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Identification of a pathogenic *PMP2* variant in a multi-generational family with CMT type 1: clinical gene panels versus genome-wide approaches to molecular diagnosis

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Abstract

Charcot-Marie-Tooth (CMT) disease type 1 is an inherited peripheral neuropathy characterized by demyelination and reduced nerve conduction velocities. We present a multi-generational family with peripheral neuropathy in whom clinical CMT panel testing failed to conclude a molecular diagnosis. We found a *PMP2* pathogenic variant c.155T>C, p.(Ile52Thr) that segregates with disease suggesting that *PMP2* variants should be considered in patients with neuropathy and that it may be prudent to include in clinical CMT gene panels.

Keywords

Charcot-Marie-Tooth disease; peripheral myelin protein 2; myelin P2 protein; peripheral neuropathy; CMT; PMP2

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CONFLICTS OF INTEREST

J.R.L. has stock ownership in 23andMe and Lasergen, is a paid consultant for Regeneron, and is a co-inventor on multiple United States and European patents related to molecular diagnostics for inherited neuropathies, eye diseases, and bacterial genomic fingerprinting. Other authors have no potential conflicts to report.

1. INTRODUCTION

Charcot-Marie-Tooth (CMT) disease, or hereditary motor and sensory neuropathy (HMSN), is an inherited peripheral neuropathy with an estimated prevalence of ~1 in 2500 [1,2]. CMT types are categorized based on clinical, electrophysiological, and histopathological features observed, i.e. demyelinating (type 1) or axonal (type 2) neuropathy [3,4]. CMT shows considerable genetic heterogeneity. To date, variants in more than 80 genes have been shown to cause CMT [5]. CMT type 1 is characterized by decreased motor nerve conduction velocities (< 38 m/s), sensory loss, progressive muscle weakness, distal limb atrophy and myelin defects [6]. Most patients with CMT have a duplication or missense variant in the *PMP22* gene that encodes peripheral myelin protein of 22 kDa [7,8].

Recent studies have reported *PMP2,* encoding peripheral myelin protein 2, a myelin structural protein of 15 kDa, as a novel CMT1 disease gene in subjects from four families with autosomal dominant peripheral neuropathy [9,10,11]. PMP2 is present in Schwann cells in the peripheral nervous system (PNS) [12] and is a part of the highly conserved fatty acid-binding proteins (FABPs) family. PMP2 is involved in lipid homeostasis of myelin and may play a role in remyelination of the injured PNS [13,14]. Homozygous knockout Pmp2(−/−) mice showed a temporary reduction in motor nerve conduction velocities but did not show any overt myelin defects [13]. We describe an additional family with CMT1 with a rare variant in *PMP2* c.155T>C, p.(Ile52Thr) identified by whole exome sequencing (WES) that segregates with disease; clinical gene panel testing was unable to conclude a molecular diagnosis

2. CASE PRESENTATION

The proband is a 30 year old female with a clinical diagnosis of hereditary demyelinating neuropathy classified as CMT. Symptom onset was at 5 years of age, with a gradual increase in lower extremity weakness. Bilateral *pes cavus* was noted at 12 years, progressing to *pes planus* by 20 years; she has undergone at least two right foot orthopedic procedures. Examination at 21 years demonstrated lower extremity weakness, particularly of dorsiflexion at the ankles, absence of deep tendon reflexes, and an abnormal steppage gait. Four generations of similarly affected relatives were noted, with male to male transmission of the disease trait, most consistent with an autosomal dominant condition (**Figure 1A**). Her nerve conduction studies were consistent with a demyelinating neuropathy (**Figure 1B**). Prior to WES, the proband underwent extensive genetic testing including deletion and duplication testing for *PMP22*, as well as clinical CMT gene panel testing through Athena diagnostics (11 genes) and Fulgent Genetics (49 genes); the list of genes tested in these panels is included in Supplementary material (Table S1).

3. METHODS

Informed consent was obtained from the proband and available family members in accordance with the Baylor-Hopkins Center for Mendelian Genomics (BHCMG) research protocol (Baylor College of Medicine IRB protocol number: H-29697). Whole exome

sequencing (WES) was performed on the proband, and affected paternal half-brothers. Sequencing and variant prioritization workflow was performed as previously described [15]. Sanger sequencing was used for confirmation and segregation of the potential disease-causing variant.

4. RESULTS

WES analysis revealed a heterozygous rare variant c.155T>C, p.(Ile52Thr) in *PMP2* (NM_002677) in the proband and affected paternal half-brothers. Sanger sequencing confirmed the variant and segregation according to Mendelian expectations for an autosomal dominant trait; i.e. multigenerational vertical transmission and evidence for male-to-male transmission **(Figure 1).** This variant has been reported as disease-causing in a family with CMT1 [11], is predicted likely damaging by multiple bioinformatic algorithms [16,17,18 19], is conserved (PhyloP) [20], and has a likely pathogenic CADD [21] score of 28.3.

5. DISCUSSION

We first reported *PMP2* as a candidate CMT gene in a family with demyelinating neuropathy [9]. The putative pathogenic variant in *PMP2* (p.Ile43Asn) segregated with disease. Further functional evidence of *PMP2* pathogenicity was shown by morpholino knockdown of *PMP2* orthologues in zebrafish leading to a motor neuron phenotype; the disease phenotype could be rescued by wild-type human PMP2 mRNA. However, overexpression of wild-type human mRNA also resulted in a motor neuron phenotype suggesting dosage sensitivity of the PMP2 transcript. Hong *et al* [10] described an autosomal dominant family with demyelinating CMT neuropathy with the same *PMP2* (p.Ile43Asn) variant. They assessed *PMP2* pathogenicity using transgenic mouse models to show that overexpression of wild-type as well as mutant PMP2 caused abnormal motor function resembling the CMT1 phenotype. Motley *et al* [11] described two families with demyelinating neuropathy with potential *de novo* dominant pathogenic variants in *PMP2* (p.Thr51Pro, p.Ile52Thr) causing disease. Ruskamo *et al* [22] studied the molecular basis of known *PMP2* disease variants by X-ray crystallography and determined that the specific variants did not alter overall folding of the protein but altered its biophysical properties and functional dynamics.

The identification of antibodies to PMP2 in animal models of Guillain-Barre syndrome [23,24], provided an intriguing molecular link between inherited and autoimmune-mediated neuropathies. As PMP2 plays a role in lipid homeostasis of myelin and may bind to cholesterol [25,26], potentially considering future therapeutic interventions with a cholesterol rich diet may be warranted [27]. Additionally, patients being treated with statins should be carefully monitored for exacerbation of neuromuscular symptoms.

Our report presents additional evidence classifying *PMP2* as an established neuropathy disease gene, and supports the inclusion of *PMP2* in routine clinical testing for distal symmetric polyneuropathy [28]. Moreover, we document limitations of disease gene panel testing for molecular diagnosis as newly described disease genes may not be part of the clinical testing panel. The continued elucidation of the molecular etiology of CMT informs

understanding of the pathogenesis of inherited neuropathy, improves molecular testing, further empowers genetic counseling, enables more robust prognostic information, and guides future development of targeted therapies for this chronic, progressive condition [29].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CMT	Charcot-Marie-Tooth disease
NCV	Nerve conduction velocity
UL	Upper limb
LL	Lower limb
CADD	Combined annotation dependent depletion

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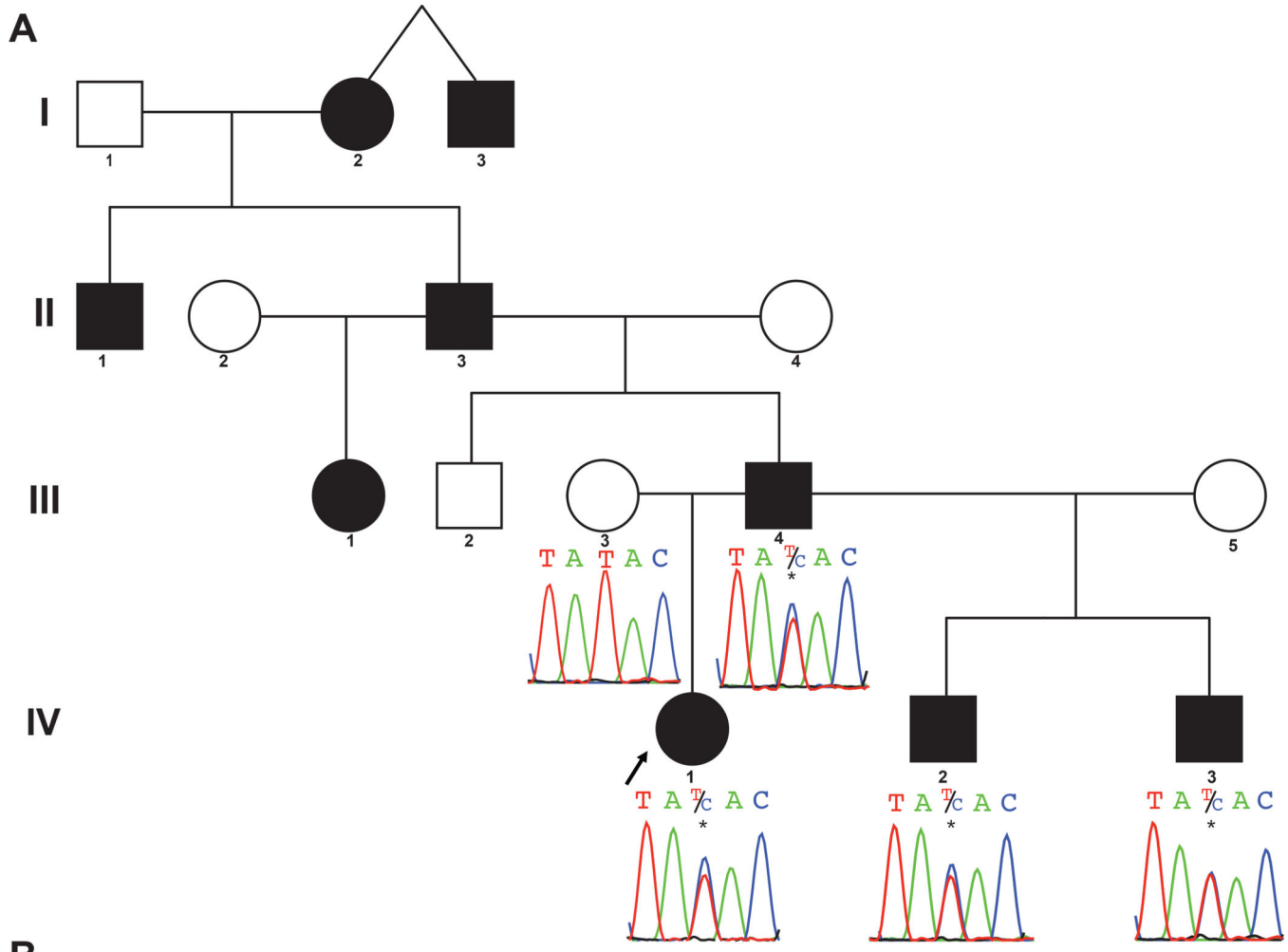

	Nerve Conduction Velocities				
Age	Right elbow	Right ulnar	Right median	Right median amplitude	Distal Latency
21 years	35.5 m/s	18 m/s	17.4 m/s	7.1 mV	5.2

Figure 1.

A. Pedigree and Sanger variants. Four-generation pedigree shows the affected proband and 8 additional paternal relatives (filled circles and squares). Sanger sequencing of the identified PMP2 c.155T>C, p.(Ile52Thr) variant demonstrates segregation of the variant with the phenotype in the proband, father, and two paternal half-brothers, and absence of the variant in the unaffected mother.

B. Nerve conduction studies of proband. Markedly reduced motor nerve conduction velocities of proband at 21 years were consistent with CMT1 (<38 m/s) demyelinating neuropathy.