T118M *PMP22* Mutation Causes Partial Loss of Function and HNPP-like Neuropathy

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Objective: **To determine the clinical consequences of the PMP22 point mutation, T118M, which has been previously considered to either cause an autosomal recessive form of Charcot-Marie-Tooth (CMT) disease or be a benign polymorphism.** ***Methods:*** **We analyzed patients from five separate kindreds and characterized their peripheral nerve function by clinical and electrophysiological methods.** ***Results:*** **All heterozygous patients had clinical and/or electrophysiological features of a neuropathy similar to hereditary neuropathy with liability to pressure palsies (HNPPs). The homozygous patient had a severe axonal neuropathy without features of demyelination.** ***Interpretation:*** **These findings suggest that T118M PMP22 retains some normal PMP22 activity, allowing the formation of compact myelin and normal nerve conduction velocities in the homozygous state. Taken together, these findings suggest that T118M is a pathogenic mutation causing a dominantly inherited form of CMT by a partial loss of PMP22 function.**

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Altered dosage of PMP22, a myelin membrane protein expressed in Schwann cells, is a major cause of inherited peripheral neuropathies.[1,2] Charcot-Marie-Tooth disease type 1A (CMT1A) results from a duplication of a region of chromosome 17 containing the *PMP22* gene.[3] Alternatively, hereditary neuropathy with liability to pressure palsies (HNPPs) is caused by a deletion of the same chromosome 17 region.[4] The existence of these diseases suggests that the level of PMP22 expression must be maintained within a critical range for proper peripheral nerve function.[1,5–7]

Although CMT1A and HNPP are caused by altered gene dosage of the same chromosomal region, their clinical phenotypes are different. Most CMT1A patients develop slowly progressive sensorimotor length-dependent neuropathies associated with uniformly slow nerve conduction velocities (NCVs).[8,9] In contrast, most HNPP patients present with transient, focal motor, or sensory deficits and have conduction slowing only at sites of mechanical compression.[7]

Missense mutations in *PMP22* cause a variety of neuropathy phenotypes.[1,5] Several mutations cause a syndrome similar to that found with HNPP, suggesting that they produce a loss of PMP22 function.[10] The majority of *PMP22* missense mutations, however, act genetically as autosomal dominants,[11–16] suggesting that they produce a gain of PMP22 function. A few mutations, such as R157W,[17] R157G,[18] and T118M[19] are autosomal recessive in nature.

A missense mutation at codon 118 of the *PMP22* gene producing a threonine to methionine amino acid substitution (T118M) has been reported in the context of familial neuropathy,[19] but both its clinical relevance and its effect on PMP22 function are controversial.[20] The mutation initially was identified in a female with severe CMT1 who was hemizygous for T118M because of an HNPP deletion at the other allele.[19] Her two sons who inherited the HNPP deletion had a typical HNPP phenotype, whereas a third son who inherited only the T118M mutation was normal, suggesting that the T118M mutation is recessive. Subsequently, several investigators identified the T118M mutation in unaffected parents of neuropathy patients[20–23] or in compound heterozygotes whose phenotype was not dis-

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tinct from either HNPP or CMT1A. The T118M mutation has also been reported in a family in which the parents have a mild phenotype and carry both a CMT1A duplication and a T118M mutation, whereas a younger family member has a more severe disease but carries only the duplication,[24] suggesting that T118M is partial loss-of-function mutation that can mitigate the effects of the duplication.

We have had the opportunity to address this controversy by evaluating five distinct kindreds with the T118M missense mutation in *PMP22*. Clinical and electrophysiological evaluation of these individuals demonstrate that T118M is a dominantly inherited pathogenic partial loss-of-function mutation that can cause severe axonal degeneration in the homozygous state.

Results

Molecular Studies

Affected individuals from the five kindreds were confirmed to have the T118M mutation in either a heterozygous (Cases 1–4) or a homozygous (Case 5) state. In kindred 4 the T118M mutation is carried on the CMT1A duplication as evidenced by their cotransmission.

Clinical Findings

CASE 1 (KINDRED 1). A 53-year-old woman developed discomfort in her right elbow followed by numbness in her right fourth and fifth finger 2 weeks later. Several months later, she noted dysesthesias in her left middle finger; 2 weeks after this she noted numbness in her left foot and numbness on the anterior surfaces of both legs. On examination, her cranial nerve and motor examinations were normal. Sensory evaluation was also normal with the exception of decreased vibratory sensation in her toes. Patellar and Achilles deep tendon reflexes were uniformly decreased. Nerve conduction studies (NCSs) showed focal slowing of the ulnar nerve around the elbow and the peroneal nerve around the fibular head, and a median mononeuropathy at the wrist (Table 1).

CASE 2 (KINDRED 2). A 35-year-old man noted tingling in his hands and feet. He maintained his normal vigorous activities, including running and weightlifting. His neurological examination showed a decrease of pain and temperature sensation in a stocking distribution but normal vibratory and position sense. Deep tendon reflexes were normal, including the Achilles reflexes. NCSs showed a prolonged peroneal distal motor latency but were otherwise normal (see Table 1).

CASE 3 (KINDRED 3). The patient is a 35-year-old woman with a history of a left peroneal palsy. She first noted weakness in her left ankle as a teenager. Currently, she cannot rotate or dorsiflex her left foot and walks with a steppage gait on the left. Recently, she had a transient episode of numbness of her right fourth and fifth fingers. Light touch and joint-position sense are normal on neurological examination, but vibration, pinprick sensation, and temperature sensations are decreased in a stocking distribution. Deep tendon reflexes were normal. Electrophysiological studies demonstrated prolonged peroneal distal motor latencies, severely reduced peroneal CMAP amplitudes on the left associated with slow conduction velocities, and borderline peroneal CMAP amplitudes on the right associated with normal conduction velocities. NCVs in the upper extremities were normal (see Table 1).

CASE 4 (KINDRED 4). The proband is a 35-year-old woman with CMT1A caused by duplication of the *PMP22* gene on chromosome 17. She also has a T118M point mutation of *PMP22*. Her 4-year-old daughter also has both the CMT1A duplication and T118M point mutation. Although she initially wore braces and had heel cord lengthening because of toe

Table 1. Nerve Conduction Velocities

				Ulnar Nerve				Median Nerve			Peroneal Nerve			
Individual	Genotype	Age (yr)	Side	DML (msec)	NCV1 (m/sec)	NCV2 (m/sec)	CMAP (mV)	DML (msec)	NCV (m/sec)	CMAP (mV)	DML (msec)	NCV3 (m/sec)	NCV4 (m/sec)	CMAP (mV)
Kindred 1-1 (BAB 2352)	m/+	50	R	3.0	61.8	**43.3**	6.5	4.4	**45.9**	6.2	4.6	50	40.0	6.6
Kindred 2-1 (BAB 2470)	m/+	35	R	2.8	56.4	57.2	9.5	—	—	—	**6.5**	48.4	49.6	5.1
Kindred 3-1 (BAB 2309)	m/−	35	R	3.0	67	50	8.2	3.2	57.0	17.9	**8.0**	42.0	48.0	**2.7**
			R	3.6	58.0	50.0	7.9	2.6	59.0	20.4	**9.1**	34.0	28.0	0.4
Kindred 4-1 (BAB 2356)	m+/+	35	L	**5.3**	34	19	5.3	**10.1**	33	6.2	**6.8**	28	27	2.0
Kindred 5-1 (BAB 2435)	m/m	11	R	2.9	56.0	63.0	5.4	3.9	59.0	6.1	**6.7**	41.0	—	**2.1**
Kindred 5-2 (BAB 2433)	m/+	45	R	2.6	62.0	59.0	7.6	3.3	60.0	12.2	**5.9**	44.0	60.0	**3.2**
Kindred 5-3 (BAB 2432)	m/−	44	R	2.8	64.0	65.0	8.4	3.8	59.0	13.0	**5.6**	55.0	56.0	6.6

CMAP = compound muscle action potential; DML = distal motor latency, NCV = nerve conduction velocity; NCV1= NCV of wrist/elbow; NCV2 = NCV around elbow; NCV3 = NCV of ankle/knee; NCV4 = NCV around knee; m = T118M mutation; t = normal or wildtype; − = deletion.
Boldface letters signify abnormal values.

walking, she was able to keep up with her peers during childhood and was on the softball and swim teams during high school. She was able to ride a bicycle and roller skate without problems. Her feet were transiently numb when she attempted to ski. As an adult, her first symptoms of neuropathy were numbness in the hands and a throbbing pain in her feet. Currently, she occasionally trips on a curb or carpet. She does not wear orthotics. She experiences temporary numbness in her extremities and reports fatigue, particularly after exercise. On neurological examination, her strength was entirely normal. Her CMT Neuropathy Score (CMTNS) was 5, suggesting an extremely mild case of CMT1A.[25] NCVs in the upper extremities were 33 to 34m/sec (see Table 1).

The proband's 4-year-old daughter was also evaluated, and a limited NCS demonstrated a prolonged ulnar distal motor latency of 5.8 milliseconds. The daughter's CMTNS was 2. She currently falls frequently, in part because of tight heel cords. Her strength in the upper and lower extremities is normal as is vibration and position sense. She had a mild decrease in cold sensation in her feet. She is areflexic.

CASE 5 (KINDRED 5). An 11-year-old girl has been followed up in the neurology clinic since she was 5 years of age because of progressive muscle weakness and gait abnormalities. She was the product of a normal gestation and C-section delivery at 37 weeks because of a breech presentation. At birth she was found to have bilateral vertical tali, as well as hip and knee flexion contractures. Her prenatal history was unremarkable, and her family history was negative for neuropathy or other neuromuscular diseases. At 6 weeks of age, serial casting was begun to place her feet in a more neutral position. At 8 months of age, she underwent bilateral vertical tali release with transfer of the anterior tibial tendon to the neck of the talus. At 18 months of age, she began to walk independently but was thought to have generalized weakness. By age 5 years, she had normal muscle bulk and strength in her upper extremities and reduced strength in her proximal and distal lower extremities with distal tapering in the lower extremities. Reflexes were +1 in the upper extremities and absent in the lower extremities. There was no evidence of pes cavus or hammertoe formation. Her creatine kinase level was normal. Electromyography and NCS were normal as were magnetic resonance images of the spine and brain. By age 8 years, she had developed pes cavus changes in her feet and heel cord tightness with a prominent first metatarsal of the right foot. She underwent Achilles tendon lengthening, plantar fascial release, and osteotomy of the first metatarsal on the right foot. Hip x-rays demonstrated varus anteverted femoral necks with lateral subluxation. She remained stable until age 10 years when she began having more difficulty ambulating and required the use of a walker. At this time, NCVs were normal, whereas the electromyogram showed chronic partial denervation in all muscles tested with high-amplitude, long-duration motor units. NCS of the lower extremity performed at age 15 years demonstrated a prolonged distal motor latency of the peroneal nerve but were otherwise normal (see Table 1). Genetic testing for spinal muscular atrophy was normal. No *PMP22* duplication or deletion was detected and no mutations were found in *GJB1/Cx32*, *MPZ*, or *EGR2*. Sequence analysis of the *PMP22* gene, however, demonstrated a homozygous T118M mutation. Both parents were heterozygous for the T118M mutation but were asymptomatic and had normal neurological examinations. However, each had prolonged distal motor latencies for the peroneal nerve (see Table 1), although the rest of their NCVs were normal.

Discussion

We describe three unrelated individuals heterozygous for the T118M mutation that have a mild demyelinating neuropathy with clinical and electrophysiological features similar to HNPP. We also describe a mother and 4-year-old daughter with both a *PMP22* duplication and T118M mutation with clinical and physiological features of a unusually mild CMT1A phenotype and electrophysiological features usually found in HNPP, not CMT1A. Finally, we describe a patient homozygous for the T118M mutation who has a severe, predominantly axonal neuropathy and whose parents had electrophysiolgical features consistent with HNPP. In addition, the T118M mutation is a nonconservative substitution as predicted by the Grantham scale,[26] and threonine 118 is conserved in PMP22 across evolution. Taken together, these results demonstrate that the T118M mutation in PMP22 is a disease-causing mutation, and not a benign polymorphism.

Individuals heterozygous for the T118M mutation in our series have areas of focal nerve conduction slowing similar to those found in patients with HNPP. Nerve conduction velocities elsewhere were normal. HNPP is caused by deletion of one of the two *PMP22* alleles leading to decreased expression of normal PMP22.[27–29] This so-called "partial loss of PMP22 function" or "deficiency" results in myelin that is more sensitive to external compression. Comparison of the clinical and electrophysiological phenotype of our patients with those with HNPP thus suggests that the T118M mutation also produces a "partial loss of function" of PMP22. Consistent with this notion, Naef and Suter[30] demonstrated that most of the T118M mutant PMP22 was sequestered in the endoplasmic reticulum (ER) in transfected COS-7 cells, whereas wild-type PMP22 protein was transported to the plasma membrane. Thus, the T118M mutant protein that is inserted into myelin may function normally, but the

amount is reduced. A similar partial loss of function could also be caused by increased protein turnover.[31]

The individual homozygous for the T118M mutation has a clinical syndrome characterized by severe distal weakness with normal nerve conduction velocities and needle electromyogram studies demonstrating widespread chronic partial denervation, consistent with either a motor axonal neuropathy or motor neuron disease. Genetic tests for mutations known to cause motor neuron disease were negative in this individual. Interestingly, mice in which both *Pmp22* alleles have been inactivated by homologous recombination, which causes a complete loss of PMP22 function, have significant clinical weakness, marked nerve conduction velocity slowing, and pathological evidence of a severe demyelinating peripheral neuropathy.[32] If the T118M mutation causes a complete loss of PMP22 function, the homozygous patient and the homozygous knockout mouse should have a similar clinical and electrophysiological phenotype. How can these data be reconciled?

One possible interpretation is that the T118M mutation produces only a "partial" loss of PMP22 function, so that the mutant protein also retains some normal PMP22 activity. Consistent with this notion, there is a clear correlation of disease phenotype with the gene dosage of the normal and mutant *PMP22* alleles (Table 2). One copy of the mutant T118M allele and one copy of the normal *PMP22* allele can produce a clinical and electrophysiological phenotype similar to mild HNPP, consistent with a partial loss of PMP22 function of T118M. Two copies of T118M thus provide sufficient residual PMP22 activity to support myelination and normal nerve conduction velocities. Patients hemizygous for the T118M who also have a *PMP22* deletion, however, have even less PMP22 protein in their myelin sheaths than does the patient who is homozygous for T118M and thus have a more severe demyelinating peripheral neuropathy, akin to *Pmp22* homozygous knockout mice. Finally, a patient with both a *PMP22* duplication and a T118M mutation on one chromosome and a normal *PMP22* gene on the other (kindred 4) has a somewhat milder phenotype than the typical CMT1A patient, as does her daughter who has the duplication and T118M mutation as well. Taken together, these data suggest that the T118M mutation causes a partial loss of PMP22 function.

Table 2. Genotype–Phenotype Correlations

No. of T118M Alleles	No. of Normal Alleles	Phenotype	Genotype
1	0	Severe, demyelinating	T118M/−
2	0	Severe, axonal	T118M/T118M
1	1	Mild, HNPP	T118M/+
1	2	Mild, demyelinating	T118M+/+

HNPP = hereditary neuropathy with liability to pressure palsies.

Although it is likely that the T118M mutation produces a partial loss of PMP22 function, at least with respect to myelin formation, this mechanism does not explain why our patient with two mutant *PMP22* alleles has pronounced denervation without overt demyelination. One possibility is that the T118M amino acid substitution, in addition to its effect on myelination, also alters a biological function of PMP22 involved in maintaining normal axonal function. The presence of two copies of the T118M mutation might disrupt normal Schwann cell axonal interactions without altering myelination, causing severe axonal neuropathy, whereas the presence of only one copy of the mutant allele in the hemizygous patient might cause severe demyelination. The observation that some MPZ mutations cause axonal loss without apparent demyelination is a precedent for this suggestion.[34] A second possibility therefore is that the homozygous T118M mutation disrupts a step or steps in normal nerve development that is independent of myelination. *Pmp22* mRNA, for example, is widely expressed in many nonneural tissues during development, including muscle and the precartilagous condensations that form vertebrae.[35] Alteration of muscle or cartilage development may explain why this patient developed hip dysplasias or vertical tali.

From a molecular diagnostics and genetic counseling perspective, the clinical interpretation of the T118M mutation has been problematic. When identified in a patient manifesting neuropathy, does this change represent a recessive mutation, a dominant allele with reduced penetrance, or a benign polymorphism?[36] We initially suggested that T118M may represent a recessive gain-of-function mutation,[37] but we now present clinical and molecular data more consistent with a partial loss-of-function mutation that may behave as a dominant allele with reduced penetrance. When considered in isolation the terms recessive and dominant imply distinct inheritance patterns for patients and families, with recessive disorders much less likely to be passed on to subsequent generations. However, in cases such as patients with the T118M mutation, the distinction appears to rest on whether heterozygous patients develop symptoms. When heterozygous patients have very mild neuropathies, as described here, the neuropathy is classified as autosomal dominant; unaffected heterozygous individuals may represent reduced penetrance for this mutant allele.[38] However, if heterozygous patients remain asymptomatic and their homozygous children develop neuropathies, then the disorder is described as recessive. Obviously, this makes genetic counseling

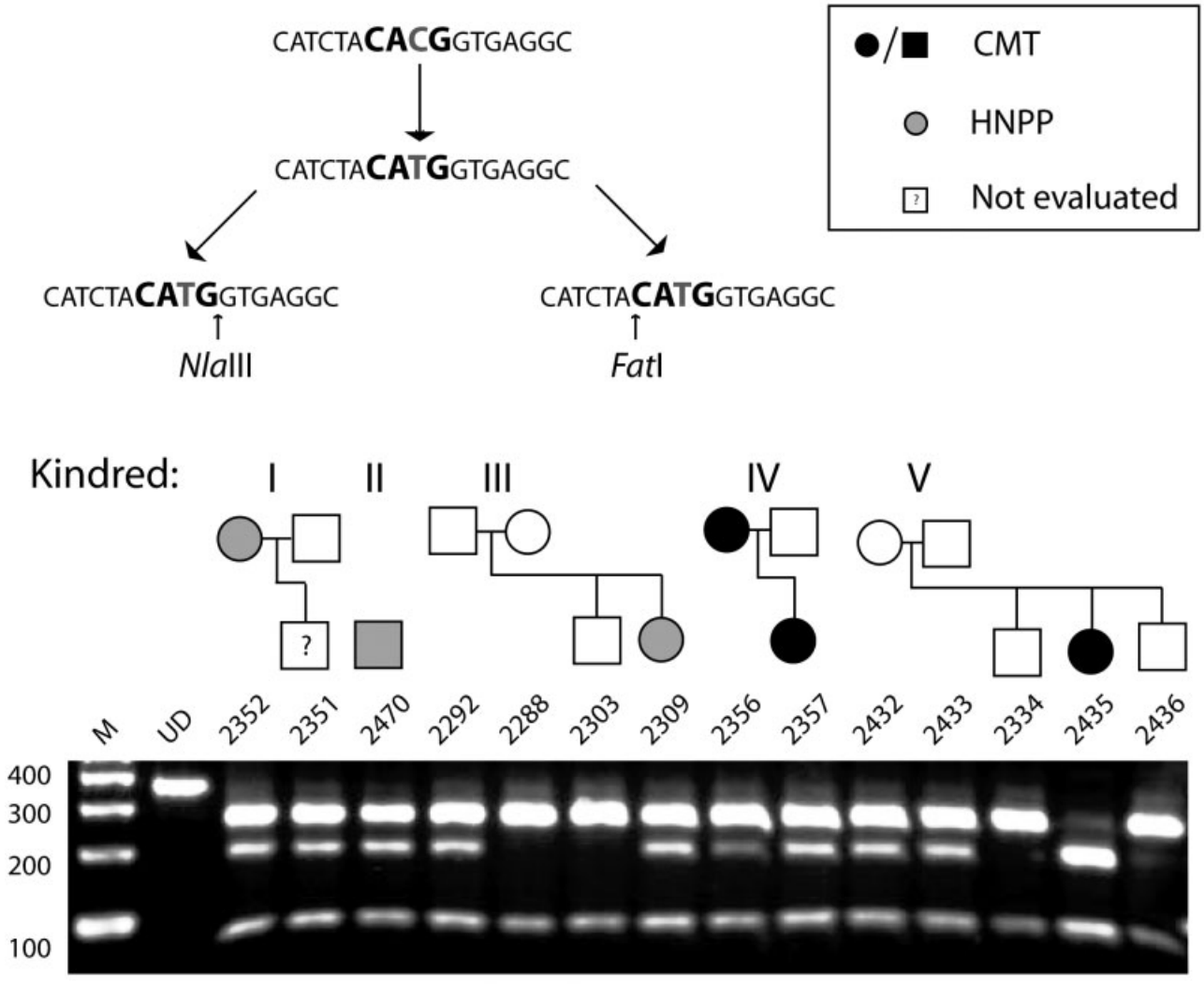

Fig. The 353C→T nucleotide change in PMP22, *which by conceptual translation leads to a Thr118Met change in the protein, creates* Nla*III and* Fat*I restriction endonuclease recognition sites. The family members are identified by four-digit identification numbers. The gel picture (1.5% metaphor agarose in 1XTBE, 5V/cm, room temperature, 1.5 hours) shows polymerase chain reaction (PCR)–amplified fragments of individual family members, each fragment separately digested with* Nla*III and* Fat*I (data not shown). The normal PCR fragment gives 255 and 57bp bands on digestion with* Nla*III, whereas the mutant gives 182, 73, and 57bp bands. Thus, in the mutant the 255bp band is split into 182 and 73bp fragments because the mutation creates a novel* Nla*III site. The 312bp band corresponds to the uncut PCR fragment. PCR was performed using the* PMP22 *primers 5F and 5R that originally were described by Lupski and colleagues.*[42] *Note that Patient 2435 is homozygous for the mutation, whereas her parents are heterozygous carriers. Lane 1 is a 100bp DNA molecular size marker and lane 2 is uncut PCR product control. A functional genomic analysis of the T118M mutation also predicts that it will cause disease. Using the Grantham scale, an estimate of chemical similarity, the T118M amino acid residue change is nonconservative and thus is potentially a phenotype-causing mutation. In addition, the T118 residue is conserved across a wide range of species (data not shown), suggesting that this amino acid is relevant to clinical outcome. One way of estimating this is by its conservation across species; disease-causing mutations are significantly more likely to occur at amino acid residues that are conserved across species.*[43] *Thus, the T118M change is a potential disease-causing change. CMT = Charcot-Marie-Tooth; HNPP = hereditary neuropathy with liability to pressure palsy.*

issues challenging. However, it also points out the limitation of using terms like recessive and dominant without considering the biological context of the disease. In a biological context, heterozygous T118M mutations mildly disrupt myelin to an extent that mild symptoms are likely to develop; homozygous mutations more severely disrupt peripheral nerve and disable patients.[39–41] Identification of additional patients homozygous for the T118M mutation or the generation of transgenic animals bearing the mutation will be necessary to more firmly establish the effect of this mutant allele.

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