Published in final edited form as:

Mol Neurobiol. 2013 April ; 47(2): 673–698. doi:10.1007/s12035-012-8370-x.

The *PMP22* Gene and Its Related Diseases

Jun Li^{1,2,3,4}, Brett Parker¹, Colin Martyn¹, Chandramohan Natarajan¹, and Jiasong Guo¹

¹Department of Neurology, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37237

²Vanderbilt Brain Institute, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37237

³Center for Human Genetics Research, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37237

⁴Tennessee Valley Healthcare System – Nashville Campus, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37237

Abstract

Peripheral myelin protein-22 (PMP22) is primarily expressed in the compact myelin of the peripheral nervous system. Levels of PMP22 have to be tightly regulated since alterations of PMP22 levels by mutations of the *PMP22* gene are responsible for >50% of all patients with inherited peripheral neuropathies, including Charcot-Marie-Tooth type-1A (CMT1A) with trisomy of *PMP22*, hereditary neuropathy with liability to pressure palsies (HNPP) with heterozygous deletion of *PMP22*, and CMT1E with point mutations of *PMP22*. While over-expression and point-mutations of the *PMP22* gene may produce gain-of-function phenotypes, deletion of *PMP22* results in a loss-of-function phenotype that reveals the normal physiological functions of the PMP22 protein. In this article, we will review the basic genetics, biochemistry and molecular structure of PMP22, followed by discussion of the current understanding of pathogenic mechanisms involving in the inherited neuropathies with mutations in *PMP22* gene.

Keywords

PMP22; myelin; peripheral nerve; CMT1A; HNPP; demyelination; conduction velocity

Nerve fibers increase their speed of action potential propagation by wrapping axons with Schwann cell membranes (myelin) in sequential segments. These segments are separated by punctuate gaps where axons are denuded of myelin, called nodes of Ranvier. This configuration permits action potentials to be conducted in an efficient saltatory fashion from one node to the next (Salzer, 2003; Hartline and Colman, 2007). To produce myelin, Schwann cells have to be precisely regulated to extend their membranes, wrap around the axons, and form a multilamellar compacted membrane structure. This process is called myelination. Myelination is not only a crucial event during development, but is also required for diseased nerves to repair demyelinated axons through remyelination (Salzer, 2003).

Once myelin is formed, its architecture is highly compartmentalized. A myelinated nerve fiber is composed of four discrete compartments (Arroyo and Scherer, 2000; Scherer and Arroyo, 2002), the node of Ranvier, the paranode, the juxtaparanode, and the internode.

Each of these compartments contains a unique, non-overlapping set of protein constituents. For instance, at the node of Ranvier, voltage-gated sodium channels are highly concentrated. The paranodal region, comprised of loops of Schwann cell membrane and interacting axonal membrane adjacent to the node of Ranvier, contains myelin associated glycoprotein (MAG) and Connexin 32 (Cx32) which are both Schwann cell proteins, as well as the axolemmal proteins Caspr. These proteins participate in Schwann cell-axon interaction and electrically isolate the nodal region. The juxtaparanodal region, the portion of the myelin and the interacting axolemma adjacent to the paranode, contains voltage-gated potassium channels (Kv1.1 and 1.2) on the axolemma. The internodes, the nerve segments flanked by two nodes of Ranvier, contain the compact myelin proteins, myelin protein zero (MPZ), *peripheral myelin protein-22 (PMP22)*, and myelin basic protein (MBP), which participate in forming the myelin sheath and in reducing capacitance of the internode. The molecular architecture of myelinated nerve fibers appears important for the functional integrity of peripheral nerves and is often disrupted in many peripheral nerve diseases.

PMP22, as an integral membrane glycoprotein of the internodal myelin, comprises an estimated 2-5% of the total myelin proteins in the peripheral nervous system (Snipes et al., 1992; Welcher et al., 1992). Its functional importance is highlighted by the fact that mutations in the *PMP22* gene are the most common causes for inherited peripheral nerve disorders, also called Charcot-Marie-Tooth diseases (CMT). CMT carries a prevalence of one in 2,500 people (Skre, 1974) and mutations of *PMP22* are responsible for >50% cases of CMT. These PMP22 related diseases disrupt the organization of myelin, and subsequently axonal integrity, which is responsible for the disabilities in patients with *PMP22* mutations. In this article, we will review the genetics, structure and basic functions of the *PMP22* gene, its encoded protein, and the pathogenic mechanisms of CMT caused by mutations in *PMP22*.

Architecture of the *PMP22* Gene

PMP22 was first isolated from mouse NIH3T3 fibroblasts as one of the growth arrest specific genes, *gas3* (Schneider et al., 1988). Serum-starved NIH3T3 cells up-regulate *gas3* (Manfioletti et al., 1990). Subsequently, *SR13*, a transcript isolated from rat nerve, was found to be strongly down-regulated in the acute phase after the sciatic nerve injury. The *SR13* has a sequence highly homologous to and is likely from the same gene as *gas3* (Welcher et al., 1992). *Gas3/SR13* (also known as PASII) was then localized to the compact myelin of the peripheral nerves with a molecular weight of 22kd. Thus, the protein was renamed as peripheral myelin protein-22 (PMP22) (Snipes et al., 1992; Kitamura et al., 1976).

In human genome, *PMP22* is located within the chromosome 17p11.2. *PMP22* is a 40kb gene that consists of six exons conserved in both humans and rodents (Figure 1A). Two alternatively transcribed exons (1a and 1b) comprise the first exon of the gene and give rise to two different transcripts (Jetten and Suter, 2000). The two transcripts are identical in their coding sequence but differ in their 5' untranslated region, suggesting that there are two different promoters (P1 and P2) which regulate the expression of each transcript. Indeed, two different *PMP22* transcripts, *exon-1a* (also called *CD25* or *1a-PMP22*) and *exon-1b* (*SR13* or *1b-PMP22*), have been isolated in rats (Spreyer et al, 1991; Welcher et al, 1991a). Analogous transcripts have also been found in humans and mice (Suter et al, 1994; van de Wetering et al, 1999). The coding region of the *PMP22* spans from exon-2 to exon-5. Exon-2 encodes the first transmembrane domain of PMP22. Exon-3 encodes the first extracellular loop. Exon-4 encodes the second transmembrane domain and half of the third transmembrane domain. Exon-5 encodes the remaining half of the third transmembrane

domain, the second extracellular domain, the fourth transmembrane domain, and the 3' untranslated region (Suter and Patel, 1994; Chen et al., 1997; Jetten and Suter, 2000).

Localization of PMP22 Expression

Detailed localizations of the PMP22 transcript and protein are listed in Table 1. Transcripts of *PMP22* express diffusely at embryonic stages, including the nervous system. They remain detectable at low levels in some adult non-neuronal tissues. Likewise, expression is seen at a relatively low level in the central nervous system, including cortex, brain stem and spinal cord. Motor nuclei of cranial nerves and spinal motor neurons have higher levels than other regions of the central nervous system. However, transcripts of *PMP22* are consistently shown to be most abundant in the myelinating Schwann cells of peripheral nerves (Snipes et al., 1992; Parmantier et al., 1995; Roux et al., 2004; Ohsawa et al., 2006).

The time-course of *PMP22* transcription in motor neurons of the central nervous system shows a rostral-caudal pattern with cranial nuclei expressing first embryonically and spinal motor neurons expressing after birth (Parmantier et al., 1995; Parmantier et al., 1997). In contrast, sensory neurons in cranial nuclei express transcripts of *PMP22* at embryonic stages, but the transcripts become undetectable in young adults. The alternative *PMP22* transcripts differ in their distribution of tissues. Exon-1b transcripts have been seen in the brainstem, spinal cord, skeletal muscle, heart, and sciatic nerve, whereas exon-1a transcripts have been found predominantly in the sciatic nerve (Parmantier et al., 1995; Parmantier et al., 1997; Lobsiger et al., 1996).

Transcription in dorsal root ganglia (DRG) is abundant during embryonic stages, but undetectable in adult DRG neurons (Parmantier et al., 1995; Parmantier et al., 1997). In contrast, another study has shown *PMP22* transcripts in adult DRG satellite cells, but no expression in DRG neurons. This data is difficult to interpret, however, since this study observed PMP22 proteins in both DRG satellite cells and neurons (De et al., 1994). Taken together, DRG cells appear to have a robust expression of *PMP22* during embryonic stages, but this expression declines dramatically in adulthood. This is in contrast to the delayed and persistently detectable PMP22 in spinal motor neurons that doesn't begin until post-natal day 1-2, but once initiated, continues to maintain expression at appreciable levels into adulthood.

In general, PMP22 proteins are localized to similar regions of *PMP22* mRNA expression. While high levels of PMP22 proteins can be seen in peripheral myelinated nerve fibers, the PMP22 protein levels in the central nervous system are much lower and are more difficult to be detected by immunohistochemistry. Depending on the quality of PMP22 antibodies, some studies have failed to detect PMP22 in the central nervous system, while others have found a low level of expression (Table 1).

The Protein Structure of PMP22

The first amino acid sequence based prediction of the PMP22 protein structure showed three putative transmembrane (TM) domains with the existence of one N-glycosylation site (Manfioletti et al., 1990). In this model, both the C-terminus and the N-glycosylation site are intracellular while the N-terminus is fully embedded in the bilayer. Since this initial proposal, structural predictions of PMP22 have been changed to include a fourth transmembrane region (Spreyer et al., 1991). However, alternative structural models of PMP22 have been proposed which suggest that TM2 and TM3 of previous tetraspan models are instead located outside the lipid bilayer, with only two transmembrane domains (TM1 and TM4 of previous tetraspan models) (Taylor et al., 2000). Unlike variable versions in the structural predictions of PMP22 transmembrane domains, the presence of an N-linked glycosylation site at Asn 41 has been confirmed several times (Manfioletti et al.,

1990;Welcher et al., 1992;Spreyer et al., 1991;Pareek et al., 1993;Snipes et al., 1992;Taylor et al., 1995). Using antibodies against different segments of PMP22, PMP22 transmembrane orientation was first examined experimentally by D'Urso et al to have an intracellular localization of its N and C termini in addition to 2 extracellular (ECL) loops, ECL1 and ECL2 (D'Urso et al., 1997). ECL1 was suggested to mediate a homophilic trans-interaction between two PMP22 proteins, while ECL2 was shown to mediate a heterophilic trans-interaction between PMP22 and myelin protein zero (Hasse et al., 2004). In Figure 1B, a current model of PMP22 structure is depicted and possesses four transmembrane domains with two extracellular domains and one intracellular domain.

One of great challenges in studying PMP22 structure has been the difficulties in purifying a large quantity of PMP22. Early studies utilized bovine peripheral nerves to attempt to increase the abundance of purified PMP22 but the procedure was laborious and did not yield PMP22 in sufficient concentration and purity necessary for structural analyses (Kitamura et al., 1976;Sedzik et al., 1998;Sedzik and Tsukihara, 2000;Sedzik et al., 2002). Recently, an alternative approach utilizing an engineered *E. coli* plasmid with a lambda repressor residue yielded PMP22 in sufficient concentration and purity for NMR analyses (Mobley et al., 2007;Sakakura et al., 2011). This technical advance, in combination with artificial lipid bilayer membrane, has allowed several important observations in the past several years. In the PMP22 extracellular loops, seven potential metal ion-coordinating sites have been identified that may be responsible for the Zn(II) binding ability of PMP22 (Myers et al., 2008). Circular dichroism (CD) analyses show transmembrane domain-1 (amino acids 1-30) as a long α -helix, ECL1 (amino acids 31-57) as a largely unstructured loop, transmembrane domain 2-4 as a molten globular helical bundle, and ECL2 as a short loop between TM3 and TM4 (Myers et al., 2008;Mobley et al., 2007;Sakakura et al., 2011).

While significant progress has been made in characterizing the structure of PMP22, details of the exact structure remain elusive. Although higher yields of PMP22 have been achieved by expressing PMP22 in *E. coli*, the purified PMP22 has to be integrated into lipid bilayer membrane. This environment may be critical for PMP22 to regain its proper folding close to its native structure (Mobley et al., 2007;Sakakura et al., 2011). Further improvement needs to be made to optimize the environment through techniques such as artificial lipid bilayer membrane.

Transcriptional Regulation of the *PMP22* Gene

A TATA-box-like DNA element has been found in the P1 and P2 promoters of *PMP22* with a high GC island. There are other putative enhancer elements that have been identified in the two promoters; however, their significance remains to be determined. Furthermore, there are multiple micro-domains around or upstream of the promoters for *PMP22*, through which PMP22 transcription is regulated both temporarily and spatially (Maier et al., 2002;Maier et al., 2003;Saberan-Djoneidi et al., 2000;Hai et al., 2001;Orfali et al., 2005). *LacZ* reporter analysis performed in a transgenic line expressing the *PMP22-1B-lacZ* gene (reporter construct with 7kb deletion at the 5' flanking region including exon1A and its upstream regulatory elements) displayed *lacZ* expression in Schwann cell, neuron, heart, intestine, lung, muscle, and brainstem (Maier et al., 2002). This indicates that the upstream regulatory elements are critical in *PMP22* gene expression. There are two sites between -1600 and -2100bp of *PMP22* which interact with cAMP response element binding (CREB) protein. CREB serves as a silencer of the *PMP22* promoter. This inhibition can be attenuated by cAMP, an effect that may be counteracted by Vitamin C to reduce PMP22 expression (Saberan-Djoneidi et al., 2000;Passage et al., 2004). The same region also contains the site for sterol regulatory element binding (SREB) protein, which enhances *PMP22* transcription in the presence of steroid hormones (Saberan-Djoneidi et al., 2000;Desarnaud et al.,

1998;Desarnaud et al., 2000). These findings have formed a theoretical basis for the treatment of CMT1A. Details of this subject will be discussed later.

Additional transcriptional regulatory DNA elements have recently been identified through investigations into several transcription factors that play important roles in regulating the myelination of Schwann cells, including EGR2, Sox10, and Oct6. These factors along with their up/down-stream factors form intricate signaling network that regulates myelin protein expression, including PMP22. For instance, activation of a G-protein coupled receptor, *gpr126*, raises cAMP levels and triggers the expression of Oct6 that in turn regulates genes encoding myelin proteins (Monk et al., 2009;Monk et al., 2011). The induction of EGR2 expression requires both POU transcription factors (Oct6 and Brn2) and Sox10, a highly mobile group-box containing transcription factor. Oct6 and Sox10 act synergistically on a 1.3kb region 35kb downstream of the EGR2 transcriptional start site (Ghislain et al., 2003;Zorick et al., 1999;Ghislain et al., 2002;Reiprich et al., 2010). Mice deficient of EGR2, a zinc-finger transcription factor, are devoid of peripheral nerve myelin (Topilko et al, 1994). Patients with EGR2 mutations may have hypo-dys/demyelinating neuropathy of different severity, including congenital hypomyelinating neuropathy, Dejerine-Sottas disease, and mild forms of CMT1 (Bellone et al., 1999;Timmerman et al., 1999;Warner et al., 1998;Warner et al., 1999). Using microarray technology, EGR2 is found to regulate expression of all major myelin proteins in rat Schwann cell culture, including Mpz, Cx32, PMP22, MBP, periaxin and Myelin Associated Glycoprotein (MAG) (Nagarajan et al., 2001).

Interestingly, when examined in a different culture system, EGR2 and Sox10 synergistically activated expression of all myelin protein genes but *PMP22* (Bondurand et al., 2001;Jang et al., 2010;LeBlanc et al., 2007;LeBlanc et al., 2006). Moreover, activation DNA elements for *EGR2* and *Sox10* are highly conserved in these myelin protein genes, and have been identified through genomic database screening. However, these DNA elements are not found in the traditional promoter region of *PMP22* (Jones et al., 2007). This data argues against a direct regulatory effect of *PMP22* by Egr2 or Sox10. This intriguing issue appears explainable now by recently identified novel distal and intronic enhancers of *PMP22* transcription in Schwann cells. Three of these enhancers are distally located upstream from the *PMP22* translation start site (at -120, -115, -91kb) (Jones et al., 2012). Additionally, an enhancer was found in the largest intronic region of *PMP22* (at +11 kb) (Jones et al., 2011). Through these enhancers, EGR2 and Sox10 act synergistically to promote the maximum level of PMP22 expression (Jones et al., 2011;Jones et al., 2012). Interestingly, several families with a mild CMT1A phenotype were recently identified who did not possess duplication of the classical trisomy of chromosome 17p12, but instead possessed a duplication of the region upstream of *PMP22* that contained the newly discovered *PMP22* enhancers (Weterman et al., 2010;Zhang et al., 2010). These clinical findings support the relevance of the new enhancers in the regulation of the *PMP22*.

In addition, EGR2 directly activates promoters of multiple sterol regulatory element binding protein (*SREBP*) genes to affect cholesterol/lipid biosynthesis, which would in turn affect myelin gene expression (LeBlanc et al., 2005). In addition, the Shh (Sonic hedgehog) protein has been shown to activate *PMP22* transcription (Ingram et al., 2002). However, it still remains to be determined whether this activation is direct or indirect.

PMP22 Synthesis and Transport

During axonal contact, myelinating Schwann cells significantly up-regulate the expression of PMP22 (Snipes et al., 1992;Bosse et al., 1999;Bosse et al., 1994). However, levels of PMP22 must be adjusted within a narrow range since reduction or increase of PMP22

expression can cause inherited neuropathies. Like other membrane proteins, newly synthesized PMP22 is transiently retained in the endoplasmic reticulum (ER) and Golgi compartments for post-translational modification, such as glycosylation. This has been demonstrated in cultured Schwann cells, in which PMP22 was first synthesized as an 18 kDa precursor protein and then post-translationally modified by N-linked glycosylation before reaching the plasma membrane (Pareek et al., 1993; Ryan et al., 2000). This trafficking process can be recapitulated in other culture cells that are transfected with plasmids encoding PMP22 (D'Urso et al., 1998). When expressed with different tags, wild-type PMP22 is still trafficked efficiently to the plasma membrane both *in vivo* and *in vitro*. These tags include myc-tagged mouse *Pmp22* in NRK cells (Liu et al., 2004) or COS7 cells (Tobler et al., 1999; Liu et al., 2004), EGFP-tagged PMP22 in HeLa or 293 cells (Shames et al., 2003), VSV-tagged PMP22 in cultured Schwann cells (Naef et al., 1997) and rat myelinating Schwann cells *in vivo* (Colby et al., 2000). However, in a gel filtration study in COS7 cells, GFP-tagged mouse PMP22 displayed a higher potential for intracellular aggregation than untagged PMP22 or myc-tagged PMP22 (Liu et al., 2004), suggesting interferences from the tag. A majority of newly synthesized PMP22 is located in ER and is sensitive to Endo-H (an enzyme that cleaves off high mannose-containing non-complex carbohydrates), confirming glycosylation of the protein as a marker of post-translational modification. Interestingly, a rapid turnover rate of the PMP22 (with a half-life of 30 to 60 minutes) has been noted in these cells (Pareek et al., 1993). A large portion of newly synthesized PMP22 is degraded (70% in rat Schwann cells and co-cultures of rat Schwann cells and neurons). Thus, only a small portion of newly synthesized PMP22 is transported to the cell surface, consisting of ~78% of all Endo-H insensitive PMP22 (Pareek et al., 1997). This indicates that complex glycosylation of the PMP22 protein likely prevents it from degradation and permits transport to the cell surface.

Functions of PMP22 in Cell Proliferation, Differentiation, and Death

A robust increase of *PMP22* during starvation implies that PMP22 may play a role in cell survival and/or proliferation (Schneider et al., 1988). This subject has been under intense investigation (Table 2). *In vitro*, over-expression of PMP22 has been consistently shown to reduce proliferation in Schwann cells or fibroblast cultures. *In vivo*, however, the over-expression of PMP22 increases Schwann cell proliferation in young adult transgenic mice, but decreases the proliferation of Schwann cells in aged human subjects with CMT1A (Table 2). Conversely, the deficiency of PMP22 produces opposite effects on Schwann cell proliferation both *in vitro* and *in vivo*. PMP22 deficiency can increase proliferation of Schwann cells and fibroblast cultures *in vitro*, and decrease Schwann cell proliferation at certain stage of development in mice. Finally, PMP22 possessing a point mutation, such as *TrJ*, suppresses Schwann cell proliferation *in vitro*, but induces an increase of Schwann cell proliferation *in vivo* (Table 2). This *in-vivo* effect could also be secondary to extensive segmental demyelination or axonal loss in the mutant peripheral nerves since Schwann cells usually proliferate to remyelinate the demyelinated or regenerating axons as a natural repairing process.

All types of *PMP22* mutations, including duplication, deletion, and point mutation, result in increased apoptosis of cultured Schwann cells, fibroblasts, and myelinating Schwann cells of mice and humans with CMT1A (Table 2). However, the apoptosis is only evident in aged Schwann cells *in vivo* and tends to be milder in PMP22 deficient cells when compared with Schwann cells with PMP22 over-expression or toxic-gain-of-function point mutations. Though the mechanisms responsible for this cell death are still elusive, several recent studies appear relevant to this issue. Perp has been identified as a mediator of p53-dependent apoptosis in fibroblasts, thymocytes, and neurons (Attardi et al., 2000; Ihrie et al., 2003; Nowak et al., 2005). Interestingly, *Perp* shows sequence similarity to PMP22 (Attardi

et al., 2000). Moreover, p53-dependent apoptosis, including neuronal cell death, is found to be mediated by another signaling protein, Siva, which directly interacts with PMP22 (Nestler et al., 2006; Jacobs et al., 2007). Additional research is needed to clarify the role of these signaling pathways in Schwann cell apoptosis *in vivo*.

These data raise the question of whether PMP22 plays a role in neuronal cell death. Although its effects on Schwann cells have been extensively studied, the effect of PMP22 on neuronal cell death has been minimally explored. This is probably due to the higher level of PMP22 expression in myelinating Schwann cells, which has been the main focus of studies. However, PMP22 is expressed in spinal motor neurons at a lower level during development and adulthood (Table 1). It is also expressed highly in sensory neurons of DRG during development, but hardly detectable in adulthood. A recent study has shown that over-expression of PMP22 increases spinal motor neuron loss in mice, but PMP22 deficiency did not alter the number of young spinal motor neurons (Nattkamper et al., 2009). Whether over- or under-expression of PMP22 affects survival of *sensory* neurons or *aged* motor neurons is unknown, and has not been examined in *Pmp22* knockout or transgenic rodents.

Patients with homozygous mutations of *PMP22* are rare. Four cases in total have been reported (Roa et al., 1993; Abe et al., 2010; Al-Thihli et al., 2008; Saporta et al., 2011). Three of the four cases are compound heterozygotes with mutations that produce deletion of *PMP22* in one allele and a different mutation (two point mutations and one with deletion of exon 3&4) in the 2nd allele. These three patients presented with severe sensory and motor deficits localized to the peripheral nervous system. However, without autopsy, it is unclear whether there was neuronal cell-body loss in the spinal cord or DRG of these cases. It is also unclear whether these deficits resulted purely from the loss of function of *PMP22* or from the toxic gain-of-function effect of mutant PMP22 proteins. We have recently reported a fourth case with deletion of both *PMP22* alleles that results in a true loss of function of PMP22 (Saporta et al., 2011). The affected proband presents a non-length dependent atrophy and weakness in the cranial nerve innervated muscles and a profound trunk sensory ataxia, suggesting neuronal loss in both sensory ganglia and cranial motor nuclei.

Four total cases of homozygous mutations are less than expected considering that CMT1A with heterozygous duplication of *PMP22* has a prevalence of 1:5000. Deletion of *PMP22* as a reciprocal mutation would be expected to be more common than just four cases. A potential explanation for this incongruity is that null function of *PMP22* produces embryonic lethality. This is consistent with the observation that homozygous *Pmp22*^{-/-} knockout mice are difficult to breed, having a reproduction rate far below that of *Pmp22*^{+/-} mice (unpublished observation).

The literature on the effect of cell differentiation by PMP22 is sparse. Motor nerves from *Pmp22*^{-/-} mice (pectineus at P4 and femoral at P24) showed excessive immature Schwann cells that established one to one relationship with axons but failed to form myelin (Adlkofer et al., 1995). Internodal length is also shortened in Schwann cell/neuron co-culture that was deficient of PMP22 (Amici et al., 2007). These observations support the theory that PMP22 plays a role in the initiation of myelination. Whether this is a direct or indirect effect remains to be determined, however. In contrast, heterozygous deletion of *Pmp22* does not affect the initiation of myelination since compact myelin is well formed in *pmp22*^{+/-} nerves (Adlkofer et al., 1995; Adlkofer et al., 1997).

Another related issue is the involvement of PMP22 in cellular junctions and interactions with the extracellular matrix. PMP22 shares >20% of amino acid homology with claudin-1 and has been considered as one of members of claudin family. PMP22 co-localizes with tight junction proteins, such as zonula occludens-1 and claudins, in epithelial cells

(Notterpek et al., 2001; Roux et al., 2004). However, it is unclear whether PMP22 interacts with these junction proteins. In addition, PMP22 has been found to complex with $\alpha 6 \beta 4$ integrin that is present in Schwann cell basal lamina, which may mediate the interaction of PMP22 with the extracellular matrix (Amici et al., 2006). It is unknown whether these molecular interactions affect Schwann cell proliferation or differentiation.

Mutations in the *PMP22* Gene and Their Related Diseases

Dyck and Lambert classified autosomal dominant inherited neuropathies into demyelinating [hereditary motor sensory neuropathy type - 1 (HMSN-1), now named as CMT1] and axonal (HMSN-2 or CMT2) groups using electrophysiological and pathological criteria (Dyck and Lambert, 1968). Nerve conduction velocities are significantly slowed in CMT1; whereas the conduction velocities in CMT2 are usually normal or minimally slowed, but amplitudes of motor and sensory responses are diminished. Pathologically, CMT1 shows prominent onion bulbs that represent excessive Schwann cell membrane processes around a myelinated nerve fiber that fail to form compact myelin. In contrast, axonal loss is the main feature of CMT2. There are now over 53 loci and 36 specific genes linked to different types of CMT. In addition to CMT types 1 and 2, patients with X-linked neuropathies are classified as CMTX. CMT4 refers to the inherited neuropathies with recessive inheritance. Finally, there is a small group of patients with CMT who do not fit well into CMT1 or CMT2 with conduction velocities ranging from 25 to 45m/s. They are often classified as the dominant intermediate form of CMT. Each of this class has been further subdivided based on differences in genetic abnormalities (Li et al., 2003; Reilly et al., 2011; Patzko and Shy, 2011).

While CMT has a high prevalence of 1:2500 (Skre, 1974), over 50% of all CMT cases are caused by mutations in *PMP22* gene, including heterozygous duplication (CMT1A), deletion (HNPP) and point mutations (CMT1E). Interestingly, these mutations produce distinct phenotypes that have revealed important biological functions of PMP22. In the sections below, we will discuss each of the three diseases.

A. CMT1A

CMT1A is an autosomal dominant disorder caused by heterozygous duplication of the chromosome 17p11.2-12, a DNA segment containing the *PMP22* gene plus more than nine additional genes (Lupski, 1992; Suter et al., 1992a; Raeymaekers et al., 1992). There is strong evidence to suggest that the *PMP22* duplication is responsible for the disease, not the other genes. First, point mutations of the *PMP22* gene are sufficient to cause peripheral neuropathy (Suter et al., 1992a). Increasing copies of *PMP22* to over-express the protein in rodents have produced pathological phenotypes resembling those of human subjects with CMT1A (Sereda et al., 1996; Huxley et al., 1998; Magyar et al., 1996). Finally, heterozygous truncation mutation inactivating one of the two *PMP22* copies also results in peripheral nerve disease (Nicholson et al., 1994; Li J et al., 2007).

Clinical Phenotype—Most CMT1A patients become symptomatic during the first two decades of their lives. They often have histories of being slow runners, being poor at sports during childhood, developing foot deformities (high arches and hammer toes) in their teenage years, and requiring orthotics for ankle support as adults. They typically have variable degrees of hand weakness that lags behind the development of foot weakness. Sensory deficits occur in both large (vibration and proprioception) and small (pain and temperature) modalities. While the combination of weak ankles and decreased proprioception often leads to problems with balance, the vast majority of patients remain ambulatory throughout their lifespans, which are not shortened by the disease. Almost all patients with CMT1A have absent deep tendon reflexes (Thomas et al., 1997; Krajewski et al., 1999). Additional features, including postural tremor (referred to as Roussy-Levy

syndrome) and muscle cramps, may also occur. While this phenotype is typical for CMT1A patients, it can be variable. Some patients develop a severe phenotype in infancy, while others have only minimal disability throughout their lives.

Nerve Electrophysiology and Pathology in CMT1A—Patients with CMT1A usually have slowed conduction velocities that can range from 7 to 40 meters/sec. However, in a given individual with CMT1A, the reduced conduction velocity varies minimally between different nerves in the same limb and between the corresponding nerves of different limbs. This feature is impressively stereotypic among patients with CMT1A and is in sharp contrast to the electrophysiological features in acquired demyelinating neuropathies. The former has been called the pattern of “uniform slowing” (Lewis et al., 2000; Lupski et al., 1991). The latter usually shows non-uniform or asymmetric changes. The conduction velocity in one nerve may be severely reduced and normal or minimally slowed in the other nerve. The non-uniform slowing is often associated with temporal dispersion of compound muscle action potentials and conduction block, which are typically not present in CMT1A. In addition, all patients with CMT1A inevitably have reduced amplitudes of motor and sensory responses. They reflect axonal loss secondary to abnormal myelination and have been shown to correlate better than conduction velocities with neurological disabilities in CMT1A patients (Krajewski et al., 1999; Krajewski et al., 2000; Thomas et al., 1997).

Pathological changes in CMT1A nerves largely explain the findings derived from electrophysiological studies. Sural nerve biopsies from patients with CMT1A show extensive onion bulb formations that affect almost every myelinated nerve fiber (Figure 2A). The onion bulbs are formed by multiple Schwann cells that extend their membrane processes around axons but fail to form compact myelin (Gabreels-Festen and Wetering, 1999; Gabreels-Festen et al., 1995). It has been suggested that onion bulbs are the result of repetitive demyelination and remyelination (Thomas et al., 1997; Robertson et al., 1997; Robertson et al., 2002), which would be expected to cause temporal dispersion and conduction block in addition to slowed conduction velocities. Yet, none of these features has been observed in CMT1A. Thus, this hypothesis may need to be revisited. In addition, there is conspicuous axonal loss in myelinated nerve fibers. Spaces between surviving myelinated nerve fibers are occupied by a large amount of collagen filaments that are presumably derived from fibroblasts. This axonal loss is consistent with severely reduced amplitudes of motor and sensory responses in patients with CMT1A.

Genetics in CMT1A—A typical CMT1A mutation is autosomal dominant and caused by heterozygous duplication of chromosome 17p11.2 with a DNA length of 1.4MB containing about 10 genes, including the *PMP22* (Raeymaekers et al., 1992; Timmerman et al., 1990; Lupski et al., 1991). As aforementioned, there is strong evidence suggesting that the duplication of *PMP22* is responsible for the disease. This DNA segment of chromosome 17p11.2 is flanked by two >17kb low copy number repetitive elements (REP) (Pentao et al., 1992). There is a 1.7kb highly conserved recombination hot-spot within the REP that is nearby a *mariner* transposon-like element (MITE). These DNA elements are postulated to mediate an unequal crossing-over during homologous recombination of meiosis between misaligned CMT1A-REP homologues, which results in a heterozygous duplication of chromosome 17p11.2. A reciprocal deletion of chromosome 17p11.2 would cause HNPP (Reiter et al., 1996).

With the advent of new genetic tools, such as array comparative genomic hybridization (aCHG), the DNA length of CMT1A duplication has been directly examined. Interestingly, the duplicated regions do not always contain the full length of 1.4MB. In a subset of patients with CMT1A, variable lengths of DNA within chromosome 17p11.2 have been found by aCHG to be duplicated, but always contain the *PMP22*. These segments may not always

associate with REP elements (Zhang et al., 2010;Choi et al., 2011). These findings also demand alternative explanations for these shortened DNA segments that are duplicated, instead of the traditional theory of the unequal crossing over.

Pathogenesis of CMT1A—Uniform slowing and axonal loss are two prominent and inevitable features of CMT1A. The later is closely correlated with disabilities in patients with CMT1A (Krajewski et al., 2000;Thomas et al., 1997). Thus, our discussion on pathogenesis will primarily focus on these two aspects.

The uniformity of CMT1A nerve conduction denotes that the slowing of nerve conduction velocities in an individual with CMT1A show minimal variations from one nerve to another and it affects nerves symmetrically between the left and right limbs (Lewis and Sumner, 1982;Lewis et al., 2000). In contrast, acquired demyelinating neuropathies, such as Guillain-Barre syndrome (GBS) and chronic demyelinating inflammatory polyradiculoneuropathy (CIDP), are often asymmetric and non-uniform, both in their clinical presentation and in their nerve conduction (Lewis et al., 2000). In general, the most common cause for the slowed conduction velocities in neuropathies is segmental demyelination. The physiological basis for reduced conduction in acquired demyelination models has been investigated by longitudinal current analysis (Bostock et al., 1981;Bostock and Sears, 1976;Bostock et al., 1978;Rasminsky and Sears, 1971;Lafontaine et al., 1982). In normal nerve fibers, this technique demonstrates a prominent inward current at the nodes of Ranvier, whereas outward current in the internode ensheathed by compact myelin is minimal. When compact myelin is stripped away during demyelination, for example by lysophosphatidyl choline, the outward current becomes excessive in the internode and shunts away the depolarizing current to reduce the action potential propagation. When the shunting current becomes sufficiently severe, conduction fails, which is called conduction block. When the whole nerve bundle is recorded, the conduction of compound nerve action potentials propagates slowly in a non-uniform format with temporal dispersion and conduction block. None of these key features is seen in CMT1A (Lewis et al., 2000). Therefore, the mechanisms derived from acquired demyelination cannot be extrapolated to explain nerve conduction changes in CMT1A.

Shortened internodes have been predicted to reduce conduction velocity in computer simulations (Brill MH et al., 1977). These predictions are further substantiated using *periaxin* knockout mice (Court FA et al., 2004). To address this issue in CMT1A, we have performed biopsies of human glabrous skin from patients with CMT1A. Due to the invasive nature of sural nerve biopsies, internodal length has not been systematically evaluated in patients with CMT1A. With the skin biopsy technique that we and others have developed, this issue can now be investigated in a systematic and minimally invasive manner. Internodes were identified by the staining of MBP. Their length was measured by confocal imaging. The mean internodal length in patients with CMT1A was $73.9 \pm 27.0 \mu\text{m}$, which was significantly shorter than that of healthy controls ($94.5 \pm 28.6 \mu\text{m}$). There was also a significant difference between CMT1A and CMT2 ($73.9 \pm 27.0 \mu\text{m}$ vs $92.0 \pm 29.1 \mu\text{m}$; $p < 0.0001$), suggesting that shortened internodal length is an intrinsic feature of CMT1A, but is not secondary to axonal loss. Interestingly, we identified no segmental demyelination in any of 117 internodes from patients with CMT1A. Segmental demyelination was found in all samples from patients with CIDP (Saporta et al., 2009). Taken together, the lack of active segmental demyelination combined with the uniformly shortened internodes questions a developmental defect of internodal lengthening in CMT1A patients, an important hypothesis to be tested in the future. This mechanism does not exclude segmental demyelination that may take place preferentially in large myelinated nerve fibers and spare the small ones like dermal myelinated nerve fibers. Remyelination in demyelinated nerve fibers should result in shorter internodes as well, but is expected to cause variable internodal length (non-uniform).

Over-expression of PMP22 may affect Schwann cells in other aspects. First, over-expression of PMP22 causes apoptosis in several types of cells, including Schwann cells (Table 2). However, this mechanism is unlikely to play a major role in dysmyelination of CMT1A. The apoptosis is only evident in aged Schwann cells with PMP22 over-expression *in vivo*, but dysmyelination is present even during the early development. Second, over-expressed PMP22 may overwhelm the protein degradation system, leading to formation of protein aggregates (Fortun et al., 2007; Dickson et al., 2002; Fortun et al., 2003; Fortun et al., 2006; Niemann et al., 2000). It may activate ERAD and/or autolysosomal systems in mutant Schwann cells (Fortun et al., 2003). These findings are usually derived from mice with a super number of *pmp22* transgenes (*7 copies*) or with missense mutations of Trembler or Trembler-J (Fortun et al., 2003; Fortun et al., 2006; Fortun et al., 2007; Rangaraju et al., 2010), while patients with CMT1A only harbor *3 copies* of wild-type *PMP22*. Indeed, sural biopsies from many patients with CMT1A showed no aggregates while aggregates were readily detectable in patients with Trembler or Trembler-J mutations (Hanemann et al., 2000). Furthermore, experiments using microarray have shown distinct mechanisms between gain-of-function point mutation mice (*Trembler* or *Trembler-J*) and mice with PMP22 over-expression. The former shows transcriptional changes in stress response, and the latter demonstrates alterations in Schwann cell proliferation (Giambonini-Brugnoli et al., 2005; Vigo et al., 2005). Together, these data suggest that caution should be taken when interpreting findings from animal models with over-expression of wild-type PMP22 versus animals with point mutations of *PMP22*. In addition, it is still unclear whether these mechanisms of abnormal protein aggregates directly relate to segmental de-/dysmyelination or internodal lengthening during development.

Interestingly, a recent investigation demonstrates that over-expressed PMP22 may augment expression of P2X7, a purinoceptor (Nobbio et al., 2009), leading to influx of extracellular Ca^{2+} into Schwann cells. Application of a P2X7 inhibitor restores dysmyelination in Schwann cell/neuronal co-culture with PMP22 over-expression. Thus, these data provide a pathogenic link between altered Ca^{2+} concentration and abnormal myelination in CMT1A.

Dysregulation of genes involved in cholesterol biosynthesis may also play a role in CMT1A pathogenesis. Expression profiling of transgenic mice with increased *pmp22* copy number revealed a strongly reduced expression of major genes involved in cholesterol metabolism, such as HMG-CoA synthase, HMG-CoA reductase, cytochrome P450, sterol-C4-methyl oxidase-like, and sterol-C5-desaturase (Giambonini-Brugnoli et al., 2005). A subsequent cDNA microarray study also found similar reductions in expression of cholesterol biosynthesis genes in transgenic rats with *pmp22* over-expression (Vigo et al., 2005). Reduction of cholesterol can have a profound effect on myelination, which would impair action potential propagation. Not only does cholesterol affect membrane dynamics by integrating into myelin membranes, it is also known to regulate the trafficking of major myelin proteins (P0) from the Schwann cell endoplasmic reticulum to myelin membrane. When this process is disturbed during cholesterol deficiency in Schwann cells, the compaction of peripheral myelin is lost and the stoichiometry of myelin membrane components is altered (Saher et al., 2009). Additionally, inhibition of cholesterol biosynthesis with the squalene epoxidase inhibitor tellurium causes mature Schwann cells to undergo demyelination (Berciano et al., 1998). The pathology of CMT1A appears related to this mechanism, since dysmyelination is evident and associated with a profound down-regulation of cholesterol synthetic pathway in CMT1A peripheral nerves. It would be important to evaluate if trafficking of additional myelin membrane proteins are affected by cholesterol dysregulation in CMT1A.

While the studies above have attempted to explain de-/dysmyelination in CMT1A, pathogenic mechanisms underlying axonal loss in this disease are largely unexplored. There

have been a variety of cellular and molecular alterations that were observed and may have pathogenic implications in the axonal loss of CMT1A. For instance, mitochondria have been found to abnormally accumulate in distal axons of patients with CMT1A (Saporta et al., 2009). Dysregulated transcription levels of glutathione S-transferase theta-2 and cathepsin-A are well correlated with disabilities of patients with CMT1A (Fledrich et al., 2012). Adhesion molecules at the interface of Schwann cells and axons are dysregulated in mouse and human nerves with PMP22 over-expression (Kinter et al., 2012). However, much work still has to be done to establish their causal roles, if any.

B. HNPP

In the vast majority of patients with HNPP, the causal mutation is a heterozygous deletion of chromosome 17p11.2, the same DNA segment duplicated in CMT1A (Chance et al., 1993). However, in a small number of patients, the disease is caused by other types of mutations that result in heterozygous loss of function of the *PMP22* gene (Nicholson et al., 1994; Shy et al., 2006). Please see Table 1 and Figure 1 for a complete list of all mutations in *PMP22* that are causal for HNPP. Thus, insufficiency of *PMP22* alone produces a phenotype identical to that in patients with heterozygous deletion of chromosome 17p11.2 (Li J et al., 2007). This finding substantiates that *PMP22* is responsible for the disease and not other genes residing in chromosome 17p11.2. Moreover, it is the loss-of-function phenotype in the disease that reveals the normal physiological functions of *PMP22*.

Clinical Phenotype—HNPP is an autosomal dominant disorder that produces episodic, recurrent focal sensory motor neuropathy (Gonnaud et al., 1995; Mouton et al., 1999; Li et al., 2004). The common symptoms of focal numbness, muscular weakness and atrophy usually present during adolescence. These focal deficits are often evoked by mechanical stress in the peripheral nerves, such as compression, limb stretch or repetitive movement of the affected limbs (Mandich et al., 1995; Mouton et al., 1999; Timmerman et al., 1996; Li et al., 2002; Li et al., 2004). Therefore, symptoms of HNPP frequently manifest at common sites of entrapment neuropathies such as peroneal nerve across the fibular head, median nerve across the wrist and ulnar nerve across the elbow. Strenuous physical activities can even result in limb paralysis with massive axonal damage, suggesting that axons can be affected if stress is sufficiently severe (Horowitz et al., 2004). Occasionally, a brachial plexopathy may be the presenting symptom and affect an upper limb unilaterally (Chance et al., 1994).

Physical examination usually only finds minimal abnormalities in young patients but significant sensory loss, weakness, and muscle atrophy in the hands and/or feet are often observed in the elderly.

Nerve Electrophysiology in HNPP—Electrophysiological findings in patients with HNPP are quite unique. Nerve conduction studies show accentuated slowing in motor distal latencies at the sites susceptible for mechanical pressure, such as median nerve at the wrist and peroneal nerve at the ankle. In addition, focal slowing of conduction velocities is almost always seen at sites subject of compression, such as ulnar nerve across the elbow and peroneal nerve across the fibular head. In contrast, conduction velocities in other nerve segments are usually normal or minimally slowed in a majority of cases (Li et al., 2002; Amato et al., 1996; Mouton et al., 1999; EARL et al., 1964; Stogbauer et al., 2000; Andersson et al., 2000). In our experience, 60-80% of patients fulfilling these electrophysiological features have positive finding in their DNA testing for HNPP deletion. The remaining patients would be caused by other etiologies. These electrophysiological features also support a nerve susceptibility to mechanical stress.

Exceptions to the typical electrophysiological pattern aforementioned have been noticed. There are HNPP patients with conspicuously slowed conduction velocities not limited to entrapment or compression sites. In some patients with long-standing disease, nerve conduction study may show a generalized symmetric polyneuropathy.

Nerve Pathology in HNPP—The presence of tomacula or “sausage”-shaped structures in peripheral nerve myelin is the pathological hallmark of HNPP upon histopathological examination of sural nerve biopsies (Figure 2B). These structures consist of redundantly overfolded layers in the myelin sheath (Madrid R and Bradley G, 1975). Mice with targeted deletion of *pmp22* also display characteristic tomacula in myelinated Schwann cells (Adlkofer et al., 1995; Amici et al., 2006; Bai et al., 2010). Tomacula are not unique to PMP22 insufficiency, however, and have been found in other neuropathies and animal models, including anti-MAG neuropathy, CMT1B, chronic inflammatory demyelinating neuropathy, Tangier's disease, and several animal models of peripheral nerve disorders (Sander et al., 2000; Cai et al., 2002; Cai et al., 2006a; Cai et al., 2006b). However, prevalence of tomacula might be different among these diseases and characteristics of abnormal myelin folding in the tomacula may be different as well. For instance, myelin folding mainly appears longitudinally orientated along the axis of axons in CMT4B1/2 (Bolino et al., 2004) while the folding is predominantly concentric around the axonal axis in HNPP (Madrid R and Bradley G, 1975). Detailed morphological analysis is required to differentiate the differences of these tomacula. Finally, axons within the tomacula are often deformed, and appear constricted or flattened (Bai et al., 2010).

Pathogenesis in HNPP—Although tomacula are a histologically significant finding in HNPP, the presence of these structures does not clearly explain the pathophysiological mechanism of HNPP—especially as they are diffusely present along nerves, but the clinical abnormalities in HNPP are multifocal. Animal models have provided key insights into how *pmp22* deletions may result in the phenotype of HNPP. Homozygous deletion (*pmp22*^{−/−}) mice develop a severe de-/dysmyelinating neuropathy with very slow conduction velocities. While heterozygous deletion (*pmp22*^{+/-}) mice are affected with minimal slowing of conduction velocities and possess little evidence of demyelination in teased nerve fibers, segmental myelination may develop in the late stage of mouse life. Like its human counterpart, peripheral nerves in *pmp22*^{+/-} mice show numerous tomacula. It has thus been hypothesized that the transient focal deficits in HNPP are caused by reversible conduction block (CB) evoked by mechanical stress (Lewis et al., 2000; Li et al., 2002, 2004). In compression susceptibility studies conducted in *pmp22*^{+/+} and *pmp22*^{+/-} mice, it has been noticed that the average time to induce conduction block was significantly shorter in *pmp22*^{+/-} mice. Moreover, recovery from nerve conduction block is also delayed in *pmp22*^{+/-} mice. These findings suggest that there is a compromised security of action potential propagation on the HNPP nerves, which predispose HNPP nerves to develop conduction block upon mechanical challenge. This notion is also supported by the fact that compound muscle action potential (CMAP) can be reduced in the *pmp22*^{+/-} nerves in the absence of significant axonal loss and segmental demyelination (Bai et al., 2010).

Constrictions of axons within tomacula have been observed. This constriction may affect the security of action potential propagation since a reduction of axonal diameter is expected to increase the longitudinal electrical resistance. It should also be emphasized that tomacula are formed by excessive myelin folding, which is loosely or not compacted. Poor myelin compaction could impair the insulation of myelin. Moreover, these decompacted tomacula typically extend beyond paranodal region, and often reach the juxtaparanode or even internode (Bai et al., 2010). The additive effect of these changes could profoundly decrease the insulation of myelin. Nevertheless, exactly how the tomacula and/or axonal constrictions affect the security of action potential propagation remains to be clarified.

Finally, axonal degeneration develops in the late stage of HNPP. Mechanisms responsible for the axonal loss are also unknown.

C. CMT1E

CMT1E represents a rare subtype of CMT1 (1-5% of all CMT1 cases) that is caused by point mutations in the *PMP22* gene (Bird, 1993). A variety of *PMP22* point mutations have been documented in humans (Table 3 and Figure 1B) with clinical manifestations ranging from mild mechanical pressure sensitive neuropathy (reminiscent of HNPP) to severe dysmyelinating neuropathy (reminiscent of Dejerine-Sottas syndrome) (Russo et al., 2011). However, mutations of *PMP22* that result in a typical HNPP phenotype should not be included in the group of CMT1E (Table 3). Additionally, unique point mutations of *PMP22* can also result in mild to severe *axonal* CMT1E (Gess et al., 2011). In the past, diseases with some point mutations of *PMP22*, including the Trembler-J mutation, have been classified as CMT1A in the literature (Roa et al., 1991; Patel et al., 1992; Devaux and Scherer, 2005). However, recent advances have demonstrated that CMT1A with over-expressed *PMP22* of wild-type is distinct from those with *PMP22* point mutations at either molecular or pathological level. For instance, sural biopsies from many patients with CMT1A showed no protein aggregates while aggregates were readily detectable in patients with Trembler or Trembler-J mutations (Hanemann et al., 2000). Furthermore, experiments using microarray have shown distinct mechanisms between toxic gain-of-function point mutation mice (*Trembler* or *Trembler-J*) and mice with *PMP22* over-expression. The former shows transcriptional changes in stress response, and the latter demonstrates alterations in Schwann cell proliferation (Giambonini-Brugnoli et al., 2005; Vigo et al., 2005). Therefore, it is reasonable to classify these point mutations of *PMP22* as CMT1E and keep its distinction from CMT1A with duplication of *PMP22*.

With such genetic and phenotypic variety, the evaluation of CMT1E pathogenesis has become an intriguing topic that has revealed important mechanisms of *PMP22* function. Utilizing two animal models of CMT1E, the Trembler and Trembler-J mice, recent research has been able to shed light on the heterogeneity of CMT1E clinical phenotypes and the degradation mechanism of *PMP22*.

Clinical Phenotype and Genetics—Patients with *PMP22* missense mutations present with clinical phenotypes often indistinguishable from those in patients with other subtypes of CMT1, ranging from a mild HNPP-like neuropathy to a severe early-onset dysmyelinating neuropathy like Dejerine-Sottas disease (Russo et al., 2011; Nicholson et al., 1994; Shy et al., 2006; Roa et al., 1993). Such phenotypic heterogeneity may be due to the broad variety of *PMP22* point mutations in CMT1E patients that could cause either a toxic gain-of-function (as seen in the Trembler-J mouse), a loss of *PMP22* function, or a combination of both. To illustrate this point, CMT1E patients can possess point mutations affecting the same amino acid residue of *PMP22*, yet present with distinct clinical manifestations. This can be observed in two patients with *PMP22* point mutation, one of whom was documented with an Ala67Thr substitution and an HNPP phenotype, while the other was documented with an Ala67Pro substitution and a more severe CMT1 phenotype accompanied by deafness (Nodera et al., 2003; Kovach et al., 1999). Conversely, CMT1E patients may possess mutations affecting the same *PMP22* amino acid residue and display similar phenotypes, as is observed in two CMT1E patients (possessing either the Gly100Arg or Gly100Glu amino acid substitution) who had been documented with a Dejerine-Sottas like neuropathy (Bort et al., 1997; Marques, Jr. et al., 1998). The known mutations of *PMP22* include 44 single base substitutions, 14 deletions, 2 insertions, 1 reciprocal translocation, several splice-site mutations, and several single base substitutions in non-coding exon1A and the 3' UTR. With only a few exceptions, almost all *PMP22* missense mutations display

autosomal dominant inheritance. The majority of these mutations are associated with either mild HNPP or severe CMT1E clinical presentations (Russo et al., 2011). Given the phenotypic variability, the definitive diagnosis of CMT1E must be accomplished primarily by genetic testing of the *PMP22* gene.

Clinically, patients with CMT1E may present with symptoms of impaired motor development, distal muscle weakness, foot deformities, and a loss of deep tendon reflex. Sensory deficits, such as diminished response to pinprick and vibration, have also been observed.

Finally, a small subset of CMT1E patients have presented with clinical symptoms indicative of an axonal neuropathy. For instance, a patient heterozygous for the *PMP22* missense mutation (R159C amino acid substitution) was shown to possess a mild axonal neuropathy that manifested later in life around 46-51 years of age. This patient demonstrates symptoms such as symmetric sensory loss in the distal limbs, foot deformities, lower limb paresis, and gait ataxia (Gess et al., 2011).

Nerve Electrophysiology/Pathology in CMT1E Patients—The majority of CMT1E mutations result in a de-/dysmyelinating electrophysiological pattern evidenced by reduced nerve conduction velocities and increased distal latencies. Depending on the severity of the CMT1E phenotype, motor neuron conduction velocities can be reduced to less than 10 m/s.

Pathologically, CMT1E mutations also produce a variety of morphological phenotypes. CMT1E mutations causing severe and early onset phenotype may show profound myelinated nerve fiber loss and abundant onion bulbs (Hanemann et al., 2000). This is comparable to that in CMT1A nerves. In contrast, sural nerves of patients with *PMP22* frameshift mutation and HNPP clinical presentation show a mildly increased loss of large diameter myelinated fibers, formation of onion bulbs, and tomacula (Lennsen et al., 1998). In a group of Italian patients with a missense mutation of *PMP22* and a mild HNPP phenotype, reductions in myelin thickness and large diameter myelinated fiber abundance were observed, but there was no noticeable appearance of onion bulb formation (Luigetti et al., 2008). Additionally, in the rare cases of axonal CMT1E, patients may have no signs of de/dysmyelination but axonal loss (Gess et al., 2011).

Pathogenesis of CMT1E—Much of the current understanding of CMT1E pathogenesis has been derived from the study of the Trembler and Trembler-J mice. These mouse models were shown to possess point mutations in the *pmp22* gene while displaying pathological phenotypes resembling those of CMT1E patients (Suter et al., 1992b; Low, 1976a; Low, 1976b). Trembler mice possess a glycine to aspartic acid substitution (G150D), while Trembler-J mice possess a leucine to proline substitution (L16P). Subsequent studies discovered CMT1E patients with *PMP22* amino acid substitutions identical to those of the Trembler and Trembler-J mice (Valentijn et al., 1992; Ionasescu et al., 1993). There are other mouse models with several different point mutations, including H12R, S72T and Y153TER (Isaacs et al., 2000; Suh et al., 1997). Although these animal models share the same mutations as only a handful of CMT1E patients, their studies have revealed critical findings regarding the potential nature of *PMP22* dysfunction relevant to the majority of CMT1E patients and even patients with point mutations in genes encoding other myelin proteins.

A majority of CMT1E mutations, such as Tr or TrJ, are believed to result in a toxic gain of function phenotype in cells. After being expressed in cells, mutant *PMP22* fails to properly transport from the endoplasmic reticulum (ER) to the plasma membrane. Instead, mutant proteins may form heterodimers with wild-type *PMP22* accumulated in the ER-Golgi intermediate compartment, leading to the formation of protein aggregates. This defect

prevents a portion of wild-type PMP22 protein from reaching the plasma membrane due to the sequestration of wild-type PMP22 protein in stabilized wild-type/TrJ heterodimers in the intermediate compartments (Colby et al., 2000; Dickson et al., 2002; Shames et al., 2003). Different CMT1E mutations show variable propensities in forming protein aggregates (Shames et al., 2003; Liu et al., 2004), but do not cause significant ER stress (Dickson et al., 2002). Surprisingly, degrees of aggregation are not correlated with severities of neuropathies among different types of CMT1E (Liu et al., 2004), suggesting that other factors, in addition to protein aggregation, are also involved in the pathogenic mechanisms.

Thus, alternative mechanisms have been explored. Studies have shown an increase of proteasomes and autolysosomes in Tr or TrJ Schwann cells *in vitro* or *in vivo* (Notterpek et al., 1997; Ryan et al., 2002; Fortun et al., 2003; Fortun et al., 2007), but proteasome activities are usually inhibited in the mutant Schwann cells (Fortun et al., 2006). By up-regulating autophagy through starvation or drugs like Rapamycin, aggregates have been largely reduced, which partially rescued phenotype in the mutant mice (Fortun et al., 2007; Madorsky et al., 2009; Rangaraju et al., 2010).

In addition, several biomechanical changes in TrJ sciatic nerves and Schwann cells have recently been documented. Type IV collagen was found to accumulate with PMP22 protein in the perinuclear region of TrJ Schwann cells. Fixed nerve fibers from TrJ mice were softer and smoother than wild-type fibers by using atomic force microscopy (Rosso et al., 2012). The disruption of F-actin cytoskeletal organization in TrJ nerve fibers has also been documented (Kun et al., 2012), which has led to the idea that *PMP22* point mutations could potentially affect protein secretory pathways of extracellular matrix components (Rosso et al., 2012).

A recent characterization of the structure, stability, and folding dynamics of wild-type and TrJ PMP22 has shed new light on the consequences of *PMP22* point mutations. Wild-type PMP22 consists of four transmembrane helices (TM1-TM4), with TM2-4 existing as a molten globular helical bundle. For proper folding of PMP22 to occur, it has been suggested that TM1 must become stably associated with the TM2-4 bundle (Sakakura et al., 2011). Additionally, it is known that wild-type PMP22 has a very low folding efficiency *in vivo* (20%) which leads to the majority of newly synthesized PMP22 being targeted for degradation in the ERAD pathway (Pareek et al., 1993; Pareek et al., 1997; Sakakura et al., 2011). Evaluation of the TrJ PMP22 protein structure reveals that the L16P mutation creates a hinge-like area within TM1 that allows for the wagging motion of the TM1 helix flanking the mutation site. This causes serious alterations to the interface between TM1 and the TM2-4 bundle, such that the folding efficiency of TrJ PMP22 is lowered to near negligible levels (Sakakura et al., 2011). Furthermore, calnexin, a protein which recognizes and sequesters both misfolded wild-type and TrJ forms of PMP22 in the ER, forms a stronger and longer lived complex with TrJ PMP22 than with wild-type PMP22 (Dickson et al., 2002). The TM1 helix is known to directly interact with calnexin (Fontanini et al., 2005), leading Sakakura et al to propose that the inability of TrJ PMP22 to properly fold results in increased sequestration by calnexin due to TM1 availability. Subsequently, the enhanced affinity between calnexin and TrJ PMP22 may increase the delivery of TrJ PMP22 to aggresomes, overloading the ERAD pathway (Sakakura et al., 2011).

The toxic gain-of-function would not explain the HNPP phenotype in a portion of patients with CMT1E mutations. Of 23 HNPP-causing mutations, 18 of them result in partial or near complete truncation of PMP22 (Table 3). Thus, they would be functionally equivalent to classical chromosome 17p11.2 deletion with loss of function of PMP22. However, the remaining five mutations are point mutations. Two of the five (V30M and T118M) have been studied in transfected cells and showed no or minimal impairment of PMP22

trafficking to cytoplasmic membrane (Shames et al., 2003; Dickson et al., 2002; Naef and Suter, 1999). Thus, these mutations do not likely cause HNPP by dominant-negative effect of wild-type PMP22. These mutations are distributed either in extracellular domains or transmembrane domains, suggesting that HNPP phenotype is also not associated with a specific domain of the PMP22. Thus, how these point mutations result in the loss of function phenotype remains to be determined in the future.

Animal Models for PMP22-Related Diseases

To explore molecular mechanisms in CMT1A and HNPP, PMP22 expression levels have been manipulated in transgenic and knockout mouse models. *Pmp22* with copy numbers ranging from 3 to 16 have been randomly inserted into rodent genome. This has resulted in several mouse models and one rat model with over-expression of *PMP22* (Sereda et al., 1996; Magyar et al., 1996; Huxley et al., 1996; Huxley et al., 1998; Robaglia-Schlupp et al., 2002). Rats with *PMP22* over-expression from three transgenic human *PMP22* copies displayed pathological features similar to that in its human counterparts, as evidenced by peripheral hypomyelination, axonal loss, Schwann cell proliferation, gait abnormalities and slowed conduction velocities. Although onion bulbs were observed, they were not as prominent as one would see in CMT1A sural nerve biopsies (Sereda et al., 1996; Hanemann et al., 2000; Robertson et al., 2002). Overall, this has been considered to be a reasonable model of CMT1A.

Thus far, there have been several *pmp22* over-expressing mouse models that have been made (Sereda et al., 1996; Magyar et al., 1996; Huxley et al., 1996; Huxley et al., 1998; Robaglia-Schlupp et al., 2002). They usually carry a very high copy number of *pmp22* while a lower copy number may not produce significant phenotype (Huxley et al., 1998). For example, TgN248 mice have 16 copies (Magyar et al., 1996) and C22 mice have 7 copies (Robaglia-Schlupp et al., 2002). While the high copy number of *pmp22* produces severe peripheral neuropathies, they are pathologically different from those in patients with CMT1A (trisomy of *PMP22*). Sciatic nerves in these mice show minimal myelin or are amyelinated (no myelin). Conduction velocities are severely slowed and appear similar to that in DSS (Sereda et al., 1996; Magyar et al., 1996; Huxley et al., 1996; Huxley et al., 1998; Robaglia-Schlupp et al., 2002; Robertson et al., 2002). Thus, it is difficult to determine whether these mice are a faithful representation of human CMT1A. Recently, a mouse model with 3-4 copies of *pmp22* has been reported. It also shows pathological changes that may better resemble human CMT1A nerves (Verhamme et al., 2011). This could prove to be a good mouse model of CMT1A in the future. In addition, using the mouse model, *pmp22* over-expression has been controlled temporally. It has been shown that the de-/dysmyelination is reversible in adult animals, when the over-expression of *pmp22* is switched off (Perea et al., 2001). This finding has therapeutic implication. It suggests that an effective therapy may still be beneficial even after the dysmyelination develops in CMT1A.

As aforementioned, mouse models with point mutations either taking place spontaneously or by chemical mutagenesis have been available. The most commonly used models in this category are *Trembler (G150D)* and *Trembler-J (L16P)* (Low, 1976a; Low, 1976b; Suter et al., 1992a; Robertson et al., 1997; Robertson et al., 2002; Henry et al., 1983). In addition, there are several other mouse models, including *Trembler-Ncnp (exon IV deletion)*, H12R, S72T, and Y153TER (Isaacs et al., 2000; Suh et al., 1997).

While these animal models with point mutations of *pmp22* have been valuable resources to characterize the toxic gain-of-function of *pmp22* mutations, they should not be considered as the animal models of CMT1A. It is clear that point mutations of *PMP22* in either humans or mice may result in severe de-/dysmyelinating neuropathies (Henry et al., 1983; Gabreels-

Festen and Wetering, 1999). However, experiments have suggested distinct pathogenic mechanisms between point mutation mice (*Trembler*) and rodents with *pmp22* over-expression (Giambonini-Brugnoli et al., 2005;Vigo et al., 2005). Thus, caution should be taken when selecting an appropriate animal model pertinent to the scientific question to be addressed.

Therapeutic Development in PMP22 Related Diseases

In an *in vitro* study, high levels of ascorbic acid has been shown to repress *PMP22* expression by affecting intracellular cAMP level through adenylate cyclase (Kaya et al., 2007). Conversely, adding vitamin A or E counteracts the effect of ascorbic acid in inhibiting PMP22 expression (Kaya et al., 2008). In addition, addition of ascorbic acid to the medium in neuron-Schwann cell co-culture promotes myelination (Clark and Bunge, 1989;Bunge et al., 1989). These findings have led a therapeutic development for CMT1A. Application of vitamin C to decrease PMP22 has successfully improved the pathological changes and clinical deficits in animal models with *PMP22* over-expression (Sereda et al., 2003;Passage et al., 2004).

These experimental successes have resulted in large clinical trials of ascorbic acid therapies conducted in Europe and USA. Although the results from the USA clinical trial are still pending, the findings of the clinical trial in Europe have raised significant questions regarding the efficacy of ascorbic acid therapy. The clinical trial in Europe enrolled 277 CMT1A patients who were randomized into either an ascorbic acid arm (138 patients) or a placebo arm (133 patients) (Pareyson et al., 2011). Treatment (1.5g per day) was well tolerated since 89% in each group completed the study. For the primary outcome measure, CMT neuropathy score was used, and there were no significant differences found between the two groups at 24 months of treatment. While these results appear negative, several important lessons can be gleaned from the study. First, the overall disease progression of patients with CMT1A is slow. For example, there was an increase of only 0.2 ± 0.7 CMT neuropathy score points over 24 months in the placebo group. This finding indicates that the primary outcome measurement (neuropathy score) is relatively insensitive for patients with CMT1A and should be optimized. Second, a detailed analysis of ascorbic acid pharmacokinetics after large doses, including its absorption rate, is still lacking in human subjects. Third, it is unclear how well ascorbic acid is delivered to its molecular targets in myelinating Schwann cells. Finally, it also raises questions whether the absolute levels of PMP22 are really pathogenic since levels of PMP22 are not well correlated with disabilities of CMT1A (Katona et al., 2009).

The negative trials demand an alternative approach for treating CMT1A. With the advent of large-scale drug screening facilities, additional therapeutic candidates are expected to emerge for future clinical trials. This approach usually requires the development of cell-lines expressing a PMP22 reporter, such as a fluorescence protein. Large libraries of candidate compounds (several thousands) are then placed in a robotic system which rapidly screens for drugs that can suppress the level of PMP22 in the cell-lines. Since increases in PMP22 levels are thought to cause CMT1A, newly identified PMP22 suppressing drugs can be selected for future clinical studies.

The peripheral nervous system possesses classical progesterone/androgen receptors as well as non-classical GABA_A steroid receptors. Neuroactive steroids such as progesterone, dihydroprogesterone, tetrahydroprogesterone, dihydrotestosterone and 3alpha-diol can activate these receptors. Among them, progesterone is a known regulator of myelin gene expression and its administration to Schwann cells increases PMP22 expression (Desarnaud et al., 1998;Melcangi et al., 1999). Administration of the progesterone receptor antagonist

onapriston in early postnatal CMT1A rats has successfully ameliorated the neuropathy phenotype (Sereda et al., 2003; Horste MG et al., 2004; Meyer zu et al., 2007), including reduced axonal loss, improved muscle strength, muscle mass, and better performance in a variety of motor function testing. However, anti-progesterone therapy failed to improve myelin sheath thickness (Meyer zu et al., 2007). Taken together, studies in progesterone antagonists could also yield an alternative treatment for CMT1A.

As discussed above, CMT1E with toxic gain-of-function mutations of *PMP22* exhibit pathological changes and mechanisms distinct from that in CMT1A with over-expression of wild-type *PMP22*. Thus, therapy development would have to take a different approach. Curcumin is a polyphenol and an active component of turmeric (*Curcuma longa*), a dietary spice in Indian cuisine. It has antioxidant, anti-inflammatory and anti-cancer properties. Interestingly, Egan et al have shown that curcumin may rescue misfolded proteins in both cell cultures and a cystic fibrosis mouse model by presumably interfering with the function of ER calcium-dependent chaperones (Egan et al., 2004). This has prompted investigators to examine its effect in CMT1E, such as TrJ mice. Oral administration of curcumin partially mitigates the neuropathy phenotype in the TrJ mice in a dose-dependent manner, including a decrease of the percentage of apoptotic Schwann cells, increased number and size of myelinated axons in sciatic nerves, and improved motor performance (Khajavi et al., 2007). This result also supports the importance of misfolded proteins in the pathogenesis of CMT1E.

Summary

Since its discovery, *PMP22* has demonstrated its importance in the peripheral nervous system through its abundant expression specifically in the peripheral myelin and being the culprit gene for the highest prevalence inherited neuropathies, including CMT1A, HNPP and CMT1E. Normal physiological functions are likely best learnt in diseases or animal models where *PMP22* gene is deficient. In these systems, *PMP22* appears involved in myelin integrity, thereby affecting security of action potential propagation. In contrast, over-expressed *PMP22* likely affects peripheral nerves by gain-of-function. Its pathogenic mechanism also appears distinct from that in neuropathies with *PMP22* point mutations. In the following section, we propose hypothetical pathogenic mechanisms that are illustrated in Figure 3. These mechanisms are likely incomplete, but would provide a platform for the future exploration.

Trisomy of *PMP22* results in highly variable levels of *PMP22* proteins (Katona et al., 2009). This variable over-expression has dys-regulated expression of a variety of proteins in myelin. Two pathways appear highly relevant to the dysmyelination in CMT1A. Enzymes that catalyze the production of cholesterol are severely suppressed (Giambonini-Brugnoli et al., 2005). Deficiency of cholesterol synthesis during development of peripheral nerves is known to impair myelination, including reduced myelin thickness and shortened internodes (Saher et al., 2005; Saher et al., 2009). These changes would explain the slowed conduction velocity in CMT1A. Trisomy of *PMP22* also appears to up-regulate the expression of P2X7, a purinergic receptor, which would increase the calcium levels in Schwann cells (Nobbio et al., 2009). High calcium level is known to induce segmental demyelination (Smith and Hall, 1988; Smith et al., 2001). However, this mechanism likely operates after myelin developed since high calcium levels are normally present in immature developing Schwann cells. In addition, dysregulation of proteins at the interface between axons and Schwann cells has been observed recently (Kinter et al., 2012). Disruption of Schwann-glia interaction may lead to axonal degeneration (Sahenk et al., 1999).

In contrast, haploinsufficiency of *PMP22* results in formation of tomacula by unknown mechanisms. The tomacula decompacts myelin beyond paranodal regions and expand into juxtaparanode or internode (Bai et al., 2010). We hypothesize that the decompaction would reduce myelin electrical resistance (insulation), leading to excessive leakage of current. Along with constricted axons in the tomacula, these abnormalities would put *PMP22*^{+/-} nerves at the risk of failure of action potential propagation. This failure would be readily precipitated by mechanical stress in patients with HNPP.

Unlike wild-type *PMP22* proteins in CMT1A and HNPP, mutations in CMT1E, such as *TrJ*, produce mutant proteins, which form aggregates in Schwann cells. These mutant proteins are prone to be misfolded in the ER (Sakakura et al., 2011), leading to ER stress, up-regulation of ERAD or perhaps autophagic activities secondarily (Ryan et al., 2002; Fortun et al., 2003; Notterpek et al., 1997). Like nerves in CMT1A, shortened internodes are observed in *TrJ* mutations (Liu et al., 2004; Liu et al., 2005). Unlike CMT1A, segmental demyelination in *TrJ* nerves is far more prominent (Devaux and Scherer, 2005). However, it is still unknown how the dysregulated protein degradation leads to segmental demyelination.

Acknowledgments

This work is, in part, supported by NIH (R01NS066927) and Veterans Affairs (BLR&D).

Reference List

- Abe A, Nakamura K, Kato M, Numakura C, Honma T, Seiwa C, Shirahata E, Itoh A, Kishikawa Y, Hayasaka K. Compound heterozygous *PMP22* deletion mutations causing severe Charcot-Marie-Tooth disease type 1. *J Hum Genet.* 2010; 55:771–773. [PubMed: 20739940]
- Adlkofer K, Frei R, Neuberg DH, Zielasek J, Toyka KV, Suter U. Heterozygous peripheral myelin protein 22-deficient mice are affected by a progressive demyelinating tomaculous neuropathy. *J Neurosci.* 1997; 17:4662–4671. [PubMed: 9169527]
- Adlkofer K, Martini R, Aguzzi A, Zielasek J, Toyka KV, Suter U. Hypermyelination and demyelinating peripheral neuropathy in *Pmp22*-deficient mice. *Nat Genet.* 1995; 11:274–280. [PubMed: 7581450]
- Al-Thihli K, Rudkin T, Carson N, Poulin C, Melancon S, Der Kaloustian VM. Compound heterozygous deletions of *PMP22* causing severe Charcot-Marie-Tooth disease of the Dejerine-Sottas disease phenotype. *Am J Med Genet A.* 2008; 146A:2412–2416. [PubMed: 18698610]
- Amato AA, Gronseth GS, Callera KJ, Kagan-Hallet KS, Bryan WW, Barohn RJ. Tomaculous neuropathy: a clinical and electrophysiological study in patients with and without 1.5-Mb deletions in chromosome 17p11.2. *Muscle Nerve.* 1996; 19:16–22. [PubMed: 8538664]
- Amici SA, Dunn WA Jr, Murphy AJ, Adams NC, Gale NW, Valenzuela DM, Yancopoulos GD, Notterpek L. Peripheral myelin protein 22 is in complex with $\alpha 6 \beta 4$ integrin, and its absence alters the Schwann cell basal lamina. *J Neurosci.* 2006; 26:1179–1189. [PubMed: 16436605]
- Amici SA, Dunn WA Jr, Notterpek L. Developmental abnormalities in the nerves of peripheral myelin protein 22-deficient mice. *J Neurosci Res.* 2007; 85:238–249. [PubMed: 17131416]
- Andersson PB, Yuen E, Parko K, So YT. Electrodiagnostic features of hereditary neuropathy with liability to pressure palsies. *Neurology.* 2000; 54:40–44. [PubMed: 10636123]
- Arroyo EJ, Scherer SS. On the molecular architecture of myelinated fibers. *Histochem Cell Biol.* 2000; 113:1–18. [PubMed: 10664064]
- Attardi LD, Reczek EE, Cosmas C, Demicco EG, McCurrach ME, Lowe SW, Jacks T. PERP, an apoptosis-associated target of p53, is a novel member of the PMP-22/gas3 family. *Genes Dev.* 2000; 14:704–718. [PubMed: 10733530]
- Bai Y, Zhang X, Katona I, Saporta MA, Shy ME, O'Malley HA, Isom LL, Suter U, Li J. Conduction block in *PMP22* deficiency. *J Neurosci.* 2010; 30:600–608. [PubMed: 20071523]

- Bellone E, Di ME, Soriani S, Varese A, Doria LL, Ajmar F, Mandich P. A novel mutation (D305V) in the early growth response 2 gene is associated with severe Charcot-Marie-Tooth type 1 disease. *Hum Mutat.* 1999; 14:353–354. [PubMed: 10502832]
- Berciano MT, Calle E, Fernandez R, Lafarga M. Regulation of Schwann cell numbers in tellurium-induced neuropathy: apoptosis, supernumerary cells and internodal shortening. *Acta Neuropathol.* 1998; 95:269–279. [PubMed: 9542592]
- Bird, TD. Charcot-Marie-Tooth Neuropathy Type 1. 1993.
- Bolino A, Bolis A, Previtali SC, Dina G, Bussini S, Dati G, Amadio S, Del Carro U, Mruk DD, Feltri ML, Cheng CY, Quattrini A, Wrabetz L. Disruption of *Mtmr2* produces CMT4B1-like neuropathy with myelin outfolding and impaired spermatogenesis. *J Cell Biol.* 2004; 167:711–721. [PubMed: 15557122]
- Bondurand N, Girard M, Pingault V, Lemort N, Dubourg O, Goossens M. Human Connexin 32, a gap junction protein altered in the X-linked form of Charcot-Marie-Tooth disease, is directly regulated by the transcription factor SOX10. *Hum Mol Genet.* 2001; 10:2783–2795. [PubMed: 11734543]
- Bort S, Nelis E, Timmerman V, Sevilla T, Cruz-Martinez A, Martinez F, Millan JM, Arpa J, Vilchez JJ, Prieto F, Van Broeckhoven C, Palau F. Mutational analysis of the MPZ, PMP22 and Cx32 genes in patients of Spanish ancestry with Charcot-Marie-Tooth disease and hereditary neuropathy with liability to pressure palsies. *Hum Genet.* 1997; 99:746–754. [PubMed: 9187667]
- Bosse F, Brodbeck J, Muller HW. Post-transcriptional regulation of the peripheral myelin protein gene PMP22/gas3. *J Neurosci Res.* 1999; 55:164–177. [PubMed: 9972819]
- Bosse F, Zoidl G, Wilms S, Gillen CP, Kuhn HG, Muller HW. Differential expression of two mRNA species indicates a dual function of peripheral myelin protein PMP22 in cell growth and myelination. *J Neurosci Res.* 1994; 37:529–537. [PubMed: 8021974]
- Bostock H, Sears TA. Continuous conduction in demyelinated mammalian nerve fibers. *Nature.* 1976; 263:786–787. [PubMed: 995194]
- Bostock H, Sears TA, Sherratt RM. The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibres. *J Physiol.* 1981; 313:301–315. [PubMed: 7277221]
- Bostock H, Sherratt RM, Sears TA. Overcoming conduction failure in demyelinated nerve fibres by prolonging action potentials. *Nature.* 1978; 274:385–387. [PubMed: 209331]
- Brill MH, Waxman SG, Moore JW, Joyner RW. Conduction velocity and spike configuration in myelinated fibers: computed dependence on internode distance. *J Neurol Neurosurg Psychiatry.* 1977; 40:769–774. [PubMed: 925697]
- Bunge RP, Bunge MB, Bates M. Movements of the Schwann cell nucleus implicate progression of the inner (axon-related) Schwann cell process during myelination. *J Cell Biol.* 1989; 109:273–284. [PubMed: 2745552]
- Cai Z, Blumbergs PC, Cash K, Rice PJ, Manavis J, Swift J, Ghabriel MN, Thompson PD. Paranodal pathology in Tangier disease with remitting-relapsing multifocal neuropathy. *J Clin Neurosci.* 2006a; 13:492–497. [PubMed: 16678735]
- Cai Z, Finnie JW, Blumbergs PC, Manavis J, Ghabriel MN, Thompson PD. Early paranodal myelin swellings (tomacula) in an avian riboflavin deficiency model of demyelinating neuropathy. *Exp Neurol.* 2006b; 198:65–71. [PubMed: 16336963]
- Cai Z, Sutton-Smith P, Swift J, Cash K, Finnie J, Turnley A, Thompson PD, Blumbergs PC. Tomacula in MAG-deficient mice. *J Peripher Nerv Syst.* 2002; 7:181–189. [PubMed: 12365566]
- Chance PF, Alderson MK, Leppig KA, Lensch MW, Matsunami N, Smith B, Swanson PD, Odelberg SJ, Distèche CM, Bird TD. DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell.* 1993; 72:143–151. [PubMed: 8422677]
- Chance PF, Lensch MW, Lipe H, Brown RH Sr. Brown RH Jr. Bird TD. Hereditary neuralgic amyotrophy and hereditary neuropathy with liability to pressure palsies: two distinct genetic disorders. *Neurology.* 1994; 44:2253–2257. [PubMed: 7991108]
- Chen Y, Medvedev A, Ruzanov P, Marvin KW, Jetten AM. cDNA cloning, genomic structure, and chromosome mapping of the human epithelial membrane protein CL-20 gene (EMP1), a member of the PMP22 family. *Genomics.* 1997; 41:40–48. [PubMed: 9126480]

- Choi BO, Kim NK, Park SW, Hyun YS, Jeon HJ, Hwang JH, Chung KW. Inheritance of Charcot-Marie-Tooth disease 1A with rare nonrecurrent genomic rearrangement. *Neurogenetics*. 2011; 12:51–58. [PubMed: 21193943]
- Clark MB, Bunge MB. Cultured Schwann cells assemble normal-appearing basal lamina only when they ensheath axons. *Dev Biol*. 1989; 133:393–404. [PubMed: 2659405]
- Colby J, Nicholson R, Dickson KM, Orfali W, Naef R, Suter U, Snipes GJ. PMP22 carrying the trembler or trembler-J mutation is intracellularly retained in myelinating Schwann cells. *Neurobiol Dis*. 2000; 7:561–573. [PubMed: 11114256]
- Court FA, Sherman DL, Pratt T, Garry EM, Ribchester RR, Cottrell DF, Fleetwood-Walker SM, Brophy PJ. Restricted growth of Schwann cells lacking Cajal bands slows conduction in myelinated nerves. *Nature*. 2004; 431:191–195. [PubMed: 15356632]
- D'Urso D, Prior R, Greiner-Petter R, Gabreels-Festen AA, Muller HW. Overloaded endoplasmic reticulum-Golgi compartments, a possible pathomechanism of peripheral neuropathies caused by mutations of the peripheral myelin protein PMP22. *J Neurosci*. 1998; 18:731–740. [PubMed: 9425015]
- De LM, Nahin RL, Mendoza ME, Ruda MA. SR13/PMP-22 expression in rat nervous system, in PC12 cells, and C6 glial cell lines. *J Neurosci Res*. 1994; 38:167–181. [PubMed: 8078102]
- Desarnaud F, Bidichandani S, Patel PI, Baulieu EE, Schumacher M. Glucocorticosteroids stimulate the activity of the promoters of peripheral myelin protein-22 and protein zero genes in Schwann cells. *Brain Res*. 2000; 865:12–16. [PubMed: 10814728]
- Desarnaud F, Do Thi AN, Brown AM, Lemke G, Suter U, Baulieu EE, Schumacher M. Progesterone stimulates the activity of the promoters of peripheral myelin protein-22 and protein zero genes in Schwann cells. *J Neurochem*. 1998; 71:1765–1768. [PubMed: 9751213]
- Devaux JJ, Scherer SS. Altered ion channels in an animal model of Charcot-Marie-Tooth disease type IA. *J Neurosci*. 2005; 25:1470–1480. [PubMed: 15703401]
- Dickson KM, Bergeron JJ, Shames I, Colby J, Nguyen DT, Chevet E, Thomas DY, Snipes GJ. Association of calnexin with mutant peripheral myelin protein-22 ex vivo: a basis for “gain-of-function” ER diseases. *Proc Natl Acad Sci U S A*. 2002; 99:9852–9857. [PubMed: 12119418]
- Dyck PJ, Lambert EH. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic, and electrophysiologic findings in hereditary polyneuropathies. *Arch Neurol*. 1968; 18:603–618. [PubMed: 4297451]
- EARL CJ, FULLERTON PM, WAKEFIELD GS, SCHUTTA HS. HEREDITARY NEUROPATHY, WITH LIABILITY TO PRESSURE PALSIES; A CLINICAL AND ELECTROPHYSIOLOGICAL STUDY OF FOUR FAMILIES. *Q J Med*. 1964; 33:481–498. [PubMed: 14212604]
- Egan ME, Pearson M, Weiner SA, Rajendran V, Rubin D, Glockner-Pagel J, Canny S, Du K, Lukacs GL, Caplan MJ. Curcumin, a major constituent of turmeric, corrects cystic fibrosis defects. *Science*. 2004; 304:600–602. [PubMed: 15105504]
- Fledrich R, Schlotter-Weigel B, Schnizer TJ, Wichert SP, Stassart RM, zu Horste GM, Klink A, Weiss BG, Haag U, Walter MC, Rautenstrauss B, Paulus W, Rossner MJ, Sereda MW. A rat model of Charcot-Marie-Tooth disease 1A recapitulates disease variability and supplies biomarkers of axonal loss in patients. *Brain*. 2012; 135:72–87. [PubMed: 22189569]
- Fontanini A, Chies R, Snapp EL, Ferrarini M, Fabrizi GM, Brancolini C. Glycan-independent role of calnexin in the intracellular retention of Charcot-Marie-tooth 1A Gas3/PMP22 mutants. *J Biol Chem*. 2005; 280:2378–2387. [PubMed: 15537650]
- Fortun J, Dunn WA Jr. Joy S, Li J, Notterpek L. Emerging role for autophagy in the removal of aggregates in Schwann cells. *J Neurosci*. 2003; 23:10672–10680. [PubMed: 14627652]
- Fortun J, Go JC, Li J, Amici SA, Dunn WA Jr. Notterpek L. Alterations in degradative pathways and protein aggregation in a neuropathy model based on PMP22 overexpression. *Neurobiol Dis*. 2006; 22:153–164. [PubMed: 16326107]
- Fortun J, Verrier JD, Go JC, Madorsky I, Dunn WA, Notterpek L. The formation of peripheral myelin protein 22 aggregates is hindered by the enhancement of autophagy and expression of cytoplasmic chaperones. *Neurobiol Dis*. 2007; 25:252–265. [PubMed: 17174099]

- Gabreels-Festen A, Wetering RV. Human nerve pathology caused by different mutational mechanisms of the PMP22 gene. *Ann N Y Acad Sci.* 1999; 883:336–343. [PubMed: 10586258]
- Gabreels-Festen AA, Bolhuis PA, Hoogendijk JE, Valentijn LJ, Eshuis EJ, Gabreels FJ. Charcot-Marie-Tooth disease type 1A: morphological phenotype of the 17p duplication versus PMP22 point mutations. *Acta Neuropathol.* 1995; 90:645–649. [PubMed: 8615087]
- Gess B, Jeibmann A, Schirmacher A, Kleffner I, Schilling M, Young P. Report of a novel mutation in the PMP22 gene causing an axonal neuropathy. *Muscle Nerve.* 2011; 43:605–609. [PubMed: 21337347]
- Ghislain J, Desmarquet-Trin-Dinh C, Gilardi-Hebenstreit P, Charnay P, Frain M. Neural crest patterning: autoregulatory and crest-specific elements co-operate for Krox20 transcriptional control. *Development.* 2003; 130:941–953. [PubMed: 12538520]
- Ghislain J, Desmarquet-Trin-Dinh C, Jaegle M, Meijer D, Charnay P, Frain M. Characterisation of cis-acting sequences reveals a biphasic, axon-dependent regulation of Krox20 during Schwann cell development. *Development.* 2002; 129:155–166. [PubMed: 11782409]
- Giambonini-Brugnoli G, Buchstaller J, Sommer L, Suter U, Mantei N. Distinct disease mechanisms in peripheral neuropathies due to altered peripheral myelin protein 22 gene dosage or a Pmp22 point mutation. *Neurobiol Dis.* 2005; 18:656–668. [PubMed: 15755691]
- Gonnaud PM, Sturtz F, Fourbil Y, Bonnebouche C, Tranchant C, Warter JM, Chazot G, Bady B, Vial C, Brechard AS. DNA analysis as a tool to confirm the diagnosis of asymptomatic hereditary neuropathy with liability to pressure palsies (HNPP) with further evidence for the occurrence of de novo mutations. *Acta Neurol Scand.* 1995; 92:313–318. [PubMed: 8848937]
- Hai M, Bidichandani SI, Patel PI. Identification of a positive regulatory element in the myelin-specific promoter of the PMP22 gene. *J Neurosci Res.* 2001; 65:508–519. [PubMed: 11550219]
- Hanemann CO, D'Urso D, Gabreels-Festen AA, Muller HW. Mutation-dependent alteration in cellular distribution of peripheral myelin protein 22 in nerve biopsies from Charcot-Marie-Tooth type 1A. *Brain.* 2000; 123(Pt 5):1001–1006. [PubMed: 10775544]
- Hartline DK, Colman DR. Rapid conduction and the evolution of giant axons and myelinated fibers. *Curr Biol.* 2007; 17:R29–R35. [PubMed: 17208176]
- Hasse B, Bosse F, Hanenberg H, Muller HW. Peripheral myelin protein 22 kDa and protein zero: domain specific trans-interactions. *Mol Cell Neurosci.* 2004; 27:370–378. [PubMed: 15555916]
- Henry EW, Cowen JS, Sidman RL. Comparison of Trembler and Trembler-J mouse phenotypes: varying severity of peripheral hypomyelination. *J Neuropathol Exp Neurol.* 1983; 42:688–706. [PubMed: 6313869]
- Horowitz SH, Spollen LE, Yu W. Hereditary neuropathy with liability to pressure palsy: fulminant development with axonal loss during military training. *J Neurol Neurosurg Psychiatry.* 2004; 75:1629–1631. [PubMed: 15489403]
- Horste, MG.; Nave, KA.; Sereda, MW. PMP22 expression in skin biopsies serves as a marker for disease severity in a model of Charcot-Marie-Tooth disease 1A (CMT1A). 2004.
- Huxley C, Passage E, Manson A, Putzu G, Figarella-Branger D, Pellissier JF, Fontes M. Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum Mol Genet.* 1996; 5:563–569. [PubMed: 8733121]
- Huxley C, Passage E, Robertson AM, Youl B, Huston S, Manson A, Saberan-Djoniedi D, Figarella-Branger D, Pellissier JF, Thomas PK, Fontes M. Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. *Hum Mol Genet.* 1998; 7:449–458. [PubMed: 9467003]
- Ihrle RA, Reczek E, Horner JS, Khachatryan L, Sage J, Jacks T, Attardi LD. Perp is a mediator of p53-dependent apoptosis in diverse cell types. *Curr Biol.* 2003; 13:1985–1990. [PubMed: 14614825]
- Ingram WJ, Wicking CA, Grimmond SM, Forrest AR, Wainwright BJ. Novel genes regulated by Sonic Hedgehog in pluripotent mesenchymal cells. *Oncogene.* 2002; 21:8196–8205. [PubMed: 12444557]
- Ionasescu VV, Ionasescu R, Searby C, Barker DF. Charcot-Marie-Tooth neuropathy type 1A with both duplication and non-duplication. *Hum Mol Genet.* 1993; 2:405–410. [PubMed: 8099303]
- Isaacs AM, et al. Identification of two new Pmp22 mouse mutants using large-scale mutagenesis and a novel rapid mapping strategy. *Hum Mol Genet.* 2000; 9:1865–1871. [PubMed: 10915775]

- Jacobs SB, Basak S, Murray JI, Pathak N, Attardi LD. Siva is an apoptosis-selective p53 target gene important for neuronal cell death. *Cell Death Differ.* 2007; 14:1374–1385. [PubMed: 17464332]
- Jang SW, Srinivasan R, Jones EA, Sun G, Keles S, Krueger C, Chang LW, Nagarajan R, Svaren J. Locus-wide identification of Egr2/Krox20 regulatory targets in myelin genes. *J Neurochem.* 2010; 115:1409–1420. [PubMed: 21044070]
- Jetten AM, Suter U. The peripheral myelin protein 22 and epithelial membrane protein family. *Prog Nucleic Acid Res Mol Biol.* 2000; 64:97–129. [PubMed: 10697408]
- Jones EA, Brewer MH, Srinivasan R, Krueger C, Sun G, Charney KN, Keles S, Antonellis A, Svaren J. Distal enhancers upstream of the Charcot-Marie-Tooth type 1A disease gene PMP22. *Hum Mol Genet.* 2012; 21:1581–1591. [PubMed: 22180461]
- Jones EA, Jang SW, Mager GM, Chang LW, Srinivasan R, Gokey NG, Ward RM, Nagarajan R, Svaren J. Interactions of Sox10 and Egr2 in myelin gene regulation. *Neuron Glia Biol.* 2007; 3:377–387. [PubMed: 18634568]
- Jones EA, Lopez-Anido C, Srinivasan R, Krueger C, Chang LW, Nagarajan R, Svaren J. Regulation of the PMP22 gene through an intronic enhancer. *J Neurosci.* 2011; 31:4242–4250. [PubMed: 21411665]
- Katona I, Wu X, Feely SM, Sottile S, Siskind CE, Miller LJ, Shy ME, Li J. PMP22 expression in dermal nerve myelin from patients with CMT1A. *Brain.* 2009; 132:1734–1740. [PubMed: 19447823]
- Kaya F, Belin S, Bourgeois P, Micallef J, Blin O, Fontes M. Ascorbic acid inhibits PMP22 expression by reducing cAMP levels. *Neuromuscul Disord.* 2007; 17:248–253. [PubMed: 17303424]
- Kaya F, Belin S, Diamantidis G, Fontes M. Ascorbic acid is a regulator of the intracellular cAMP concentration: old molecule, new functions? *FEBS Lett.* 2008; 582:3614–3618. [PubMed: 18835269]
- Khajavi M, Shiga K, Wiszniewski W, He F, Shaw CA, Yan J, Wensel TG, Snipes GJ, Lupski JR. Oral curcumin mitigates the clinical and neuropathologic phenotype of the Trembler-J mouse: a potential therapy for inherited neuropathy. *Am J Hum Genet.* 2007; 81:438–453. [PubMed: 17701891]
- Kinter J, Lazzati T, Schmid D, Zeis T, Erne B, Lutzelschwab R, Steck AJ, Pareyson D, Peles E, Schaeren-Wiemers N. An essential role of MAG in mediating axon-myelin attachment in Charcot-Marie-Tooth 1A disease. *Neurobiol Dis.* 2012
- Kitamura K, Suzuki M, Uyemura K. Purification and partial characterization of two glycoproteins in bovine peripheral nerve myelin membrane. *Biochim Biophys Acta.* 1976; 455:806–816. [PubMed: 999941]
- Kovach MJ, Lin JP, Boyadjiev S, Campbell K, Mazzeo L, Herman K, Rimer LA, Frank W, Llewellyn B, Jabs EW, Gelber D, Kimonis VE. A unique point mutation in the PMP22 gene is associated with Charcot-Marie-Tooth disease and deafness. *Am J Hum Genet.* 1999; 64:1580–1593. [PubMed: 10330345]
- Krajewski K, Turansky C, Lewis R, Garbern J, Hinderer S, Kamholz J, Shy ME. Correlation between weakness and axonal loss in patients with CMT1A. *Ann N Y Acad Sci.* 1999; 883:490–492. [PubMed: 10586281]
- Krajewski KM, Lewis RA, Fuerst DR, Turansky C, Hinderer SR, Garbern J, Kamholz J, Shy ME. Neurological dysfunction and axonal degeneration in Charcot-Marie-Tooth disease type 1A. *Brain.* 2000; 123(Pt 7):1516–1527. [PubMed: 10869062]
- Kun A, Canclini L, Rosso G, Bresque M, Romeo C, Hanusz A, Cal K, Calliari A, Sotelo SJ, Sotelo JR. F-actin distribution at nodes of Ranvier and Schmidt-Lanterman incisures in mammalian sciatic nerves. *Cytoskeleton (Hoboken).* 2012; 69:486–495. [PubMed: 22328339]
- Lafontaine S, Rasminsky M, Saida T, Sumner AJ. Conduction block in rat myelinated fibres following acute exposure to anti-galactocerebroside serum. *J Physiol.* 1982; 323:287–306. [PubMed: 7097575]
- LeBlanc SE, Jang SW, Ward RM, Wrabetz L, Svaren J. Direct regulation of myelin protein zero expression by the Egr2 transactivator. *J Biol Chem.* 2006; 281:5453–5460. [PubMed: 16373334]

- LeBlanc SE, Srinivasan R, Ferri C, Mager GM, Gillian-Daniel AL, Wrabetz L, Svaren J. Regulation of cholesterol/lipid biosynthetic genes by Egr2/Krox20 during peripheral nerve myelination. *J Neurochem.* 2005; 93:737–748. [PubMed: 15836632]
- LeBlanc SE, Ward RM, Svaren J. Neuropathy-associated Egr2 mutants disrupt cooperative activation of myelin protein zero by Egr2 and Sox10. *Mol Cell Biol.* 2007; 27:3521–3529. [PubMed: 17325040]
- Lenßen PP, Gabreels-Festen AA, Valentijn LJ, Jongen PJ, van Beersum SE, van Engelen BG, van Wensen PJ, Bolhuis PA, Gabreels FJ, Mariman EC. Hereditary neuropathy with liability to pressure palsies. Phenotypic differences between patients with the common deletion and a PMP22 frame shift mutation. *Brain.* 1998; 121(Pt 8):1451–1458. [PubMed: 9712007]
- Lewis RA, Sumner AJ. The electrodiagnostic distinctions between chronic familial and acquired demyelinating neuropathies. *Neurology.* 1982; 32:592–596. [PubMed: 6283420]
- Lewis RA, Sumner AJ, Shy ME. Electrophysiological features of inherited demyelinating neuropathies: A reappraisal in the era of molecular diagnosis. *Muscle Nerve.* 2000; 23:1472–1487. [PubMed: 11003782]
- Li J, Ghandour K, Radovanovic D, Shy RR, Krajewski KM, Shy ME, Nicholson GA. Stoichiometric alteration of PMP22 protein determines the phenotype of HNPP. *Archives of Neurology.* 2007; 64:974–978. [PubMed: 17620487]
- Li, J.; Lewis, R.; Shy, M. Charcot-Marie-Tooth Disease.. In: Aminoff, M.; Daroff, R., editors. *Encyclopedia of the Neurological Sciences.* Academic Press; 2003. p. 676-686.
- Li J, Krajewski K, Lewis RA, Shy ME. Loss-of-function phenotype of hereditary neuropathy with liability to pressure palsies. *Muscle Nerve.* 2004; 29:205–210. [PubMed: 14755484]
- Li J, Krajewski K, Shy ME, Lewis RA. Hereditary neuropathy with liability to pressure palsy: the electrophysiology fits the name. *Neurology.* 2002; 58:1769–1773. [PubMed: 12084875]
- Liu N, Varma S, Shooter EM, Tolwani RJ. Enhancement of Schwann cell myelin formation by K252a in the Trembler-J mouse dorsal root ganglion explant culture. *J Neurosci Res.* 2005; 79:310–317. [PubMed: 15605381]
- Liu N, Yamauchi J, Shooter EM. Recessive, but not dominant, mutations in peripheral myelin protein 22 gene show unique patterns of aggregation and intracellular trafficking. *Neurobiol Dis.* 2004; 17:300–309. [PubMed: 15474367]
- Lobsiger CS, Magyar JP, Taylor V, Wulf P, Welcher AA, Program AE, Suter U. Identification and characterization of a cDNA and the structural gene encoding the mouse epithelial membrane protein-1. *Genomics.* 1996; 36:379–387. [PubMed: 8884260]
- Low PA. Hereditary hypertrophic neuropathy in the trembler mouse. Part 1. Histopathological studies: light microscopy. *J Neurol Sci.* 1976a; 30:327–341. [PubMed: 187739]
- Low PA. Hereditary hypertrophic neuropathy in the trembler mouse. Part 2. Histopathological studies: electron microscopy. *J Neurol Sci.* 1976b; 30:343–368. [PubMed: 187740]
- Luigetti M, Conte A, Madia F, Mereu ML, Zollino M, Marangi G, Pomponi MG, Liberatore G, Tonali PA, Sabatelli M. A new single-nucleotide deletion of PMP22 in an HNPP family without recurrent palsies. *Muscle Nerve.* 2008; 38:1060–1064. [PubMed: 18642376]
- Lupski JR. An inherited DNA rearrangement and gene dosage effect are responsible for the most common autosomal dominant peripheral neuropathy: Charcot-Marie-Tooth disease type 1A. *Clin Res.* 1992; 40:645–652. [PubMed: 1486731]
- Lupski JR, Oca-Luna RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo-Cardenas O, Barker DF, Killian JM, Garcia CA. DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell.* 1991; 66:219–232. [PubMed: 1677316]
- Madorsky I, Opalach K, Waber A, Verrier JD, Solmo C, Foster T, Dunn WA Jr. Notterpek L. Intermittent fasting alleviates the neuropathic phenotype in a mouse model of Charcot-Marie-Tooth disease. *Neurobiol Dis.* 2009; 34:146–154. [PubMed: 19320048]
- Madrid R, Bradley G. The pathology of neuropathies with focal thickening of the myelin sheath (tomaculous neuropathy): studies on the formation of the abnormal myelin sheath. *Journal of The Neurological Sciences.* 1975; 25:415–448.

- Magyar JP, Martini R, Ruelicke T, Aguzzi A, Adlkofer K, Dembic Z, Zielasek J, Toyka KV, Suter U. Impaired differentiation of Schwann cells in transgenic mice with increased PMP22 gene dosage. *J Neurosci.* 1996; 16:5351–5360. [PubMed: 8757248]
- Maier M, Berger P, Nave KA, Suter U. Identification of the regulatory region of the peripheral myelin protein 22 (PMP22) gene that directs temporal and spatial expression in development and regeneration of peripheral nerves. *Mol Cell Neurosci.* 2002; 20:93–109. [PubMed: 12056842]
- Maier M, Castagner F, Berger P, Suter U. Distinct elements of the peripheral myelin protein 22 (PMP22) promoter regulate expression in Schwann cells and sensory neurons. *Mol Cell Neurosci.* 2003; 24:803–817. [PubMed: 14664827]
- Mandich P, James R, Nassani S, Defferrari R, Bellone E, Mancardi G, Schenone A, Abbruzzese M, Rocchi M, Ajmar F. Molecular diagnosis of hereditary neuropathy with liability to pressure palsies (HNPP) by detection of 17p11.2 deletion in Italian patients. *J Neurol.* 1995; 242:295–298. [PubMed: 7643137]
- Manfioletti G, Ruaro ME, Del SG, Philipson L, Schneider C. A growth arrest-specific (gas) gene codes for a membrane protein. *Mol Cell Biol.* 1990; 10:2924–2930. [PubMed: 1692961]
- Marques W Jr, Thomas PK, Sweeney MG, Carr L, Wood NW. Dejerine-Sottas neuropathy and PMP22 point mutations: a new base pair substitution and a possible “hot spot” on Ser72. *Ann Neurol.* 1998; 43:680–683. [PubMed: 9585367]
- Melcangi RC, Magnaghi V, Cavarretta I, Zucchi I, Bovolin P, D'Urso D, Martini L. Progesterone derivatives are able to influence peripheral myelin protein 22 and P0 gene expression: possible mechanisms of action. *J Neurosci Res.* 1999; 56:349–357. [PubMed: 10340743]
- Meyer zu HG, Prukop T, Liebetanz D, Mobius W, Nave KA, Sereda MW. Antiprogestosterone therapy uncouples axonal loss from demyelination in a transgenic rat model of CMT1A neuropathy. *Ann Neurol.* 2007; 61:61–72. [PubMed: 17262851]
- Mobley CK, Myers JK, Hadziselimovic A, Ellis CD, Sanders CR. Purification and initiation of structural characterization of human peripheral myelin protein 22, an integral membrane protein linked to peripheral neuropathies. *Biochemistry.* 2007; 46:11185–11195. [PubMed: 17824619]
- Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C, Moens CB, Talbot WS. A G protein-coupled receptor is essential for Schwann cells to initiate myelination. *Science.* 2009; 325:1402–1405. [PubMed: 19745155]
- Monk KR, Oshima K, Jors S, Heller S, Talbot WS. Gpr126 is essential for peripheral nerve development and myelination in mammals. *Development.* 2011; 138:2673–2680. [PubMed: 21613327]
- Mouton P, Tardieu S, Gouider R, Birouk N, Maisonnobe T, Dubourg O, Brice A, LeGuern E, Bouche P. Spectrum of clinical and electrophysiologic features in HNPP patients with the 17p11.2 deletion. *Neurology.* 1999; 52:1440–1446. [PubMed: 10227632]
- Myers JK, Mobley CK, Sanders CR. The peripheral neuropathy-linked Trembler and Trembler-J mutant forms of peripheral myelin protein 22 are folding-destabilized. *Biochemistry.* 2008; 47:10620–10629. [PubMed: 18795802]
- Naef R, Adlkofer K, Lescher B, Suter U. Aberrant protein trafficking in Trembler suggests a disease mechanism for hereditary human peripheral neuropathies. *Mol Cell Neurosci.* 1997; 9:13–25. [PubMed: 9204477]
- Naef R, Suter U. Impaired intracellular trafficking is a common disease mechanism of PMP22 point mutations in peripheral neuropathies. *Neurobiol Dis.* 1999; 6:1–14. [PubMed: 10078969]
- Nagarajan R, Svaren J, Le N, Araki T, Watson M, Milbrandt J. EGR2 mutations in inherited neuropathies dominant-negatively inhibit myelin gene expression. *Neuron.* 2001; 30:355–368. [PubMed: 11394999]
- Nattkamp H, Halfter H, Khazaei MR, Lohmann T, Gess B, Eisenacher M, Willscher E, Young P. Varying survival of motoneurons and activation of distinct molecular mechanism in response to altered peripheral myelin protein 22 gene dosage. *J Neurochem.* 2009; 110:935–946. [PubMed: 19493167]
- Nestler M, Martin U, Hortschansky P, Saluz HP, Henke A, Munder T. The zinc containing proapoptotic protein siva interacts with the peroxisomal membrane protein pmp22. *Mol Cell Biochem.* 2006; 287:147–155. [PubMed: 16683188]

- Nicholson GA, Valentijn LJ, Cherryson AK, Kennerson ML, Bragg TL, DeKroon RM, Ross DA, Pollard JD, McLeod JG, Bolhuis PA. A frame shift mutation in the PMP22 gene in hereditary neuropathy with liability to pressure palsies. *Nat Genet.* 1994; 6:263–266. [PubMed: 8012388]
- Niemann S, Sereda MW, Suter U, Griffiths IR, Nave KA. Uncoupling of myelin assembly and schwann cell differentiation by transgenic overexpression of peripheral myelin protein 22. *J Neurosci.* 2000; 20:4120–4128. [PubMed: 10818147]
- Nobbio L, Sturla L, Fiorese F, Usai C, Basile G, Moreschi I, Benvenuto F, Zocchi E, De FA, Schenone A, Bruzzone S. P2X7-mediated increased intracellular calcium causes functional derangement in Schwann cells from rats with CMT1A neuropathy. *J Biol Chem.* 2009; 284:23146–23158. [PubMed: 19546221]
- Nodera H, Nishimura M, Logigian EL, Herrmann DN, Kaji R. HNPP due to a novel missense mutation of the PMP22 gene. *Neurology.* 2003; 60:1863–1864. [PubMed: 12796555]
- Notterpek L, Roux KJ, Amici SA, Yazdanpour A, Rahner C, Fletcher BS. Peripheral myelin protein 22 is a constituent of intercellular junctions in epithelia. *Proc Natl Acad Sci U S A.* 2001; 98:14404–14409. [PubMed: 11717414]
- Notterpek L, Shooter EM, Snipes GJ. Upregulation of the endosomal-lysosomal pathway in the trembler-J neuropathy. *J Neurosci.* 1997; 17:4190–4200. [PubMed: 9151736]
- Nowak M, Koster C, Hammerschmidt M. Perp is required for tissue-specific cell survival during zebrafish development. *Cell Death Differ.* 2005; 12:52–64. [PubMed: 15529176]
- Ohsawa Y, Murakami T, Miyazaki Y, Shirabe T, Sunada Y. Peripheral myelin protein 22 is expressed in human central nervous system. *J Neurol Sci.* 2006; 247:11–15. [PubMed: 16626749]
- Orfali W, Nicholson RN, Guiot MC, Peterson AC, Snipes GJ. An 8.5-kb segment of the PMP22 promoter responds to loss of axon signals during Wallerian degeneration, but does not respond to specific axonal signals during nerve regeneration. *J Neurosci Res.* 2005; 80:37–46. [PubMed: 15723356]
- Pareek S, Notterpek L, Snipes GJ, Naef R, Sossin W, Laliberte J, Iacampo S, Suter U, Shooter EM, Murphy RA. Neurons promote the translocation of peripheral myelin protein 22 into myelin. *J Neurosci.* 1997; 17:7754–7762. [PubMed: 9315897]
- Pareek S, Suter U, Snipes GJ, Welcher AA, Shooter EM, Murphy RA. Detection and processing of peripheral myelin protein PMP22 in cultured Schwann cells. *J Biol Chem.* 1993; 268:10372–10379. [PubMed: 8486695]
- Pareyson D, Reilly MM, Schenone A, Fabrizi GM, Cavallaro T, Santoro L, Vita G, Quattrone A, Padua L, Gemignani F, Visioli F, Laura M, Radice D, Calabrese D, Hughes RA, Solari A. Ascorbic acid in Charcot-Marie-Tooth disease type 1A (CMT-TRIAAL and CMT-TRAUK): a double-blind randomised trial. *Lancet Neurol.* 2011; 10:320–328. [PubMed: 21393063]
- Parmantier E, Braun C, Thomas JL, Peyron F, Martinez S, Zalc B. PMP-22 expression in the central nervous system of the embryonic mouse defines potential transverse segments and longitudinal columns. *J Comp Neurol.* 1997; 378:159–172. [PubMed: 9120057]
- Parmantier E, Cabon F, Braun C, D'Urso D, Muller HW, Zalc B. Peripheral myelin protein-22 is expressed in rat and mouse brain and spinal cord motoneurons. *Eur J Neurosci.* 1995; 7:1080–1088. [PubMed: 7613613]
- Passage E, Norreel JC, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X, Robaglia-Schlupp A, Pellissier JF, Fontes M. Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. *Nat Med.* 2004; 10:396–401. [PubMed: 15034573]
- Patel PI, Roa BB, Welcher AA, Schoener-Scott R, Trask BJ, Pentao L, Snipes GJ, Garcia CA, Francke U, Shooter EM, Lupski JR, Suter U. The gene for the peripheral myelin protein PMP-22 is a candidate for Charcot-Marie-Tooth disease type 1A. *Nat Genet.* 1992; 1:159–165. [PubMed: 1303228]
- Patzko A, Shy ME. Update on Charcot-Marie-Tooth disease. *Curr Neurol Neurosci Rep.* 2011; 11:78–88. [PubMed: 21080241]
- Pentao L, Wise CA, Chinault AC, Patel PI, Lupski JR. Charcot-Marie-Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. *Nat Genet.* 1992; 2:292–300. [PubMed: 1303282]

- Raeymaekers P, Timmerman V, Nelis E, Van Hul W, De Jonghe P, Martin JJ, Van Broeckhoven C. Estimation of the size of the chromosome 17p11.2 duplication in Charcot-Marie-Tooth neuropathy type 1a (CMT1a). HMSN Collaborative Research Group. *J Med Genet.* 1992; 29:5–11. [PubMed: 1552545]
- Rangaraju S, Verrier JD, Madorsky I, Nicks J, Dunn WA Jr, Notterpek L. Rapamycin activates autophagy and improves myelination in explant cultures from neuropathic mice. *J Neurosci.* 2010; 30:11388–11397. [PubMed: 20739560]
- Rasminsky M, Sears TA. Internodal conduction in normal and demyelinated mammalian single nerve fibres. *J Physiol.* 1971; 217:66P–67P.
- Reilly MM, Murphy SM, Laura M. Charcot-Marie-Tooth disease. *J Peripher Nerv Syst.* 2011; 16:1–14. [PubMed: 21504497]
- Reiprich S, Kriesch J, Schreiner S, Wegner M. Activation of Krox20 gene expression by Sox10 in myelinating Schwann cells. *J Neurochem.* 2010; 112:744–754. [PubMed: 19922439]
- Reiter LT, Murakami T, Koeuth T, Pentao L, Muzny DM, Gibbs RA, Lupski JR. A recombination hotspot responsible for two inherited peripheral neuropathies is located near a mariner transposon-like element. *Nat Genet.* 1996; 12:288–297. [PubMed: 8589720]
- Roa BB, Dyck PJ, Marks HG, Chance PF, Lupski JR. Dejerine-Sottas syndrome associated with point mutation in the peripheral myelin protein 22 (PMP22) gene. *Nat Genet.* 1993; 5:269–273. [PubMed: 8275092]
- Roa BB, Garcia CA, Lupski JR. Charcot-Marie-Tooth disease type 1A: molecular mechanisms of gene dosage and point mutation underlying a common inherited peripheral neuropathy. *Int J Neurol.* 1991; 25-26:97–107. [PubMed: 11980069]
- Robaglia-Schlupp A, Pizant J, Norreel JC, Passage E, Saberan-Djoneidi D, Ansaldi JL, Vinay L, Figarella-Branger D, Levy N, Clarac F, Cau P, Pellissier JF, Fontes M. PMP22 overexpression causes dysmyelination in mice. *Brain.* 2002; 125:2213–2221. [PubMed: 12244079]
- Robertson AM, King RH, Muddle JR, Thomas PK. Abnormal Schwann cell/axon interactions in the Trembler-J mouse. *J Anat.* 1997; 190(Pt 3):423–432. [PubMed: 9147228]
- Robertson AM, Perea J, McGuigan A, King RH, Muddle JR, Gabreels-Festen AA, Thomas PK, Huxley C. Comparison of a new pmp22 transgenic mouse line with other mouse models and human patients with CMT1A. *J Anat.* 2002; 200:377–390. [PubMed: 12090404]
- Rosso G, Negreira C, Sotelo JR, Kun A. Myelinating and demyelinating phenotype of Trembler-J mouse (a model of Charcot-Marie-Tooth human disease) analyzed by atomic force microscopy and confocal microscopy. *J Mol Recognit.* 2012; 25:247–255. [PubMed: 22528185]
- Roux KJ, Amici SA, Notterpek L. The temporospatial expression of peripheral myelin protein 22 at the developing blood-nerve and blood-brain barriers. *J Comp Neurol.* 2004; 474:578–588. [PubMed: 15174074]
- Russo M, Laura M, Polke JM, Davis MB, Blake J, Brandner S, Hughes RA, Houlden H, Bennett DL, Lunn MP, Reilly MM. Variable phenotypes are associated with PMP22 missense mutations. *Neuromuscul Disord.* 2011; 21:106–114. [PubMed: 21194947]
- Ryan MC, Notterpek L, Tobler AR, Liu N, Shooter EM. Role of the peripheral myelin protein 22 N-linked glycan in oligomer stability. *J Neurochem.* 2000; 75:1465–1474. [PubMed: 10987826]
- Ryan MC, Shooter EM, Notterpek L. Aggresome formation in neuropathy models based on peripheral myelin protein 22 mutations. *Neurobiol Dis.* 2002; 10:109–118. [PubMed: 12127149]
- Saberan-Djoneidi D, Sanguedolce V, Assouline Z, Levy N, Passage E, Fontes M. Molecular dissection of the Schwann cell specific promoter of the PMP22 gene. *Gene.* 2000; 248:223–231. [PubMed: 10806367]
- Sahenk Z, Chen L, Mendell JR. Effects of PMP22 duplication and deletions on the axonal cytoskeleton. *Ann Neurol.* 1999; 45:16–24. [PubMed: 9894872]
- Saher G, Brugger B, Lappe-Siefke C, Mobius W, Tozawa R, Wehr MC, Wieland F, Ishibashi S, Nave KA. High cholesterol level is essential for myelin membrane growth. *Nat Neurosci.* 2005; 8:468–475. [PubMed: 15793579]
- Saher G, Quintes S, Mobius W, Wehr MC, Kramer-Albers EM, Brugger B, Nave KA. Cholesterol regulates the endoplasmic reticulum exit of the major membrane protein P0 required for peripheral myelin compaction. *J Neurosci.* 2009; 29:6094–6104. [PubMed: 19439587]

- Sakakura M, Hadziselimovic A, Wang Z, Schey KL, Sanders CR. Structural basis for the Trembler-J phenotype of Charcot-Marie-Tooth disease. *Structure*. 2011; 19:1160–1169. [PubMed: 21827951]
- Salzer JL. Polarized domains of myelinated axons. *Neuron*. 2003; 40:297–318. [PubMed: 14556710]
- Sander S, Ouvrier RA, McLeod JG, Nicholson GA, Pollard JD. Clinical syndromes associated with tomacula or myelin swellings in sural nerve biopsies. *J Neurol Neurosurg Psychiatry*. 2000; 68:483–488. [PubMed: 10727485]
- Saporta MA, Katona I, Lewis RA, Masse S, Shy ME, Li J. Shortened internodal length of dermal myelinated nerve fibres in Charcot-Marie-Tooth disease type 1A. *Brain*. 2009
- Saporta MA, Katona I, Zhang X, Roper HP, McClelland L, Macdonald F, Brueton L, Blake J, Suter U, Reilly MM, Shy ME, Li J. Neuropathy in a human without the PMP22 gene. *Arch Neurol*. 2011; 68:814–821. [PubMed: 21670407]
- Scherer SS, Arroyo EJ. Recent progress on the molecular organization of myelinated axons. *J Peripher Nerv Syst*. 2002; 7:1–12. [PubMed: 11939347]
- Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. *Cell*. 1988; 54:787–793. [PubMed: 3409319]
- Sedzik J, Kotake Y, Uyemura K. Purification of PASII/PMP22--an extremely hydrophobic glycoprotein of PNS myelin membrane. *Neuroreport*. 1998; 9:1595–1600. [PubMed: 9631472]
- Sedzik J, Tsukihara T. Solubilization of PNS myelin membrane proteins by detergents. *Neuroreport*. 2000; 11:2559–2563. [PubMed: 10943722]
- Sedzik J, Uyemura K, Tsukihara T. Towards crystallization of hydrophobic myelin glycoproteins: P0 and PASII/PMP22. *Protein Expr Purif*. 2002; 26:368–377. [PubMed: 12460760]
- Sereda M, Griffiths I, Puhlhofer A, Stewart H, Rossner MJ, Zimmerman F, Magyar JP, Schneider A, Hund E, Meinck HM, Suter U, Nave KA. A transgenic rat model of Charcot-Marie-Tooth disease. *Neuron*. 1996; 16:1049–1060. [PubMed: 8630243]
- Sereda MW, Meyer zu HG, Suter U, Uzma N, Nave KA. Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). *Nat Med*. 2003; 9:1533–1537. [PubMed: 14608378]
- Shames I, Fraser A, Colby J, Orfali W, Snipes GJ. Phenotypic differences between peripheral myelin protein-22 (PMP22) and myelin protein zero (P0) mutations associated with Charcot-Marie-Tooth-related diseases. *J Neuropathol Exp Neurol*. 2003; 62:751–764. [PubMed: 12901701]
- Shy ME, Scavina MT, Clark A, Krajewski KM, Li J, Kamholz J, Kolodny E, Szigeti K, Fischer RA, Saifi GM, Scherer SS, Lupski JR. T118M PMP22 mutation causes partial loss of function and HNPP-like neuropathy. *Ann Neurol*. 2006; 59:358–364. [PubMed: 16437560]
- Skre H. Genetic and clinical aspects of Charcot-Marie-Tooth's disease. *Clin Genet*. 1974; 6:98–118. [PubMed: 4430158]
- Smith KJ, Hall SM. Peripheral demyelination and remyelination initiated by the calcium-selective ionophore ionomycin: in vivo observations. *J Neurol Sci*. 1988; 83:37–53. [PubMed: 3126271]
- Smith KJ, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. *Ann Neurol*. 2001; 49:470–476. [PubMed: 11310624]
- Snipes GJ, Suter U, Welcher AA, Shooter EM. Characterization of a novel peripheral nervous system myelin protein (PMP-22/SR13). *J Cell Biol*. 1992; 117:225–238. [PubMed: 1556154]
- Spreyer P, Kuhn G, Hanemann CO, Gillen C, Schaal H, Kuhn R, Lemke G, Muller HW. Axon-regulated expression of a Schwann cell transcript that is homologous to a 'growth arrest-specific' gene. *EMBO J*. 1991; 10:3661–3668. [PubMed: 1935894]
- Stogbauer F, Young P, Kühlenbaumer G, De Jonghe P, Timmerman V. Hereditary recurrent focal neuropathies: clinical and molecular features. *Neurology*. 2000; 54:546–551. [PubMed: 10680781]
- Suh JG, Ichihara N, Saigoh K, Nakabayashi O, Yamanishi T, Tanaka K, Wada K, Kikuchi T. An in-frame deletion in peripheral myelin protein-22 gene causes hypomyelination and cell death of the Schwann cells in the new Trembler mutant mice. *Neuroscience*. 1997; 79:735–744. [PubMed: 9219937]
- Suter U, Moskow JJ, Welcher AA, Snipes GJ, Kosaras B, Sidman RL, Buchberg AM, Shooter EM. A leucine-to-proline mutation in the putative first transmembrane domain of the 22-kDa peripheral

- myelin protein in the trembler-J mouse. *Proc Natl Acad Sci U S A*. 1992a; 89:4382–4386. [PubMed: 1374899]
- Suter U, Patel PI. Genetic basis of inherited peripheral neuropathies. *Hum Mutat*. 1994; 3:95–102. [PubMed: 7515304]
- Suter U, Welcher AA, Ozcelik T, Snipes GJ, Kosaras B, Francke U, Billings-Gagliardi S, Sidman RL, Shooter EM. Trembler mouse carries a point mutation in a myelin gene. *Nature*. 1992b; 356:241–244. [PubMed: 1552943]
- Taylor V, Welcher AA, Program AE, Suter U. Epithelial membrane protein-1, peripheral myelin protein 22, and lens membrane protein 20 define a novel gene family. *J Biol Chem*. 1995; 270:28824–28833. [PubMed: 7499407]
- Taylor V, Zraggen C, Naef R, Suter U. Membrane topology of peripheral myelin protein 22. *J Neurosci Res*. 2000; 62:15–27. [PubMed: 11002284]
- Thomas PK, Marques W Jr, Davis MB, Sweeney MG, King RH, Bradley JL, Muddle JR, Tyson J, Malcolm S, Harding AE. The phenotypic manifestations of chromosome 17p11.2 duplication. *Brain*. 1997; 120(Pt 3):465–478. [PubMed: 9126058]
- Timmerman V, De Jonghe P, Ceuterick C, De Vriendt E, Lofgren A, Nelis E, Warner LE, Lupski JR, Martin JJ, Van Broeckhoven C. Novel missense mutation in the early growth response 2 gene associated with Dejerine-Sottas syndrome phenotype. *Neurology*. 1999; 52:1827–1832. [PubMed: 10371530]
- Timmerman V, Lofgren A, Le Guern E, Liang P, De Jonghe P, Martin JJ, Verhalle D, Robberecht W, Gouider R, Brice A, Van Broeckhoven C. Molecular genetic analysis of the 17p11.2 region in patients with hereditary neuropathy with liability to pressure palsies (HNPP). *Hum Genet*. 1996; 97:26–34. [PubMed: 8557256]
- Timmerman V, Raeymaekers P, De Jonghe P, De Winter G, Swerts L, Jacobs K, Gheuens J, Martin JJ, Vandenberghe A, Van Broeckhoven C. Assignment of the Charcot-Marie-Tooth neuropathy type 1 (CMT 1a) gene to 17p11.2-p12. *Am J Hum Genet*. 1990; 47:680–685. [PubMed: 2220808]
- Tobler AR, Notterpek L, Naef R, Taylor V, Suter U, Shooter EM. Transport of Trembler-J mutant peripheral myelin protein 22 is blocked in the intermediate compartment and affects the transport of the wild-type protein by direct interaction. *J Neurosci*. 1999; 19:2027–2036. [PubMed: 10066256]
- Valentijn LJ, Baas F, Wolterman RA, Hoogendijk JE, van den Bosch NH, Zorn I, Gabreels-Festen AW, de VM, Bolhuis PA. Identical point mutations of PMP-22 in Trembler-J mouse and Charcot-Marie-Tooth disease type 1A. *Nat Genet*. 1992; 2:288–291. [PubMed: 1303281]
- Verhamme C, King RH, ten Asbroek AL, Muddle JR, Nourallah M, Wolterman R, Baas F, van Schaik IN. Myelin and axon pathology in a long-term study of PMP22-overexpressing mice. *J Neuropathol Exp Neurol*. 2011; 70:386–398. [PubMed: 21487305]
- Vigo T, Nobbio L, Hummelen PV, Abbruzzese M, Mancardi G, Verpoorten N, Verhoeven K, Sereda MW, Nave KA, Timmerman V, Schenone A. Experimental Charcot-Marie-Tooth type 1A: a cDNA microarrays analysis. *Mol Cell Neurosci*. 2005; 28:703–714. [PubMed: 15797717]
- Warner LE, Mancias P, Butler IJ, McDonald CM, Keppen L, Koob KG, Lupski JR. Mutations in the early growth response 2 (EGR2) gene are associated with hereditary myelinopathies. *Nat Genet*. 1998; 18:382–384. [PubMed: 9537424]
- Warner LE, Svaren J, Milbrandt J, Lupski JR. Functional consequences of mutations in the early growth response 2 gene (EGR2) correlate with severity of human myelinopathies. *Hum Mol Genet*. 1999; 8:1245–1251. [PubMed: 10369870]
- Welcher AA, De LM, Suter U, Snipes GJ, Meakin SO, Shooter EM. Isolation of transcriptionally regulated sequences associated with neuronal and non-neuronal cell interactions. *Prog Brain Res*. 1992; 94:163–176. [PubMed: 1287719]
- Weterman MA, van RF, de WM, Bordewijk L, Samijn JP, van der Pol WL, Meggouh F, Baas F. Copy number variation upstream of PMP22 in Charcot-Marie-Tooth disease. *Eur J Hum Genet*. 2010; 18:421–428. [PubMed: 19888301]
- Zhang F, Seeman P, Liu P, Weterman MA, Gonzaga-Jauregui C, Towne CF, Batish SD, De VE, De JP, Rautenstrauss B, Krause KH, Khajavi M, Posadka J, Vandenberghe A, Palau F, Van ML, Baas F, Timmerman V, Lupski JR. Mechanisms for nonrecurrent genomic rearrangements

- associated with CMT1A or HNPP: rare CNVs as a cause for missing heritability. *Am J Hum Genet.* 2010; 86:892–903. [PubMed: 20493460]
- Zorick TS, Syroid DE, Brown A, Gridley T, Lemke G. Krox-20 controls SCIP expression, cell cycle exit and susceptibility to apoptosis in developing myelinating Schwann cells. *Development.* 1999; 126:1397–1406. [PubMed: 10068633]
- Adlkofer K, Martini R, Aguzzi A, Zielasek J, Toyka KV, Suter U. Hypermyelination and demyelinating peripheral neuropathy in Pmp22-deficient mice. *Nat Genet.* 1995; 11:274–80. [PubMed: 7581450]
- Amici SA, Dunn WA Jr, Murphy AJ, Adams NC, Gale NW, Valenzuela DM, Yancopoulos GD, Notterpek L. Peripheral myelin protein 22 is in complex with alpha6beta4 integrin, and its absence alters the Schwann cell basal lamina. *J Neurosci.* 2006; 26(4):1179–89. [PubMed: 16436605]
- Brancolini C, Edomi P, Marzinotto S, Schneider C. Exposure at the cell surface is required for gas3/PMP22 to regulate both cell death and cell spreading: implication for the Charcot-Marie-Tooth type 1A and Dejerine-Sottas diseases. *Mol Biol Cell.* 2000; 11(9):2901–14. [PubMed: 10982389]
- Brancolini C, Marzinotto S, Edomi P, Agostoni E, Fiorentini C, Müller HW, Schneider C. Rho-dependent regulation of cell spreading by the tetraspan membrane protein Gas3/PMP22. *Mol Biol Cell.* 1999; 10(7):2441–59. [PubMed: 10397775]
- D'Urso D, Schmalenbach C, Zoidl G, Prior R, Müller HW. Studies on the effects of altered PMP22 expression during myelination in vitro. *J Neurosci Res.* 1997; 48(1):31–42. [PubMed: 9086179]
- Erdem S, Mendell JR, Sahenk Z. Fate of Schwann cells in CMT1A and HNPP: evidence for apoptosis. *J Neuropathol Exp Neurol.* 1998; 57(6):635–42. [PubMed: 9630241]
- Fabbretti E, Edomi P, Brancolini C, Schneider C. Apoptotic phenotype induced by overexpression of wild-type gas3/PMP22: its relation to the demyelinating peripheral neuropathy CMT1A. *Genes Dev.* 1995; 9(15):1846–56. [PubMed: 7649472]
- Hanemann CO, Gabreëls-Festen AA, Stoll G, Müller HW. Schwann cell differentiation in Charcot-Marie-Tooth disease type 1A (CMT1A): normal number of myelinating Schwann cells in young CMT1A patients and neural cell adhesion molecule expression in onion bulbs. *Acta Neuropathol.* 1997; 94(4):310–5. [PubMed: 9341930]
- Hanemann CO, Rosenbaum C, Kupfer S, Wosch S, Stoegbauer F, Müller HW. Improved culture methods to expand Schwann cells with altered growth behaviour from CMT1A patients. *Glia.* Jun; 1998 23(2):89–98. [PubMed: 9600378]
- Magyar JP, Martini R, Ruelicke T, Aguzzi A, Adlkofer K, Dembic Z, Zielasek J, Toyka KV, Suter U. Impaired differentiation of Schwann cells in transgenic mice with increased PMP22 gene dosage. *J Neurosci.* 1996; 16:5351–60. [PubMed: 8757248]
- Niemann S, Sereda MW, Suter U, Griffiths IR, Nave KA. Uncoupling of myelin assembly and schwann cell differentiation by transgenic overexpression of peripheral myelin protein 22. *J Neurosci.* 2000; 20:4120–8. [PubMed: 10818147]
- Nobbio L, Vigo T, Abbruzzese M, Levi G, Brancolini C, Mantero S, Grandis M, Benedetti L, Mancardi G, Schenone A. Impairment of PMP22 transgenic Schwann cells differentiation in culture: implications for Charcot-Marie-Tooth type 1A disease. *Neurobiol Dis.* 2004; 16:263–73. [PubMed: 15207283]
- Perkins CS, Aguayo AJ, Bray GM. Schwann cell multiplication in Trembler mice. *Neuropathol Appl Neurobiol.* 1981; 7(2):115–26. [PubMed: 7231639]
- Robertson AM, Huxley C, King RH, Thomas PK. Development of early postnatal peripheral nerve abnormalities in Trembler-J and PMP22 transgenic mice. *J Anat.* 1999; 195(Pt 3):331–9. [PubMed: 10580849]
- Robertson AM, Perea J, McGuigan A, King RH, Muddle JR, Gabreëls-Festen AA, Thomas PK, Huxley C. Comparison of a new pmp22 transgenic mouse line with other mouse models and human patients with CMT1A. *J Anat.* 2002; 200(4):377–90. [PubMed: 12090404]
- Sahenk Z, Serrano-Munuera C, Chen L, Kakabadze I, Najagara HN. Evidence for impaired axonal regeneration in PMP22 duplication: studies in nerve xenografts. *J Peripher Nerv Syst.* 2003; 8:116–27. [PubMed: 12795716]

- Sancho S, Young P, Suter U. Regulation of Schwann cell proliferation and apoptosis in PMP22-deficient mice and mouse models of Charcot-Marie-Tooth disease type 1A. *Brain*. 2001; 124(Pt 11):2177–87. [PubMed: 11673320]
- Schuijter MM, Mann CJ, Bildsoe H, Huxley C, Hughes SM. Analyses of the differentiation potential of satellite cells from myoD^{-/-}, mdx, and PMP22 C22 mice. *BMC Musculoskelet Disord*. 2005; 6:15. [PubMed: 15762989]
- Snipes GJ, Suter U, Shooter EM. Human peripheral myelin protein-22 carries the L2/HNK-1 carbohydrate adhesion epitope. *J Neurochem*. 1992; 61(5):1961–4. [PubMed: 7693873]
- Suh JG, Ichihara N, Saigoh K, Nakabayashi O, Yamanishi T, Tanaka K, Wada K, Kikuchi T. An in-frame deletion in peripheral myelin protein-22 gene causes hypomyelination and cell death of the Schwann cells in the new Trembler mutant mice. *Neuroscience*. 1997; 79(3):735–44. [PubMed: 9219937]
- Verrier JD, Lau P, Hudson L, Murashov AK, Renne R, Notterpek L. Peripheral myelin protein 22 is regulated post-transcriptionally by miRNA-29a. *Glia*. 2009; 57:1265–79. [PubMed: 19170179]
- Zoidl G, Blass-Kampmann S, D'Urso D, Schmalenbach C, Müller HW. Retroviral-mediated gene transfer of the peripheral myelin protein PMP22 in Schwann cells: modulation of cell growth. *EMBO J*. 1995; 14(6):1122–8. [PubMed: 7720703]
- Zoidl G, D'Urso D, Blass-Kampmann S, Schmalenbach C, Kuhn R, Müller HW. Influence of elevated expression of rat wild-type PMP22 and its mutant PMP22Trembler on cell growth of NIH3T3 fibroblasts. *Cell Tissue Res*. 1997; 287(3):459–70. [PubMed: 9023077]

Figure 1. PMP22 gene structure and predicted protein structure

A. A schematic representation of a human *PMP22* gene consists of six exons. Exon-1a containing transcripts predominantly express in myelinating Schwann cells. Exon-1b containing transcripts express in non-neuronal cells. B. A schematic representation of human PMP22 protein topology shows extracellular, intracellular and transmembrane domains. A variety of mutations causal for CMT4E are marked at the amino acid sites where the mutations take place.

Figure 2. Pathological findings in CMT1A and HNPP

A. A sural nerve biopsy was performed in a 70-year-old man with CMT1A. A semithin preparation shows numerous onion bulbs scattered over the entire visual field (from Berciano et al, JNNP 2006; 77: 1169-1176 with permission). B. Photomicrograph of sural nerve biopsy-teased nerve fiber preparation from a 23-year-old woman with HNPP, showing areas of myelin thickening (tomaculum). C. Photomicrograph of sural nerve biopsy epoxy semithin preparation, transverse section (methyl blue), from the patient illustrates multiple myelinated fibers with excessively thick myelin (tomaculum). B&C are from Crum et al, Muscle & Nerve 2000; 23: 979-983 with permission.

Figure 3.
Hypothetical mechanisms in CMT1A, HNPP and CMT1E.

Table 1

Localization of PMP22 transcripts and protein

mRNA	Species	Embryonic		Postnatal / Young adults		Reference
		Non neuronal	Neuronal	Non neuronal	Neuronal	
	Human	N/A	N/A	N/A	(Br) + (BS) +	Ohsawa et al 2006
	Rat	N/A	(Br) +	N/A	(Br) + (SN) +++ (SC) ++	Snipes et al (1992); Roux et al (2004);
	Rat	N/A	N/A	(Ki) + (Te) + (SM) ++ (He) ++ (Re) +++ (Ce) +++ (Lu) +++ (Co) ++ (Il) ++	(BS)-motor nuclei ++; (SC)-MN ++ (SN) +++; BS-sensory nuclei & DRG –	Taylor et al (1995); Parmantier et al (1995)
	Rat	N/A	N/A	N/A	(Br) + (BS) + (SC) ++ (SN) +++ (DRG)-Neu – (DRG)-Sat ++	De Leon et al (1994)
	Mouse		(Br) +++		(Br) +	Wulf and Suter (1999)
	Mouse	N/A	(Ventral Brain) ++ (SC) + (SC)-MN – (DRG) ++	(Co) +++ (Ce) +++ (Re) +++ (Lu) +++ (Il) ++ (Je) ++ (He) +	(BS)-motor nuclei ++; (SC)-MN ++ (SN) +++; BS-sensory nuclei & DRG –	Parmantier et al (1995 and 1997); Lobsiger et al (1996)
Protein	Mouse	(Li) +++ (Gu) +++ (CaV) +++ (Lu) ++ (Le) ++	(SG) ++	N/A	(CN) +++	Beachner, et al., (1995)
	Human (adult)	N/A	N/A	N/A	(FN) +++	Erme et al (2002)
	Human (Adult)	N/A	N/A	N/A	(VR) +++ (MN) + (GN) +	Ohsawa et al 2006
	Rat	N/A	N/A	N/A	(SN) +++ (SC) –	Roux et al (2004); D'Urso and Muller (1997); Erme et al (2002); Dickson et al (2002); Snipes et al (1992)

mRNA	Species	Embryonic		Postnatal / Young adults		Reference
		Non neuronal	Neuronal	Non neuronal	Neuronal	
	Rat	N/A	(Br) +	N/A	(Br) +	Roux et al (2004)
	Rat (adult)	N/A	N/A	N/A	(DRG)-Neu ++ (DRG)-Sat ++ SC (DH and VW) ++; MN -	De Leon et al (1994)
	Rat (adult)	N/A	N/A	(Li) + (Co) +	N/A	Notterpek et al (2001)
	Mouse	N/A	N/A	N/A	(SN-compact myelin) +++	Haney et al (1996); Carenini et al (1999); Notterpek et al (1997);

N/A = not addressed; Br = Brain; BS = Brain stem; CaV = Cartilage of the forming vertebrae; Ce = Caecum; Co = Colon; DRG = Dorsal root ganglion; CN = cranial nerve; FN = Femoral nerve; Gu = Gut; He = Heart; Il = Ileum; Ki = Kidney; Le = Lens; Li = Liver; Lu = Lung; Re = Rectum; SC = Spinal cord; SG = Smooth muscle; SM = Sciatic nerve; Te = Testis; GN = preganglionic sympathetic neuron; DH = dorsal horn; VH = Ventral horn; VW = ventral white matter;

* Roots were removed from these spinal cords

Table 2**PMP22 effect in cell proliferation**

Genotype	Type of Cell or Tissue	Change of Proliferation	Reference
overexpression	Retroviral transferred Schwann cells	Decreased	Zoidl, et al, 1995 D'urso, et al, 1997
	Cultivated Schwann cells from nerve biopsies of CMT1A patients	Decreased	Hanemann, et al, 1998
	Cultivated Schwann cells from homogygous and heterozygous pmp22 transgenic rat	Decreased	Nobbi, et al, 2004
	Nerve biopsies from CMT1A patients, age of 26-72 years (Schwann cells)	Decreased	Erderm, et al, 1998
	Nerve from heterozygous pmp22 transgenic mice (Schwann cells)	No difference at postnatal day 1(P1), but increased at P4, P10, P21 and 10 weeks of age	Sancho, et al, 2001
	Nerve biopsies of CMT1A patients, age of 3-6 years, (Schwann cells)	No difference	Hanemann, et al, 1997
	Retroviral transferred NIH 3T3 cells (Fibroblast)	Decreased	Zoidl, et al, 1997
underexpression	Retroviral transferred Schwann cells	Increased	Zoidl, et al, 1995
	Nerve from HNPP mice (Schwann cells)	Increased	Amici, et al. 2006
	Nerve biopsies from HNPP patients, age of 24-50 years (Schwann cells)	Increased	Erderm, et al, 1998
	Nerve from homozygous pmp22 knockout mice (Schwann cells)	No difference at P1, P4, P21, but increased at P10 and 10 weeks of age	Sancho, et al, 2001
	Retroviral transferred Schwann cells	No difference	D'urso, et al, 1997
Point mutant	Nerve of Trembler mice (Schwann cells)	Increased	Ferkins, et al, 1981
	Nerve of Trembler mice (Schwann cells)	Increased	Robertson, et al, 2002
	Nerve from trembler mice (Schwann cells)	No difference at P1, P4, P10, but increased at P21 and 10 weeks of age	Sancho, et al, 2001
	Retroviral transferred NIH 3T3 cells (Fibroblast)	Decreased	Zoidl, et al, 1997
PMP22 effect in apoptosis			
Genotype	Type of Cell or Tissue	Change of Apoptosis	Reference
overexpression	NIH 3T3 cells microinjected with expression construct (Fibroblasts)	Increased	Fabbretti, et al, 1995 Brancolini, et al, 1999, 2000
	Retroviral transferred NIH 3T3 cells (Fibroblast)	Increased	Zoidl, et al, 1997
	Schwann cells microinjected with expression construct	Increased	Brancolini, et al, 1999
	Nerve biopsies from CMT1A patients, age of 26-72 years (non-myelinating Schwann cells)	Increased	Erderm, et al, 1998
	Nerve from heterozygous pmp22 transgenic mice (Schwann cells)	No difference at postnatal day 1(P1), but increased at P4, P10, P21 and 10 weeks of age	Sancho, et al, 2001
	Nerve biopsies of young CMT1A patients, age of 3-6 years, (Schwann cells)	No difference	Hanemann, et al, 1997
underexpression	Nerve biopsies from HNPP patients, age of 24-50 years (Schwann cells)	Apoptosis was found in 3 of 6 patientss	Erderm, et al, 1998

Genotype	Type of Cell or Tissue	Change of Proliferation	Reference
	Nerve from the homozygous pmp22 knockout mice (Schwann cells)	No difference at P1, P4, P10, but increased at P21 and 10 weeks of age	Sancho, et al, 2001
point mutant	Retroviral transferred NIH 3T3 cells (Fibroblast)	No difference	Zoidl, et al, 1997
	Nerve from trembler mice (Schwann cells)	Apoptosis was found at 6 months age	Soh, et al, 1997
	Nerve from trembler mice (Schwann cells)	No difference at P1, P4, P10, but significantly increased at P21 and 10 weeks of age	Sancho, et al, 2001
PMP22 effect in differentiation or myelination			
Genotype	Type of Cell or Tissue	Differentiation or Myelination	Reference
wild type	Nerve from the wild type rat (Schwann cells)	PMR22 expression correlated with myelination during nerve development. After nerve injury, PMP22 expression decreased	Snipes, et al, 1992
	Nerve from the wild type mice (Schwann cells)	PMP22 expression decreased during Schwann cell dedifferentiation	Varrier, et al, 2009
overexpression	Nerve from the transgenic mice with 16 and 30 copies of the pmp22 gene (Schwann cells)	Increased number of nonmyelinating Schwann cell which characterized by premyelinating state	Magyer, et al, 1996
	Nerve from the transgenic mice with 4 and 7 copies of the pmp22 gene (Schwann cells)	Many Schwann cells failed to differentiate fully into the myelinating phenotype, especially in the mice with 7 copies of pmp22 gene (>40%)	Robertson, et al, 1999
	Nerve from the heterozygous pmp22 transgenic rat (Schwann cells)	Schwann cells were arrested at promyelinating stage but the molecular differentiation was not blocked	Niemann, et al, 2000
	Nerve from the CMT1A patients xenografted to nude mice(Schwann cells)	the onset of myelination was delayed	Sahenk, et al, 2003
	Schwann cells isolated from the homogygous and heterozygous pmp22 transgenic rat were co-culture with DRG's neurons or treated with forskolin	Part of hemizygous transgenic Schwann cells remained in undifferentiated phenotype. Instead, homozygous transgenic Schwann cells remain all in an undifferentiated stage	Nobbio, et al, 2004
	Muscle Satellite Cells isolated from the transgenic mice with 7 copies of the pmp22 gene	No difference	Schuieler, et al, 2005
underexpression	Nerve from homozygous and heterozygous pmp22 knockout mice (Schwann cells)	Myelination rate decreased	Adlkofer, et al, 1995
Point mutant	Nerve from homozygous and heterozygous Trembler-J mice (Schwann cells)	Many Schwann cells failed to differentiate into the myelinating phenotype, especially in the homozygous mice (~98%)	Robertson, et al, 1999

Note: Data were shown with BrdU labeled positive rate *in vitro* and Schwann cell density *in vivo*.

Table 3List of *PMP22* mutations causing CMT1E or HNPP

Author	Journal	Mutation	Phenotype	Location
Muglia et al	J Neurol Sci. 2007; 263:194-7.	Deletion in coding region (c. 11delT) --creates a frame shift and premature stop at codon 6.	HNPP	N-Terminal
Nicholson et al	Nat Genet. 1994; 6:263-6.	two base pair deletion in exon2 (c.19_20del AG) causing a frame shift	HNPP	
Kleopas et al Russo et al	Neurogenetics 2004; 5:171-175 Neuromuscul Disord 2011; 21: 106-114	Point mutation (c.65 C>T) that results in amino acid substitution Ser22Phe	HNPP/CMT1	1 st Trans-Membrane Domain
Bort et al	Hum Genet. 1997; 99: 746-54.	5' splice site mutation at c.78 G>T	HNPP	
Brozkova et al	Muscle & Nerve. 2011; 44: 819-22	Splice site mutation at c. 78+5G>A	HNPP	1 st Extracellular loop
Sahenk et al	Neurology 1998; 51: 702-707	Point mutation (c.89 G>A) that results in amino acid substitution Val30Met [*]	HNPP	
Bellone et al	J Neurol Neurosurg Psychiatry. 2006; 77:538-40	5' splice site mutation at c.178 G>C	HNPP	
Meulemann et al	Neuromuscul Disord. 2001; 11:400-3	3' splice site mutation at c.179 G>C	HNPP	2 nd Trans-Membrane Domain
Haite et al	Neuromuscul Disord. 1998; 8: 591-603	Point mutation (c.183 G>A) that results in amino acid deletion TRP 61 causing a stop mutation.	HNPP	
Nodera et al	Neurology. 2003; 60: 1863-4.	Point mutation (c.199G>A) that results in amino acid substitution Ala67Thr	HNPP	
Luigetti et al	Muscle & Nerve 2008; 38: 1060-1064	Deletion in coding region (c. 227delG) causing a frameshift at Ser76 and a premature stop codon	HNPP	1 st Intracellular loop
Young et al Lenssen et al Johnson et al	Neurology. 1997;48: 450-2. Brain. 1998;121:1451-8. J Neurosci Res 2005; 82:743-752	Insertion in coding region (c. 281insG) causing a frameshift mutation at Gly94	HNPP	
Moszyńska et al	Acta Biochimica Polonica 2009; 56: 627-630	Deletion in coding region (c. 297delT) causing a frameshift at Thr99fs and a premature stop codon	HNPP	3 rd Trans-Membrane Domain
Brozkova et al	Muscle & Nerve. 2011; 44: 819-22	Splice site mutation at c. 320G>C	HNPP	
Shy et al Russo et al	Ann Neurol. 2006; 59:358-64 Neuromuscul Disord 2011; 21: 106-114	Point mutation(c.353C>T that results in amino acid substitution (Thr118Met)	HNPP	
Bissar-Tadmouri et al	Clin Genet. 2000; 58:396-402	Deletion in coding region (c. 364_365delCC) creates a frameshift at Pro122fs	HNPP	2 nd Extracellular loop
Pareyson and Taroni	Curr Opin Neurol. 1996; 9: 348-54.	Point mutation (c.372G>A) that results in a premature stop codon Trp124X	HNPP	

Author	Journal	Mutation	Phenotype	Location
Pegoraro et al	Neuromuscul Disord. 2005;15: 858-62	Point mutation (c.372G>A) that results in a premature stop codon Trp124X and a point mutation in LMNA c.1535T>C)	HNPP	4 th Trans-Membrane Domain
Russo et al	Neuromuscul Disord 2011; 21: 106-114	Point mutation (c.392C > G) that results in amino acid substitution Ser131Cys	HNPP/CMT 1	
Zephir et al	Neuromuscul Disord. 2005;15: 493-7	Insertion in coding region(c. 433_434) causing a frameshift at Leu145fs and a premature stop codon	HNPP	
Taroni et al	Am J Hum Genet 1995; 57: A229	Deletion in coding region (c. 434delT) causing a frameshift at Leu145fs and a premature stop codon	HNPP	
Cassanovas et al	Muscle & Nerve 2012; 45: 135-138	Deletion in coding region of exon 5	HNPP	
Sutton et al	Neuromuscul Disord 2004; 14: 804-809	Deletion in coding region of exons 4 and 5	HNPP	
van de Wetering et al	Neuromuscul Disord 2002; 12: 651-655	Deletion in coding region between exon 1a and exon 4	HNPP	
Valentijn et al	Hum Mutat. 1995; 5: 76-80.	Point mutation (c.36C >A) that results in amino acid substitution His12Gln	Dejerine-Sottas syndrome (DSS)	1 st Trans-Membrane Domain
Valentijn et al	Nat Genet. 1992; 2: 288-91.	Point mutation (c.47T >C) that results in amino acid substitution Leu19Pro	CMT1	
Kleopa et al	Neurogenetics. 2004; 5: 171-5	Point mutation(c.65C>T) that results in amino acid substitution Ser22Phe	CMT1/HNPP	
Joo et al	Neuromuscul Disord. 2004; 14: 325-8.	Point mutation (c.68C>G) that results in amino acid substitution Thr23Arg	CMT1 and deafness	
Mersijanova et al	Hum Mutat. 2000;15: 340-7	Double point mutation (c.73_78) resulting in double amino acid deletion Val25_Ser26del	CMT1	
Boerkoel et al	Ann Neurol. 2002; 51: 190-201.	Point mutation (c.82T>C) that results in amino acid substitution Trp28Arg	CMT+Deafness	
Fabrizi et al	Neurology. 1999; 53: 846-51.	Point mutation (c.110A>T) that results in amino acid substitution Asp37Val	CMT1	1 st Extracellular loop
Ekici et al Park et al	Neurogenetics. 2000; 3: 49-50 Clin Genet. 2006; 70: 253-6.	3' splice site mutation at c.179 2A>C	CMT1	
Huehne et al	Hum Mutat. 2003; 21: 100	Point mutation (c.193G>T) that results in amino acid substitution Val65Phe	CMT1 and deafness	
Kovach et al	Am J Hum Genet. 1999; 64: 1580-93.	Point mutation (c.199G>C) that results in amino acid substitution Ala67Pro	CMT1 and deafness	
Roe et al	Nat Genet. 1993; 5: 269-73.	Point mutation (c.206T>A) that results in amino acid substitution Met69Lys	DSS	2 nd Trans-Membrane Domain

Author	Journal	Mutation	Phenotype	Location
Russo et al	Neuromuscul Disord 2011; 21: 106–114	Point mutation (c.206T > G). that results in amino acid substitution Met69Arg	CMT	
Boerkoel et al Jen et al	Ann Neurol. 2002; 51: 190-201. J Neurol Sci. 2005; 237: 21-4.	Point mutation (c.212T>C) that results in amino acid substitution Leu71 Pro	CMT/DSS(+vestibular/hearing loss)	
Ainsworth et al Ekici et al	Hum Genet. 1998; 103: 242-4 Hum Mutat. 2001;17: 81	Point mutation (c.214T>C) that results in amino acid substitution Ser72Pro	DSS	
Tyson et al	Brain. 1997; 120: 47-63.	Point mutation (c.215C>G) that results in amino acid substitution Ser72Trp	DSS	
Roa et al Ionasescu et al Marques et al Simonati et al Bissar-Tadmouri et al Ceuterick-de groote et al Mostacciuolo et al Pante- Bordeneuve et al Numakura et al Kochanski et al Marques et al	Neurology. 1995; 45: 1766-7. Nat Genet. 1993; 5: 269-73. Clin Genet. 2000; 58: 396-402. Pathol Res Pract. 2001; 197:193-8. Hum Mutat. 2001; 18: 32-41. J Neurol. 2001; 248: 795-803. Ann Neurol. 2002; 51: 190-201. Hum Mutat. 2002; 20: 392-8. Acta Biochim Pol. 2004; 51:1047-50. Neurol. 1998; 43: 680-3.	Point mutation (c.215C>T) that results in amino acid substitution Ser72Leu	CMT1/DSS (+deafness)	
Tyson et al Russo et al	Brain. 1997; 120: 47-63. Neuromuscul Disord 2011; 21: 106–114	Point mutation (c.227G>T) that results in amino acid substitution Ser76Ile	DSS (+deafness)	
Bort et al	Hum Mutat. 1998; S1:S95-8	Point mutation (c.235T>C) that results in amino acid substitution Ser79Pro	DSS	
Roa et al	N Engl J Med. 1993; 329: 96-101.	Point mutation (c.236C>G) that results in amino acid substitution Ser79Cys	CMT1	
Brozkova et al	Muscle & Nerve. 2011; 44: 819-22.	Point mutation that results in amino acid substitution Ser79Thr	DSS	
Ikegami et al	Hum Genet. 1998; 102: 294-8.	Deletion in coding region (c. 238_239delCT) causing a frameshift and premature stop at Leu80	DSS	
Tyson et al	Brain. 1997;120: 47-63.	Point mutation (c.239T>C) that results in amino acid s substitution Leu80Pro	DSS	
Silander et al Yener et al	Hum Mutat. 1998; 12: 59-68. J Neurol. 2001; 248:193-6.	Point mutation (c251_253) resulting in amino acid deletion Phe84del	DSS (+cranial nerve involvement)	
Numakura et al	Hum Mutat. 2002; 20: 392-8.	Point mutation(c.256C>T) causing premature stop at Gln86	CMT 1	

Author	Journal	Mutation	Phenotype	Location
Ohnishi et al	Rinsho Shinkeigaku. 1995; 35: 788-92.	Point mutation (c.277G>C) that results in amino acid substitution Gly93Arg	CMT1	1 st Intracellular loop
Ionasescu et al Boerkoel et al	Muscle Nerve. 1997; 20: 1308-10. Ann Neurol. 2002; 51: 190-201.	Deletion in coding region (c. 281delG) causing a frameshift mutation at Gly94	CMT1/DSS	
DeVries et al Johnson et al	JPNS 2011; 16:113–118 J Neurosci Res 2005; 82:743–752	Point insertion c.281_282insG resulting in frameshift mutation at Gly94	CMT1	
Bort et al	Hum Genet. 1997; 99: 746-54.	Point mutation (c.298G>A) that results in amino acid substitution Gly100Arg	DSS	3 rd Trans-Membrane Domain
Marques et al	Ann Neurol. 1998; 43: 680-3.	Point mutation (c.299) that results in amino acid substitution Gly100Glu	DSS	
Numakura et al	Hum Mutat. 2002; 20: 392-8.	Deletion in coding region (c.312 delT) causing a frameshift mutation at Ile104	CMT1	
Gabreels-Festen et al	Acta Neuropathol. 1995; 90: 645-9.	Point mutation (c314 T>G) that results in amino acid substitution Leu105Arg	CMT 1	
Choi et al	Hum Mutat. 2004; 24: 185-6.	Deletion in coding region (c. 318delT) causing a frameshift mutation at Ala106	CMT 1	
Nellis et al Numakura et al	Hum Mol Genet. 1994; 3: 515-6. Hum Mutat. 2002; 20: 392-8.	5' splice site mutation at c.319 G>A	CMT1	
Marrosu et al	Neurology. 1997; 48: 489-93.	Point mutation (c.320G>T) that results in amino acid substitution Gly107Val	CMT1	
Fabrizi et al	JPNS 1999; 4: 288-289	Point mutation (c.325T>C) that results in amino acid substitution Cys109Arg	CH	
Abe et al	Neuromuscul Disord. 2004; 14: 313-20	Point mutation(c.327C>A) causing premature stop at Cys109	CMT1	
Sambuughin et al	Neurology. 2003; 60: 506-8.	Deletion in coding region (c. 343_354delGCCATCTACACG) causing deletion of Ala115_Thr118)	CMT1 + deafness	
Roa et al Nelis et al Haite et al Seeman et al Meriyanova et al Young et al	Nat Genet. 1993; 5: 189-94. Nat Genet. 1997; 15: 13-4. Neuromuscul Disord. 1998; 8: 591-603. Ann N Y Acad Sci. 1999; 14: 883:485-9; J Neurol. 2000; 247: 696-700.Hum Mut. 2000;15: 340-7	Point mutation(c.353C>T that results in amino acid substitution (Thr118Met)	CMT 1 (hemizygous)/ polymorphism (heterozygous)	
Russo et al	Neuromuscul Disord 2011; 21: 106–114	Point mutation (c.392C > G) that results in amino acid substitution Ser131Cys	HNPP/CMT1	2 nd Extracellular loop
Navon et al	Hum Genet. 1996; 97: 685-7.	Point mutation (c.440T>G) that results in amino acid substitution (Leu147Arg)	CMT 1	4 th Trans-Membrane Domain

Author	Journal	Mutation	Phenotype	Location
Mersiyanova et al	Hum Mutat. 2000;15: 340-7.			
Ohnishi et al	Acta Neuropathol. 2000; 99: 327-30.	Point mutation (c.447C>A) that results in amino acid substitution Ser149Arg	DSS	
Ikegami et al	Hum Genet. 1998; 102: 294-8.	Point mutation (c.448G>T) that results in amino acid substitution Gly150Cys	DSS	
Ionasescu et al.	Muscle Nerve. 1997; 20: 97-9	Point mutation (c.449G>A) that results in amino acid substitution Gly150Asp	DSS	
Numakura et al	Ann Neurol. 2000; 47: 101-3.	Point mutation (c.469C>G) that results in amino acid substitution Arg157Gly and HNPP deletion	CMT1(hemizygous)	C-Terminal
Parman et al	Ann Neurol. 1999; 45: 518-22.	Point mutation (c469C>T) that results in amino acid substitution Arg157Trp	DSS	
Abe et al	J Hum Genet 2010; 55: 771–773	Compound heterozygote with deletion of the whole PMP22 and a deletion of exon 5 in the other PMP22 allele	DSS	Other

* In this publication, the mutation is numbered 202 based on cDNA sequence, using conventional numbering starting at the codon for the first amino acid making this mutation (c.89 G>A)