 1. Nerve Conduction Studies (NCS) of proband (IIA) at age 55. Sensory NCS (top) and Motor NCS (bottom). Results suggest neuropathy progression with reduced (red font) or absent sensory amplitudes, and normal-range CMAP amplitudes that were decreased from prior study (at age 52). LE = Lower Extremity, Lat. = Latency (for sensory studies, onset latency is used), Amp. = Amplitude, SCV = Sensory Conduction Velocity, MCV = Motor Conduction Velocity, EDB = Extensor Digitorum Brevis muscle, AH = Abductor Hallicus muscle, A = Ankle, FH = Fibular Head, PF = Popliteal Fossa.

Case Description & Results

Targeted gene sequencing of the proband (IIA) was obtained using the 72-gene Comprehensive Neuropathies Panel through Invitae commercial laboratories (San Francisco, CA). Results returned heterozygous variants of uncertain significance (VUS) in 3 genes: KIF1A (c.3584G>A; p.Arg1195His), **MFN2 (c.1979C>A; p.Ala660Asp)**, PLEKHG5 (c.823G>A; pGly275Ser).

Confirmatory familial testing revealed all 3 variants in his mother (IB), and MFN2 and PLEKHG5 variants in his sister (IIB). Both individuals had electrodiagnostically-confirmed axonal polyneuropathy and reported additional symptoms (Table 2). MFN2-related disease was suspected in this family.

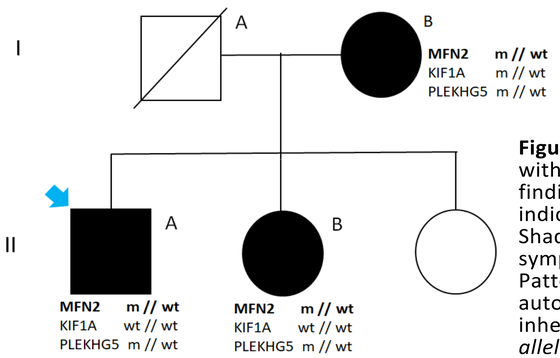


Figure 1. Family pedigree with summarized variant findings. **Proband (IIA)** is indicated by blue arrow. Shaded shapes represent symptomatic individuals. Pattern suggests likely autosomal dominant inheritance. *m = mutated allele, wt = wild type (normal) allele.*

Patient (Pedigree)	Eval age	Onset age	Sensorimotor features	Autonomic features	Additional features
Proband (IIA)	55	44	• Distal LL (below ankles), bilateral: numbness, parasthesias, "burning" pain, hyperesthesia, hot/cold sensory changes	• Orthostatic hypotension, exertional syncope • Hyper-/hypo- hidrosis • Dry eyes, bilateral	• Subacute ↓ visual acuity (right eye: 20/60, left eye: 20/25) • Paracentral scotoma, right eye
Mother (IB)	85	67	• Distal LL (below knees), bilateral: numbness, parasthesias, "burning" pain, hyperesthesia, hot/cold sensory changes • Diaphragm paralysis, bilateral	• Dry eyes, bilateral	• Wheelchair for ambulation • NIV for respiratory insufficiency • Chronic ↓ visual acuity (right eye: 20/100, left eye: 20/100)
Sister (IIB)	60	47	• Distal LL (below ankles), bilateral: numbness, parasthesias, "burning" pain, hyperesthesia, hot/cold sensory changes	• Early satiety, constipation • Orthostatic hypotension, presyncope	• Scintillating scotomas, bilateral

Table 2. Clinical features of proband (IIA) and symptomatic family members (IB, IIB). In all 3 individuals, sensorimotor symptoms were the presenting symptoms at onset. In patients IIA and IIB, autonomic symptoms began in 5th decade of life and were confirmed with quantitative autonomic function testing. *LL = Lower Limb*

Discussion & Conclusions

We describe the case of a 55-year-old man (proband, IIA) with axonal polyneuropathy, associated autonomic and visual symptoms, and a family history of the same symptoms. Clinical and genomic evaluation of the proband and 2 relatives (IB, IIB) suggests that the shared variant in **MFN2 (c.1979C>A; p.Ala660Asp) is the most likely punitive mutation in this family.**

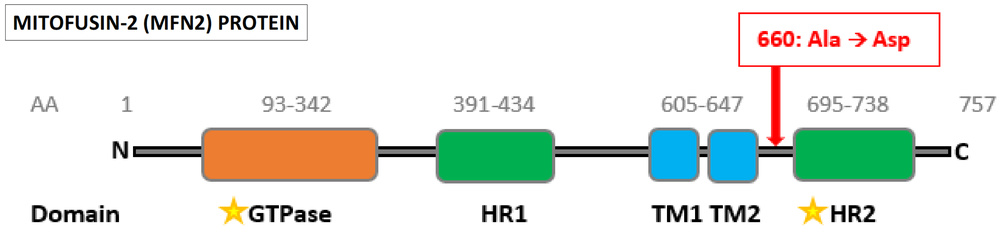
- All 3 patients had electrodiagnostic and clinical features of length-dependent axonal polyneuropathy (CMT2). All developed symptoms in adulthood, although onset ranged from 4th decade (IIA, IIB) to 6th decade (IB) of life. All had additional symptoms, which were consistent with those commonly reported in MFN2-related disease - neuropathic pain and visual symptoms (IIA, IB, IIB), autonomic dysfunction (IIA, IIB), diaphragm weakness with respiratory difficulty (IB) - although severity was variable between family members. Pedigree suggests autosomal dominant inheritance.
- The identified MFN2 sequence variant yields a dominantly-inherited missense mutation, resulting in an amino acid substitution between C-terminal TM2 and HR2 domains. This alteration disrupts physiologic MFN2 protein function according to Invitae's *in silico* functional protein modeling. This mutation is located far from the highly-conserved GTPase domain; such C-terminal (non-GTPase) mutations are associated with adult-onset, non-severe disease and phenotypic heterogeneity, consistent with this family's phenotype. [21-25]
- The other variant shared by all 3 patients, in PLEKHG5, is unlikely to be the culprit given its typical autosomal recessive inheritance pattern.

Despite an established link between MFN2 mutations and CMT2A, the molecular mechanisms by which MFN2 mutations lead to disease are largely unknown. This is likely due to the complex, multifunctional role of MFN2 in neurons. [11,14]

Targeted multigene panels have allowed for efficient, detailed genomic investigation in CMT patients. When VUS result, interpretation is challenging. Confirmatory family studies can help to clarify variant pathogenicity. [5-10]

The genetic and clinical data in this report help to broaden the spectrum of MFN2-related disease and strengthrn genotype-phenotype correlations in CMT. This understanding can improve genetic counseling of patients and support future disease-focused therapies. [22,23]

MITOFUSIN-2 (MFN2) PROTEIN



This MFN2 sequence variant **(c.1979C>A, p.Ala660Asp)** leads to: a moderately conserved **Alanine (Ala)** is replaced with an **Aspartic acid (Asp)** at position **660** of MFN2 protein. This alteration may affect protein function.

- There is a moderate physiochemical difference between Asp and Ala residues.
- *In silico* functional protein modeling suggest this residue change is likely to disrupt MFN2 protein function.

Figure 2. Schematic representation of MFN2 protein structure. MFN2 is a 757 AA protein with 4 major functional domains: 1 GTPase domain, 2 Heptad Repeat (HR) coiled-coil domains, and 2 transmembrane (TM) domains. The GTPase and HR2 domains interact to execute GTP hydrolysis during mitochondrial fusion; pathogenic MFN2 mutations are most commonly located in these domains (noted by star). GTPase mutations result in severe, childhood-onset CMT2A. Non-GTPase mutations are associated with less severe, adult-onset CMT2A with broad phenotypic heterogeneity.[14,15,21-24] The...