

# Update on Charcot-Marie-Tooth Disease

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**Abstract** Charcot-Marie-Tooth disease (CMT) disease encompasses a genetically heterogeneous group of inherited neuropathies, also known as hereditary motor and sensory neuropathies. CMT results from mutations in more than 40 genes expressed in Schwann cells and neurons causing overlapping phenotypes. The classic CMT phenotype reflects length-dependent axonal degeneration characterized by distal sensory loss and weakness, deep tendon reflex abnormalities, and skeletal deformities. Recent articles have provided insight into the molecular pathogenesis of CMT, which, for the first time, suggest potential therapeutic targets. Although there are currently no effective medications for CMT, multiple clinical trials are ongoing or being planned. This review will focus on the underlying pathomechanisms and diagnostic approaches of CMT and discuss the emerging therapeutic strategies.

**Keywords** Charcot-Marie-Tooth · Hereditary · Neuropathy · Genetics · Therapy · Disease pathomechanism

## Introduction

Charcot-Marie-Tooth (CMT) disease is the most common inherited neurologic disorder with an estimated prevalence of 17 to 40/10,000. CMT can be separated into autosomal-dominant demyelinating (CMT1) and axonal (CMT2), X-linked (CMT1X), and autosomal-recessive neuropathies. A list of the disorders together with the causative mutation

and clinical phenotype is provided in Table 1. The rapidly increasing number of genes and loci identified (<http://www.molgen.ua.ac.be/CMTMutations/Mutations/MutByGene.cfm>) presents a challenge to clinicians. In spite of the surprising variability of genes involved in the pathogenesis of CMT, common molecular pathways have been identified within Schwann cells and axons that cause these genetic neuropathies (Fig. 1). The historical clinical classification based on nerve conduction velocity (NCV) is important and very useful, especially when combined with natural history data, facilitating the genetic diagnosis (Table 2). Uniformly slow NCV less than 38 m/s in the arms is characteristic of demyelinating CMT1 and NCV above this cutoff is typical of axonal CMT2. Intermediate conduction velocities (25–45 m/s) are often found in male patients with CMT1X and in individuals with other intermediate forms of CMT.

## The Different Forms of CMT

CMT1: Autosomal-Dominant Demyelinating Neuropathy

### *CMT Type 1A*

CMT type 1A (CMT1A) is the most common form of CMT and is caused by a 1.4-Mb duplication on chromosome 17p11.2 [1, 2]. The slowly progressive disorder usually starts in the legs beginning in the first two decades of life. Patients present with a “classical CMT phenotype” that is characterized by distal muscle weakness and atrophy, sensory loss, hyporeflexia, and skeletal deformity. Life span is not shortened and patients usually remain ambulatory throughout their life, although often need ankle-foot

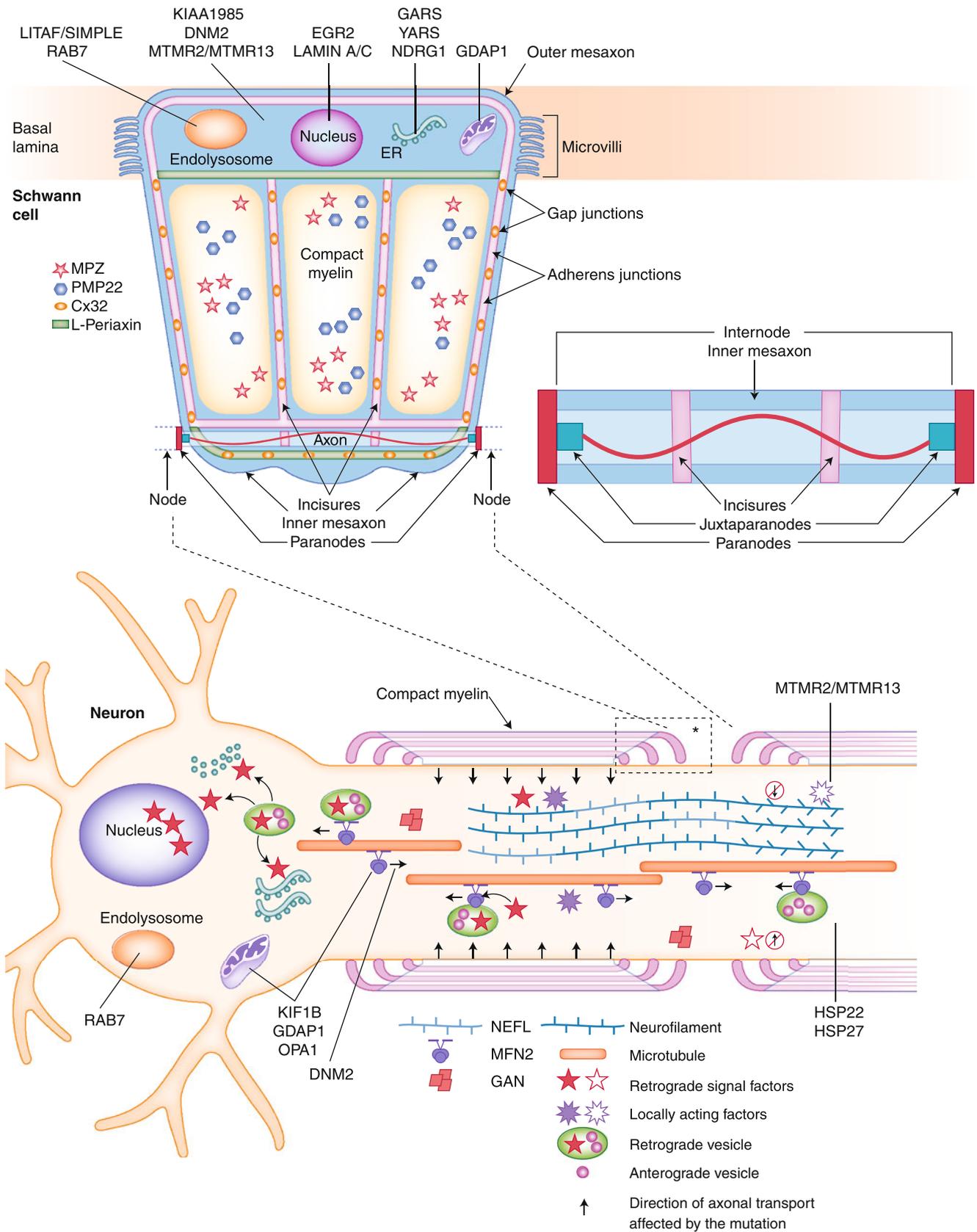
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**Table 1** Classification of CMT disease

Type	Gene/locus	Specific phenotype
<b>AD CMT1:</b>		
CMT1A	Dup 17p ( <i>PMP22</i> )	Classic CMT1
CMT1B	<i>PMP22</i> (point mutation) <i>MPZ</i>	Classic CMT1/DSD/CHN/HNPP CMT1/DSD/CHN/intermediate/CMT2
CMT1C	<i>LITAF</i>	Classic CMT1
CMT1D	<i>EGR2</i>	Classic CMT1/DSD/CHN
CMT1	<i>NEFL</i>	CMT2 but can have slow MCVs in CMT1 range ± early-onset severe disease
<b>HNPP:</b>		
HNPP	Del 17p ( <i>PMP22</i> ) <i>PMP22</i> (point mutation)	Typical HNPP Typical HNPP
<b>CMT1X:</b>		
CMT1X	<i>GJB1</i>	Intermediate ± patchy MCVs /male MCVs < female MCVs
<b>AR CMT4:</b>		
CMT4A	<i>GDAP1</i>	Demyelination or axonal, usually early onset and severe/vocal cord and diaphragm paralysis described/rare AD CMT2 families described
CMT4B1	<i>MTMR2</i>	Severe CMT1/facial/bulbar/focally folded myelin
CMT4B2	<i>MTMR13</i>	Severe CMT1/glaucoma/focally folded myelin
CMT4C	KIAA1985 ( <i>SH3TC2</i> )	Severe CMT1/scoliosis/cytoplasmic expansions
CMT4D (HMSNL)	<i>NDRG1</i>	Severe CMT1/gypsy/deafness/tongue atrophy
CMT4E	<i>EGR2</i>	Classic CMT1/DSD/CHN
CMT4F	Periaxin	CMT1/more sensory/focally folded myelin
CMT4H	<i>FGD4</i>	CMT1
CMT4J	<i>FIG4</i>	CMT1
CCFDN	<i>CTDP1</i>	CMT1/gypsy/cataracts/dysmorphic features
HMSN Russe	10q22-q23	CMT1
CMT1	<i>PMP22</i> (point mutation)	Classic CMT1/DSD/CHN/HNPP
CMT1	<i>MPZ</i>	CMT1/DSD/CHN/intermediate/CMT2
<b>AD CMT2:</b>		
CMT2A	<i>MFN2</i>	CMT2/usually severe/optic atrophy
CMT2B	<i>RAB7</i>	CMT2 with predominant sensory involvement and sensory complications
CMT2C	<i>TRPV4</i>	CMT2 with vocal cord and respiratory involvement
CMT2D	<i>GARS</i>	CMT2 with predominant hand wasting /weakness or dHMN-V
CMT2E	<i>NEFL</i>	CMT2 but can have slow MCVs in CMT1 range ± early-onset severe disease
CMT2F	HSP27 ( <i>HSPB1</i> )	Classic CMT2 or dHMN-II
CMT2G	12q12-q13.3	Classic CMT2
CMT2L	HSP22 ( <i>HSPB8</i> )	Classic CMT2 or dHMN-II
CMT2	<i>MPZ</i>	CMT1/DSD/CHN/intermediate/CMT2
CMT2 (HMSNP)	3q13.1	CMT2 with proximal involvement
<b>AR CMT2<sup>a</sup>:</b>		
AR CMT2A	<i>LMNA</i>	CMT2 proximal involvement and rapid progression described/also causes muscular dystrophy/cardiomyopathy/lipodystrophy
AR CMT2B	19q13.1-13.3	Typical CMT2
AR CMT2	<i>GDAP1</i>	CMT1 or CMT2 usually early onset and severe/vocal cord and diaphragm paralysis described/rare AD CMT2 families described
<b>DI-CMT:</b>		
DI-CMTA	10q24.1-25.1	Typical CMT
DI-CMTB	<i>DNM2</i>	Typical CMT
DI-CMTC	<i>YARS</i>	Typical CMT
<b>HNA:</b>		
HNA	<i>SEPT9</i>	Recurrent neuralgic amyotrophy

AD autosomal dominant; AR autosomal recessive; CHN congenital hypomyelinating neuropathy; CMT Charcot-Marie-Tooth; CMT1X X-linked CMT1; Del deletion; dHMN distal hereditary motor neuropathy; DI dominant intermediate; DSD Dejerine-Sottas disease; Dup duplication; HMSN hereditary motor and sensory neuropathy; HMSNL hereditary motor and sensory neuropathy-Lom; HMSNP hereditary motor and sensory neuropathy and neuronopathy; HNA hereditary neuralgic amyotrophy; HNPP hereditary neuropathy with liability to pressure palsies; MCV motor conduction velocity

<sup>a</sup> Also called AR CMT4



**Fig. 1** Shows proteins that are mutated in Charcot-Marie-Tooth (CMT) disease. Proteins have been assigned to Schwann cell and/or neuron organelles, and intracellular pathways involved in CMT are depicted. ER—endoplasmic reticulum

**Table 2** Distribution of the five most common CMT disease subtypes based on age of onset and physiology observed at the CMT Clinic of Wayne State University

Ulnar MNCV, <i>m/s</i>	Disease onset and walk-age onset	CMT subtypes	%
Very slow: $\leq 15$	1. Childhood onset Walk-age onset $\geq 15$ months	CMT1A	68
		CMT1B	32
	2. Childhood onset Walk-age onset $< 15$ months	CMT1A	100
	3. Adult onset <sup>a</sup>	CMT1A	100
	Slow: $15 <$ and $\leq 25$	1. Childhood onset Walk-age onset $\geq 15$ months	CMT1A
CMT1B			6
CMT1X males			6
2. Childhood onset Walk-age onset $< 15$ months		CMT1A	98
		CMT1X males	2
3. Adult onset <sup>a</sup>		CMT1A	94
		CMT1B	3
		CMT1X males	3
Slow: $25 <$ and $\leq 35$		1. Childhood onset Walk-age onset $\geq 15$ months	CMT1A
	CMT1X males		33
	2. Childhood onset Walk-age onset $< 15$ months	CMT1A	55
		CMT1B	3
		CMT1X males	42
	3. Adult onset <sup>a</sup>	CMT1A	88
		CMT1B	6
		CMT1X males	6
	Intermediate: $35 <$ and $\leq 45$	1. Childhood onset Walk-age onset $\geq 15$ months	CMT1X males
2. Childhood onset Walk-age onset $< 15$ months		CMT1B	17
		CMT1X males	41
		CMT1X females	17
		HNPP	25
3. Adult onset <sup>a</sup>		CMT1B	55
		CMT1X males	18
		CMT1X females	18
	HNPP	9	
Normal: $> 45$	1. Childhood onset Walk-age onset $\geq 15$ months	CMT1X females	33
		CMT2A	33
		HNPP	33

**Table 2** (continued)

Ulnar MNCV, m/s	Disease onset and walk-age onset	CMT subtypes	%
	2. Childhood onset	CMT1B	3
	Walk-age onset <15 months	CMT1X males	9
		CMT1X females	15
		CMT2A	15
		HNPP	58
	3. Adult onset <sup>a</sup>	CMT1B	28
		CMT1X males	7
		CMT1X females	26
		CMT2A	2 <sup>b</sup>
		HNPP	37

CMT Charcot-Marie-Tooth; HNPP hereditary neuropathy with liability to pressure palsies; MNCV motor nerve conduction velocity

<sup>a</sup> Adult onset: If symptoms onset was  $\geq$  third decade of life

<sup>b</sup> All patients with CMT2A have more severe phenotypes compared with the other patients with childhood onset who began walking before 15 months of age

Patients with unobtainable compound muscle action potential amplitudes in the upper extremities were not included in this table

orthotics. Median or ulnar NCVs that are uniformly slowed below 38 m/s (usually around 20 m/s) are highly suggestive of CMT1A [3••]. Although not necessary to make the diagnosis, nerve biopsies reveal demyelination and onion bulb formation.

### CMT1B

CMT1B, accounting for 10% of CMT1, is caused by mutations in myelin protein zero (*MPZ*). Most patients can be separated into two distinct phenotypes: a severe, early-onset form with delayed walking and NCV less than 10 m/s or a late-onset mild axonal neuropathy [4]. Rare causes of CMT1 (*EGR2*, and *LITAF/SIMPLE* mutations comprise less than 1% of patients [5, 6] and are described in Table 1.

### HNPP: Hereditary Neuropathy with Liability to Pressure Palsies

HNPP is characterized by recurrent episodes of focal compression of individual nerves or plexi leading to focal weakness or sensory loss. Common presentations, which usually occur for the first time in the second or third decade, include carpal tunnel syndrome and peroneal palsy with foot drop. HNPP represents about 6% of the CMT population [3••]; however, its prevalence may be underestimated, because mildly affected patients may not seek medical attention. A contiguous gene deletion of chromosome 17p11.2 that includes *PMP22* (same region that is duplicated in CMT1A) is present in approximately 80% of affected individuals [7], whereas

*PMP22* nonsense or frame shift mutations are rare causes of HNPP. Nerve conduction studies demonstrate focal slowing at common sites of entrapment [8] and nerve biopsy reveals tomacula (focal, sausage-shaped enlargement of the nerve).

### CMT1X: X-Linked CMT1

CMT1X represents the second most frequent form of CMT and results from mutations in the gap junction protein beta 1 (*GJB1*) gene encoding connexin 32 (Cx32) on the X chromosome [9]. Therefore, there is no male to male transmission in pedigrees and males are usually more impaired than females. Virtually all amino acid changing mutations are pathogenic (thus far >300 described), and most male patients have similar phenotypes to patients with complete deletions of the gene. Females also present with a wide range of symptoms, probably due to random X inactivation. NCVs are in the intermediate range (25–40 m/s) in both men and women [10]. Cx32 is expressed in myelinating Schwann cells and also in oligodendrocytes, but not in neurons. Central nervous system (CNS) involvement is rare (mild deafness, abnormal brainstem-evoked potentials) [11], but occasionally can be transiently debilitating, and is characterized by ataxia and dysarthria [12, 13].

### CMT2: Autosomal-Dominant Axonal Neuropathies

Most CMT2 patients present with the “classical phenotype,” but have a wider range of age of onset than CMT1A patients. NCVs in the upper extremities are

greater than 38 m/s and compound muscle action potential amplitudes are reduced, sometimes even unobtainable in severely affected patients. To date, causative mutations have been identified in 25% to 35% of CMT2 cases.

Mutations in the mitofusin 2 gene (*MFN2*) located on chromosome 1p36 cause CMT2A, the most common form of CMT2, which accounts for about 20% of CMT2 cases. CMT2A patients often develop severe, early-onset axonal neuropathy; a recent paper has shown that this *MFN2* mutation accounted for 91% of all severely impaired patients with CMT2 [14•]. Patients with neuropathy, optic atrophy, and additional pyramidal tract or other CNS abnormalities have been described with *MFN2* mutations [15]. Studies have found that *MFN2* mutations are present in 3.4% to 4% of all genetically defined CMT patients in Norwegian [16•] and North American [3••] populations.

CMT2E (*NEFL* mutations), CMT2F (*HSP27* mutations), CMT2G, CMT2L (*HSP22* mutations), and CMT2I patients may also present with the “classical CMT phenotype.” Late-onset patients with *MPZ* mutations (CMT1B) are sometimes classified as CMT2J because their NCV can be greater than 38 m/s. However, *MPZ* is expressed in myelinating Schwann cells, not neurons [15].

Recognition of two additional phenotypically distinct CMT2 subtypes can be helpful in diagnosing patients. The first phenotype is profound sensory loss, often with ulcerations or mutilations, and minimal clinical evidence of weakness. CMT2B is caused by mutations in the *RAB7* gene [17] or in the *SPTLC1* gene (hereditary sensory neuropathy type 1 [HSN1]). The second distinct phenotype is weakness, with minimal or no sensory loss. Patients with this phenotype include those with CMT2D, caused by mutations in the glycine aminoacyl tRNA synthetase (*GARS*) gene [18]. Patients with Silver syndrome, caused by mutations in the *BSCL2* gene, can also present in this manner. Both of these disorders are also unusual in that they may present with severe hand weakness prior to leg weakness. Other pure motor forms are sometimes also classified as distal hereditary motor neuropathies (dHMN). Sometimes mutations in the same gene may be classified as dHMN or CMT. For example, dHMN2 results from mutations in the *HSP22* or *HSP27* genes, which can also cause CMT2F and CMT2L.

Mutations in a cation channel transient receptor potential vanilloid 4 (*TRPV4*) have been recently identified as the cause of CMT2C [19••]. Zimon et al. [20•] reviewed five such patients with *TRPV4* missense mutations and demonstrated the pronounced phenotypic variability caused by *TRPV4* mutations. In fact, patients may present with scapuloperoneal dystrophy or sensorimotor neuropathy.

## CMT4: Autosomal-Recessive CMT (ARCMT)

Less than 10% of CMT cases in Europe and North America result from ARCMT; in contrast, in the Mediterranean basin and Middle East communities with a high percentage of consanguineous marriages, ARCMT is likely to account for 30–50% of all CMT cases. Diagnosing isolated cases in small kindred can be challenging, especially because polymorphisms are frequent and compound heterozygous mutations might be pathogenic. ARCMT are usually demyelinating and generally are more severe than cases of CMT with classical phenotypes. In many patients, conduction studies of proximal nerve segments may be necessary. In some cases, nerve biopsy may be helpful in guiding genetic testing. For example, focally misfolded myelin is characteristic of *MTMR2* (CMT4B1) or *MTMR13* (CMT4B2) mutations. Ethnic origin is a useful guideline, as CMT4D and CCFDN (congenital cataract, facial dysmorphism, and neuropathy syndrome) are mostly confined to the Balkan gypsy population. Recently published cases demonstrate that *SH3TC2* mutations, causing CMT4C, are the most frequent cause of ARCMT in the European and North American populations. *SH3TC2* is required for proper myelination and integrity of the node of Ranvier [21••]. Patients with CMT4C often manifest with severe early scoliosis [22]. Two causative genes, *LMNA* and *GDAP1*, have been proven to cause autosomal-recessive axonal CMT4; the spectrum of severity is highly variable [23]. Selected *GDAP1* mutations may also result in autosomal-dominant CMT2, whereas *LMNA* mutations have been associated with a broad clinical spectrum, such as cardiomyopathy, muscular dystrophies, mandibuloacral dysplasia, and restrictive dermopathy.

## Intermediate CMT

In certain CMT subgroups NCVs overlap the axonal and demyelinating range (25–45 m/s). These forms of CMT may be associated with unique disease mechanisms affecting both Schwann cells and axons. The intermediate group includes distinct gene mutations in dynamin2 (DI-CMTB) [24] and YARS (DI-CMTC) [25]; linkage to chromosome 10q24.1-25.1 has been identified in DI-CMTA. Moreover CMT1X, CMT2E, late-onset CMT1B and CMT4A patients often fall into this category.

## Diagnosis and Management

### Genetic Testing

Some basic principals should be followed in pursuing genetic testing in a patient or family with CMT.

Choosing individual genes based on the patients phenotype, family history, and the prevalence of the CMT type instead of simply ordering a screening panel of CMT can dramatically reduce the costs and focus the testing [3••, 26••]. Moreover, results from testing are not always easy to interpret since, for example, alterations in genes may occur that are not disease causing; in these cases one should feel free to contact the testing laboratory for clarification.

### Reasons to Pursue Genetic Testing

Not every patient with a genetic neuropathy wants or needs testing to identify the genetic cause of their disease. We maintain that is always the patient's decision whether or not to pursue genetic testing. Reasons that patients give for obtaining testing include identifying the inheritance pattern of their CMT, making family planning decisions, and obtaining knowledge about the cause and natural history of their form of CMT. Natural history data are available for some forms of CMT, such as CMT1A [27•] and CMT1X [28], that can provide guidance for prognosis, recognizing that there can be phenotypic variability in these subtypes. There are also reasons why patients do not want genetic testing. These include the high costs of commercial testing and fears of discrimination in the workplace or in obtaining health insurance. Because there are currently no medications to reverse any form of CMT, many patients decide against testing since their therapies will not depend on the results.

Once a genetic diagnosis has been made in a patient, other family members usually do not need genetic testing but can be identified by clinical evaluation and neurophysiology. It is our current policy to only consider genetic testing in clinically affected family members if their phenotype is atypical for the type of CMT in the family. In addition, we do not test asymptomatic minors with a family history of CMT, either by electrophysiology or genetic testing, due to the chance for increased psychological harm to the child [29]. We do routinely perform limited nerve conduction studies, although not needle electromyography, on symptomatic children with CMT. Because nerve conduction changes, including slowing, are often uniform, and detectable in early childhood in CMT [10], testing of a single nerve is often adequate to guide genetic testing or determine whether a symptomatic child is affected in a family with CMT.

### Commercial Testing Versus Research Testing

Testing for inherited neuropathies is available in two different settings: the clinical laboratory and the research laboratory. It is important that patients and physicians

understand the differences between the two options. Clinical laboratories provide commercial diagnostic testing for many inherited neuropathies. All clinical, but not the research, laboratory genetic testing done on humans in the United States is subject to regulation by the Clinical Laboratory Improvement Amendments, whose purpose is to insure quality laboratory testing. For inherited neuropathies in which the gene is not yet known or was only recently identified, research laboratories may be the only option for testing. For some diseases, even when clinical laboratories do offer testing, research laboratories may perform similar testing to try to identify rare or unusual mutations that are not screened by the clinical laboratory. Typically there is no charge to the patient for research testing. However, research laboratories may deny testing some patients if they do not fit the criteria set forth and they will not guarantee a time frame for results.

### Genetic Counseling

Competent genetic counseling is an extremely important element in the management of patients with inherited neuropathy and their families. Many clinicians may be unfamiliar with the preferred approach to be taken with a patient who has an inherited condition. Although neurologists are well trained to give advice regarding prognosis and therapy, a nondirective counseling approach is best for handling the complex issues that can arise when the diagnosis of an inherited condition is made. It is based on the principle of autonomy and the belief that an individual is the person who knows what decisions are best for his or her life. Patients with inherited conditions often seek further information regarding various decisions, including those concerning family planning. All options available to the patient, including prenatal testing, preimplantation genetic diagnosis, sperm and egg donation, adoption, having children without any testing, and having no children, should be explored to find what best fits with patient's beliefs, values, culture, and lifestyle. Contributing to a multidisciplinary approach, the presence of a physician geneticist or genetic counselor can greatly enhance the ability of a neuromuscular clinic to provide quality care to patients with inherited disease.

### Pathomechanisms and Potential Therapies

The growing number of CMT genes serves as a living "microarray" of molecules involved in the maintenance of normal peripheral nerve function. Determining the molecular pathways through which these molecules act and interact helps identify their function in Schwann cells or neurons (Fig. 1). This knowledge should eventually contribute to the development of rational therapy for many

forms of CMT. Among pathways already identified to play a role in CMT are transcriptional regulation, protein turnover, Schwann cell axonal interactions, axonal transport and mitochondrial fusion and fission. Some causal mutations are found in PNS-specific proteins (*PMP22*, *MPZ*, *periaxin*), whereas other genes encode widely expressed proteins that were not known to have a PNS-specific role before identification of mutations, which result in peripheral neuropathy (*GARS*, *HSP27*, *Cx32*). Some forms of CMT cause predominantly motor neuropathy: they disturb protein synthesis (*GARS*, *YARS*), stress response (*HSP22*, *HSP27*), apoptosis (*HSP27*), and axonal transport (*HSP27*) [30]; in contrast, other mutations lead primarily to sensory symptoms (*SPTLC1*, *RAB7*). Taken together, several mechanisms of CMT might even provide insight into the pathogenesis of neurodegenerative disorders in general.

#### *PMP22* Gene Dosage Change: Potential Therapy for CMT1A

Alterations of *PMP22* gene dosage result in two different disease entities. Approximately 50% of CMT cases are accounted for by CMT1A, which is caused by a 1.4-Mb duplication on chromosome 17 containing the *PMP22* gene; conversely, deletion of the same region causes HNPP. Multiple studies have shown that it is the dosage of *PMP22* that determines the type and extent of the neuropathy in these disorders. Consequently, treatment strategies are currently targeting the regulation of *PMP22* gene dosage.

Two compounds that have been shown to alter *PMP22* mRNA levels in rodents are progesterone and ascorbic acid. Both neurons and Schwann cells produce progesterone [31]. *PMP22* complementary DNA overexpressor rats, presenting with clinical, neurophysiologic, and pathologic features of CMT1A [32] became more severely affected when receiving a daily dose of progesterone. In contrast, administration of a selective progesterone receptor antagonist, onapristone, improved the phenotype and reduced *PMP22* mRNA levels. Unfortunately, onapristone is not safe in humans; further research is ongoing to develop less toxic progesterone antagonists suitable for future clinical trials. Ascorbic acid is necessary for PNS myelination in Schwann cells and dorsal root ganglion cocultures, it has an essential role in Schwann cell basal lamina formation [33]. Therefore, investigators treated a mouse model of CMT1A with ascorbic acid and demonstrated an improvement in myelination and a reduction of *Pmp22* to levels below those necessary to induce the disease phenotype [34]. The aforementioned studies provide proof of principle that drugs can alter *PMP22* gene dosage in cellular and animal models. Multiple international clinical trials are evaluating the potential of various doses of ascorbic acid to treat patients with CMT1A.

High-throughput screens are feasible new tools to rapidly select candidate medications from large numbers of existing compounds. Hundreds of thousands of compounds are being screened in an automated fashion on cell lines expressing *PMP22* reporter constructs, in a project supported by the Charcot Marie Tooth Association. Candidates who effectively reduce *PMP22* levels in the screen will then be further investigated in animal models and hopefully in clinical trials.

#### Schwann Cell-Axonal Interactions

Axonal degeneration occurs in all demyelinating forms of CMT and has frequently been shown to be more responsible for neurologic impairment than the demyelination itself [35–37]. Also, mutations in some genes (eg, *PO*, *GJB1*, and *GDAP1*) can result in both demyelinating and axonal forms of CMT. This phenomenon suggests that Schwann cell pathology damages the delicate myelin-axon interaction and can lead to axonal degeneration [35].

A therapeutic strategy focusing on preserving this intimate connection is providing trophic factor support to degenerating axons. Several families of trophic factors have been thoroughly investigated for the treatment of neurodegenerative diseases, including CMT. Unfortunately, no studies have been successful in humans, despite the promising animal studies. Poor delivery and short half-lives of the growth factors may contribute to some of these problems. Targeting the correct combination of trophic factors to neurons or Schwann cells at the optimal time may be necessary to achieve meaningful results.

Manipulation of ion channels is also being investigated as a means to improve axonal function in demyelinating neuropathies. It has been hypothesized that demyelination places increased energy demands on neurons and reduces their ability to maintain a charge separation. Consequently, potassium ions leak down their gradient, making depolarization less likely to occur at the node of Ranvier. Therefore, investigators have considered using potassium channel blockers to ameliorate demyelinating neuropathies. Despite the expectations, 3,4 diaminopyridine treatment did not result in significant improvement in a population of CMT patients, most of whom had CMT1 [38]. Nevertheless, more specific potassium channel blockers are being developed and may prove more effective.

Another potentially exciting approach involves the manipulation of Schwann cell–axonal signal transduction pathways. Axons express neuregulin-1 type III on their surface, which binds to ErbB receptors on Schwann cells as part of a process that initiates myelination. Transgenic overexpression of neuregulin-1 type III induces Schwann cell hypermyelination [39]. To date, neuregulin mutations have not been described to cause peripheral neuropathy.

Nevertheless, altering myelin thickness through this pathway may be a promising approach. A current study is investigating whether hypomyelination in severe, early-onset CMT1B could be due to impaired or inadequate responsiveness to neuregulin-1 type III and whether its overexpression could improve the neuropathy [40].

#### Protein Misfolding and Impaired Protein or Membrane Trafficking

*PMP22* missense mutations Leu16Pro [41] and Leu147Arg [42] cause a demyelinating neuropathy in humans and the naturally occurring demyelinating trembler J (TrJ) [43] and trembler (Tr) [44] mouse mutants. Because both of these mutations cause much more severe neuropathy than HNPP, where there is a 50% reduction in *PMP22* protein levels, the mutant *PMP22* must be causing abnormal functions within the cell rather than just leading to reduced functional *PMP22*. When epitope-tagged Tr, TrJ, and wild-type *Pmp22* were microinjected into sciatic nerves of rats and analyzed by immunohistochemistry, wild-type *Pmp22* was transported to compact myelin, but both Tr and TrJ and *Pmp22* were retained in a cytoplasmic compartment that colocalized with the endoplasmic reticulum (ER) [45]. Recent cell-based studies showed that mutant MPZ could accumulate in the ER and induce apoptosis. This aggregation-induced apoptosis was abrogated by pretreatment with curcumin. Curcumin, a component of turmeric, acts as a sarcoplasmic/ER  $\text{Ca}^{2+}$  ATPase (SERCA) inhibitor that can mitigate ER retention. In addition, oral curcumin administration partially mitigated the phenotype of the TrJ mouse [46]. Presently, an MPZ knock-in mutant mouse model of CMT1B is being treated with oral curcumin to investigate whether curcumin could be a candidate medication in other forms of CMT caused by ER retention of the misfolded protein [47]. A recent paper reported that the unfolded protein response (UPR) activated by overload of misfolded proteins in the ER was responsible for demyelination in a CMT1B mouse model [48•]. Deletion of the UPR mediator transcription factor CHOP completely rescued the motor deficit and ameliorated the neuropathy phenotype [48•]. Active areas of research include disrupted proteasome activity, regulation of intracellular membrane trafficking, and lysosomal function.

Protein turnover from the cell surface to the lysosome also appears to play an important role in some forms of CMT. To name a few examples, CMT1C (LITAF/SIMLE mutation) [6] and CMT2B (Rab7 mutation) [17] result from the impairment of lysosomal transport or degradation. Also, myotubularin-related proteins (MTMR) 2 and 13 as well as *FIG4* mutations cause CMT4B1, CMT4B2, and CMT4J by disrupting phosphoinositol-mediated trafficking of vesicles within the cell [49, 50].

#### Mitochondrial Function

Mitochondrial transport provides essential energy support to distal axons that are far away from their cell body. Mitochondria undergo dynamic cycles of fission and fusion that are regulated, in part, by mitofusins (mitofusin 1 and mitofusin 2). These nuclear encoded outer mitochondrial membrane proteins are highly conserved and are involved in embryonic development. Mitochondria missing both MFN1 and MFN2 cannot fuse [51]. Mitofusin 2 has been reported to accumulate at tethering sites between the mitochondria and the ER, facilitating intercommunication during signaling [52•]. This pathway involves calcium uptake and consequently the regulation of apoptosis. Neurodegeneration in CMT2A could be a consequence of impaired MFN2 activity in any of these functions, all of which are currently under investigation. Additionally, mutations in another mitochondrial outer membrane protein, ganglioside-induced differentiation-associated protein (GDAP)-1, lead to early-onset recessive demyelinating or axonal CMT4A [53]. The numerous examples of mitochondrial dysfunction suggest that targeting mitochondrial function might be therapeutically important for many types of CMT.

#### Gene Therapy

Gene therapy studies have been carried out for more than two decades in CMT and, despite the upcoming challenges, they hold therapeutic promise for hereditary neuropathies. Gene therapy can be defined as a strategy to transfer biologically relevant genetic material (usually genes or proteins) into affected cells in the body to treat disease. Approaches have focused on identifying appropriate therapeutic molecules and designing delivery systems, or vectors, to target them to the diseased neurons or Schwann cells. Viral vectors and plasmid DNA are the most common forms of gene therapy delivery systems. Viral vectors have been modified, so that they are unable to cause disease, but they carry efficiently the therapeutic gene to the cells that the virus infects. Unfortunately, they have often caused immunologic reactions, which currently limit their use. In contrast, plasmid DNA is nonimmunogenic, but it is characterized by poor delivery efficiency, and proteins made from it have only been produced in target organs for a short time.

Gene replacement and silencing are also emerging options to regulate gene expression and treat CMT. Single loss-of-function mutations, such as deletion of one of the *PMP22* alleles in HNPP, might be targets for gene replacement. In addition, nonsense mutations causing premature termination of a protein or even *GJB1* mutations may prove susceptible to this technique.

As a contrasting approach, gene silencing could be taken in CMT1A to reduce the amount of *PMP22* or in cases of missense mutations causing gain-of-function abnormalities. Small double-stranded RNAs or antisense oligonucleotides—that are short, single-stranded RNA or DNA sequences that bind to mRNA—inhibit translation and lead to the degradation of target mRNA. Similar naturally occurring small inhibitory RNAs can be genetically engineered to reduce the expression of target mRNAs. Catalytic RNA molecules known as ribozymes also have the potential to downregulate levels of mRNAs in a sequence-specific manner [54]. In fact, an antisense oligonucleotide to *Pmp22* mRNA has been combined with an inducible promoter to generate transgenic mice in which *Pmp22* mRNA levels, and peripheral neuropathy, can be modulated by feeding the animals tetracycline [55].

The use of stem cells is an exciting new option; however, researchers need to overcome serious difficulties before stem cells can actually be used for the treatment of inherited neuropathies. It will be a formidable challenge for stem cells to differentiate into neurons and then generate axons that need to travel down limbs more than a meter in length to reach their appropriate neuromuscular junction or sensory target. Similarly, it will be a challenge for stem cells to differentiate into Schwann cells that contact and ensheath all demyelinated axons in patients with demyelinating CMT. Nevertheless, stem cells could be engineered to secrete trophic factors that might provide support for the damaged nerves.

## Conclusions

Studying the biology of CMT has revealed a stunning variety of mechanisms involved in the pathology of the peripheral nervous system and has provided insights into the process of neurodegeneration in general. These discoveries have revolutionized our understanding and led to the identification of common pathways, which can provide a rational basis for therapeutic strategies. Genetic diagnosis of CMT is becoming increasingly available. However, accurate phenotypic evaluation is of high importance for natural history studies and the elaboration of reliable outcome measures for future clinical trials. Finding adequate therapeutic options for patients with CMT remains a challenge; however, ongoing clinical trials represent the recent rapid advances that dominate the field.

## Disclosure

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