

DISEASE MECHANISMS IN INHERITED NEUROPATHIES

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Inherited neuropathies are caused by dominant or recessive mutations in genes that are expressed by neurons and/or Schwann cells. In demyelinating neuropathies, the deleterious effects originate primarily in myelinating Schwann cells. In axonal neuropathies, neurons (axons) are initially affected. In demyelinating neuropathies, the axonal cytoskeleton is altered and axonal transport is disrupted. In some axonal neuropathies, genes that are directly involved in axonal transport are mutated. So, a common consequence of inherited neuropathies is disruption of the ability of neurons to transport cargo efficiently along the entire length of their axons. These findings correlate with the observations that axonal atrophy and/or loss are primarily responsible for the clinical disability in hereditary neuropathies.

ONION BULB

A concentric arrangement of supernumerary Schwann cells around an incompletely remyelinated axon, which is thought to represent repeated cycles of demyelination and remyelination.

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Hereditary neuropathies are among the most common inherited neurological diseases^{1–3}. They typically affect only myelinated peripheral axons (FIG. 1a; TABLE 1), but can also be part of inherited syndromes. The non-syndromic inherited neuropathies, originally named according to their genetic and clinical characteristics, are referred to as Charcot–Marie–Tooth disease (CMT) or hereditary motor and sensory neuropathy (HMSN) or different kinds of neuropathy are recognized clinically, especially in conjunction with electrophysiological testing of peripheral nerves. If the forearm motor nerve conduction velocities (NCVs) are greater or less than 38 m s⁻¹, the neuropathy is traditionally considered to be ‘axonal’ (CMT2/HMSNII) or ‘demyelinating’ (CMT1/HMSNI), respectively^{4,5}. CMT1 is more common and has an earlier age of onset (first or second decade of life). Nerve biopsies of CMT1 patients show segmental demyelination and remyelination, as well as ONION BULBS and axonal loss (FIG. 1c). CMT2 has a later onset and is associated with loss of myelinated axons. In addition to CMT, there are milder (hereditary neuropathy with liability to pressure palsies (HNPP); FIG. 1b) and more severe (Dejerine–Sottas syndrome (DSS) and congenital hypomyelinating neuropathy (CHN)) inherited neuropathies (FIG. 1d). If the neuropathy is clinically recognized at birth, it is often called CHN; if it is recognized later in infancy, it is called DSS.

Patients with CHN or DSS typically have NCVs less than 10 m s⁻¹ and dysmyelinated axons (myelin sheaths that have not formed properly), although these criteria exclude cases of severe axonal neuropathies, which could be considered ‘DSS-like’.

CMT1, CMT2 and DSS/CHN are caused by mutations in different genes (TABLE 1). Moreover, different mutations in the same gene cause different phenotypes. For example, deletion of the gene encoding peripheral myelin protein 22 kDa (*PMP22*) causes HNPP, duplication of *PMP22* causes CMT1A, and various missense mutations of *PMP22* cause a CMT1-like syndrome or even DSS/CHN. Different mutations in myelin protein zero (*MPZ*), early growth response 2 (*EGR2*) and neurofilament light (*NEFL*) also cause diverse phenotypes. For most of these genes, there is compelling evidence that gain-of-function phenotypes are the most severe, and that (heterozygous) loss-of-function alleles are associated with milder phenotypes. It was anticipated from transplantation experiments that demyelinating neuropathies would be Schwann cell autonomous⁶; that is, that the myelinating Schwann cells express the mutated gene and are solely responsible for initial damage. This hypothesis has been confirmed, but it is still possible that the expression of the mutant protein by other cell types, especially neurons, contributes to the phenotype.

Conversely, one might anticipate that axonal neuropathies are caused by mutations in genes that are expressed, and have their main functional role in neurons. Here we review the mutations that are known to cause inherited neuropathies, and emphasize how such mutations affect the biology of myelinated axons.

It will become apparent that the mutations of different genes have not only taught us important lessons about the underlying disease mechanisms, but have also provided fundamental new insights into the development and maintenance of peripheral nerves, in particular the interactions between neurons and Schwann cells.

Table 1 | Non-syndromic inherited neuropathies with a genetically identified cause

Disease	Genetic mutations	Clinical features
Autosomal or X-linked dominant demyelinating neuropathies		
HNPP	Usually deletion of one <i>PMP22</i> allele	Episodic mononeuropathies at typical sites of nerve compression. Underlying mild demyelinating neuropathy.
CMT1A	Usually duplication of one <i>PMP22</i> allele	Onset between first and second decades of life. Weakness, muscle atrophy and sensory loss, beginning in the feet and progressing proximally.
CMT1B	Dominant <i>MPZ</i>	Similar to CMT1A, but earlier onset and more severe manifestations.
CMT1C	Dominant <i>LITAF/SIMPLE</i>	Similar to CMT1A. Motor nerve conductance velocities about 20 m s ⁻¹ .
CMT1D	Dominant <i>EGR2</i>	Severity varies, according to mutation, from 'severe' to 'mild' CMT1.
CMT1X	Mainly loss-of-function <i>GJB1</i>	Similar to CMT1A, but distal atrophy is more pronounced. At every age, men are more affected than women.
Autosomal dominant axonal neuropathies		
CMT2A	Dominant <i>KIF1Bβ</i>	Onset of neuropathy by 10 years of age. Progression to weakness, atrophy in distal leg segments and mild sensory disturbance.
CMT2B	Dominant <i>RAB7</i>	Onset between the second and third decades of life. Severe sensory loss with distal ulcerations, and length-dependent weakness.
CMT2D	Dominant <i>GARS</i>	Onset of weakness between the second and third decades of life. Weakness affects arms more than legs, and sensory axons are involved.
CMT2E	Dominant <i>NEFL</i>	Variable onset and severity, ranging from DSS to CMT3 phenotype.
CMT2-P0	Dominant <i>MPZ</i>	Late onset (30 years of age or older). Progressive neuropathy with pain, hearing loss and abnormally reactive pupils.
Severe demyelinating neuropathies (autosomal dominant or recessive; CMT3)		
DSS	Dominant (<i>PMP22</i> , <i>MPZ</i> , <i>GJB1</i> , <i>EGR2</i> and <i>NEFL</i>) and recessive (<i>MTMR2</i> and <i>PRX</i>)	Delayed motor development before 3 years of age. Severe weakness and atrophy affecting distal muscles more than proximal muscles. Severe sensory loss, particularly of modalities subserved by large myelinated axons.
CHN	Dominant (<i>EGR2</i> , <i>PMP22</i> and <i>MPZ</i>) and recessive (<i>EGR2</i>)	Clinical presentation often similar to that of DSS, but hypotonic at birth.
Autosomal recessive demyelinating neuropathies (CMT4)		
CMT4A	Recessive <i>GDAP1</i>	Early childhood onset. Progression to wheelchair-dependency. Mixed demyelinating and axonal features.
CMT4B1	Recessive <i>MTMR2</i>	Early childhood onset. Might progress to wheelchair-dependency. Focally-folded myelin sheaths.
CMT4B2	Recessive <i>MTMR13</i>	Childhood onset. Progression to assistive devices for walking. Focally-folded myelin sheaths. Glaucoma.
CMT4D	Recessive <i>NDRG1</i>	Childhood onset. Progression to severe disability by 50 years of age. Hearing loss and dysmorphic features.
CMT4F	Recessive <i>PRX</i>	Childhood onset. Usually progression to severe disability. Prominent sensory symptoms.
CMT4	Recessive <i>EGR2</i>	Infantile onset. Progression to wheelchair-dependency.
Autosomal recessive axonal neuropathies (AR-CMT2)		
AR-CMT2A	Recessive <i>LMNA</i>	Onset of neuropathy in second decade of life. Progression to severe weakness and atrophy in distal muscles.
Hereditary sensory (and autonomic) neuropathies/neuronopathies (HSN or HSAN)		
HSN1	Dominant <i>SPTLC1</i>	Onset between second and third decades of life. Severe sensory loss with distal ulcerations. Length-dependent weakness.

AR, autosomal recessive; CHN, congenital hypomyelinating neuropathy; CMT, Charcot-Marie-Tooth disease; DSS, Dejerine-Sottas syndrome; EGR, early growth response; GARS, glycyI transfer RNA synthetase; GDAP, ganglioside-induced differentiation-associated protein; GJB, gap junction membrane channel protein; HNPP, hereditary neuropathy with liability to pressure palsies; HSN, hereditary sensory neuropathy; HSAN, hereditary sensory and autonomic neuropathy; KIF, kinesin family member; LITAF, lipopolysaccharide-induced tumour-necrosis factor (TNF)-α factor; LMNA, lamins A/C; MPZ, myelin protein zero; MTMR, myotubularin-related protein; NDRG, N-myc downstream-regulated gene; NEFL, neurofilament light; PMP, peripheral myelin protein; PRX, periaxin; SPTLC, serine palmitoyltransferase, long chain.

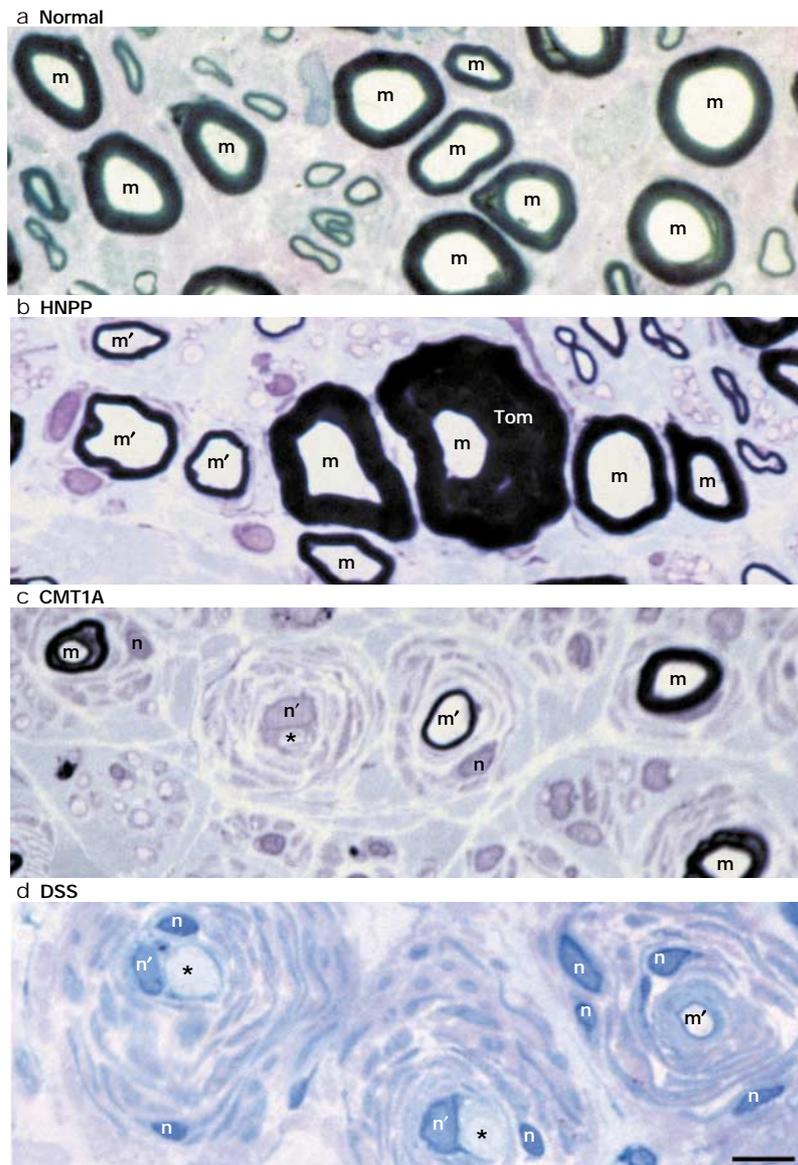


Figure 1 | The pathological features of HNPP, CMT1A and DSS. These are photomicrographs of semi-thin (0.5 μm thick) sections of sural nerve biopsies from a 27-year-old patient with normal findings (a), a 32-year-old patient with hereditary neuropathy with liability to pressure palsies (HNPP) (b), a 46-year-old patient with Charcot–Marie–Tooth disease (CMT) 1A (c), and a 16-year-old patient with dominantly inherited Dejerine–Sottas syndrome (DSS) (d). In b, note the tomaculum (Tom). In c & d, note the supernumerary Schwann cell processes and their nuclei (n) that form ‘onion bulbs’ around demyelinated and remyelinated axons, and the reduced density of myelinated axons (m). m’, thinly myelinated axons; asterisk indicates demyelinated axons and n’ indicates their associated Schwann cell nuclei. Scale bar: 10 μm .

PMP22 mutations

Myelin is a multi-lamellar spiral of specialized cell membrane that ensheathes axons larger than 1 μm in diameter. Schwann cells in the peripheral nervous system (PNS) and oligodendrocytes in the central nervous system (CNS) are the myelinating cell types. By reducing the capacitance and/or increasing the resistance, myelin reduces current flow across the internodal axonal membrane, thereby facilitating saltatory conduction at nodes of Ranvier. Most of the myelin membrane is composed of compact myelin, which contains mainly cholesterol

HYPOMYELINATED AXON
A remyelinated axon with a myelin sheath that is inappropriately thin for the axonal calibre.

and sphingolipids and some specialized lipids such as galactocerebroside and sulfatide. A smaller proportion of compact myelin is made up of proteins, including P0 (encoded by *MPZ*), PMP22 and myelin basic protein (MBP) in the PNS, and proteolipid protein (PLP) and MBP in the CNS.

The inheritance of a deletion that includes *PMP22* is the usual cause of HNPP⁷. Two homologous DNA sequences flanking *PMP22* are the molecular basis for its deletion¹. Their high degree of homology promotes unequal crossing over during meiosis, simultaneously generating a duplicated and a deleted allele. In rare instances, HNPP is caused by mutations in *PMP22* itself, presumably resulting from loss-of-function alleles. Episodic mononeuropathies at typical sites of nerve compression, with recovery within days to months, are the hallmark of HNPP⁸. Biopsies of unpalsied nerves show focal thickenings (tomacula) caused by folding of the myelin sheath, as well as segmental demyelination and remyelination (FIG. 1b). These findings are corroborated in *Pmp22*^{-/-} mice, a genetically authentic animal model of HNPP⁹.

CMT1A is the most common form of inherited neuropathy and is usually caused by the heterozygous inheritance of a duplication that includes *PMP22* (REF 1). Most patients are clinically affected by the end of their second decade of life^{10,11}. Weakness, atrophy and sensory loss in the lower limbs, foot deformities and loss of reflex are invariably present in affected patients. NCVs are abnormally slow — typically about 20 m s^{-1} — even before the clinical onset of disease^{10,11}. Reduced motor amplitudes and the loss of motor units are correlated with clinical disability, indicating that axonal loss, and not reduced conduction velocity *per se*, causes weakness¹² (FIG. 2). Demyelination is more prevalent in children, and ‘HYPOMYELINATED’ AXONS become more numerous with age^{11,13}.

PMP22 is a small intrinsic membrane protein (FIG. 3). On the basis of genetic evidence, *PMP22* is involved in early steps of myelination and has a structural role in compact myelin¹⁴. How deletion and duplication of *PMP22* cause HNPP and CMT1A, respectively, is not completely understood, but the deleterious effects are related to gene dosage, which, in turn, is correlated with the relative amounts of *PMP22* protein that are found in compact myelin¹⁵. *PMP22* can form dimers and multimers¹⁶. It might also interact with P0 (REFS 17,18), so that an altered *PMP22*/P0 ratio might destabilize the myelin sheath^{14,19,20}. Overexpressed *PMP22* might also lead to an altered endosomal recycling pathway for plasma membranes, resulting in the accumulation of membranes in late endosomes²¹. These findings are reminiscent of proteolipid protein 1 (PLP1) overexpression, which might saturate the myelin RAFT transport pathway and, together with associated cholesterol, misdirect PLP1 to endosomes/lysosomes²². As a result, lipid raft-associated signalling molecules might be disturbed, thereby affecting myelination and the viability of oligodendrocytes. It is conceivable that *PMP22* overexpression has similar effects, as *PMP22* is also associated with rafts^{23,24}, although it is not a classic PLP.

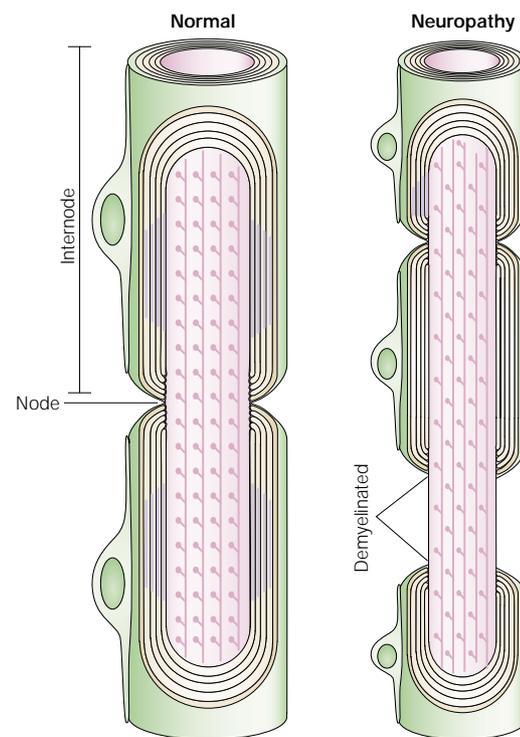


Figure 2 | Schematic overview of pathological changes in Charcot-Marie-Tooth disease 1 (CMT1). As the original myelin internodes are lost, they are replaced by one or more internodes of lesser length, and some regions of the axon remain demyelinated. Neurofilaments are more tightly packed and less phosphorylated. These changes are accompanied by a progressive, length-dependent loss of sensory and motor axons.

RAFTS

Domains of the plasma membrane enriched in sphingolipids and cholesterol. They incorporate lipid-conjugated proteins and therefore serve to assemble proteins involved in signal transduction.

TETRASPAN

Proteins with four membrane-spanning domains.

INTEGRINS

A large family of transmembrane proteins that act mainly as receptors for extracellular matrix molecules.

DOMINANT-NEGATIVE

A mutant molecule that can form a heteromeric complex with the normal molecule, reducing the activity of the entire complex.

EPITOPE-TAG

The immunological determinant of an antigen that has been fused to a protein of interest for its subsequent localization with specific antibodies.

In vitro, PMP22 overexpression can reduce Schwann cell proliferation^{25,26} and increase the rate of apoptosis^{25,27,28}. *In vivo*, the specificity of these effects has not been distinguished from the Schwann cell proliferation and apoptosis that are features of many neuropathies²⁹. In any case, neither Schwann cell proliferation nor apoptosis are strikingly altered early in the pathogenesis of PMP22-overexpressing or *Pmp22*-null (*Pmp22*^{-/-}) mice²⁹, but the effects might be subtle or involve even earlier steps of development. The finding that PMP22 associates with P2X7 receptors (ATP-gated ion channels), leading to apoptosis in cultured HEK-293 cells³⁰, might be relevant because activity-dependent ATP release from premyelinated axons can inhibit the proliferation and differentiation of Schwann cells through P2 receptors³¹.

Based on *in vitro* studies, PMP22 might also be involved in the regulation of cell spreading in a Rho-dependent manner²⁸. Such effects could lead to defects in either axonal ensheathment or in Schwann cell differentiation. Indeed, myelinating Schwann cells express markers of non-myelinating/denervated Schwann cells (p75 neurotrophin receptor (p75^{NTR}) and neural cell-adhesion molecule (NCAM)), even in young CMT1A patients in which most myelin sheaths seem to be normal²⁶. However, this might not be a specific response to PMP22 overexpression, since similar molecular changes have been described in other myelin mutants^{32,33}.

The transgenic *Pmp22* rat that carries additional copies of the *Pmp22* gene ('the CMT rat') provides an interesting twist on this theme^{20,34}. Homozygous CMT rats have almost no myelin sheaths. As expected, the amounts of PMP22 and P0 proteins in these mutants are markedly reduced, but the steady-state levels of their cognate messenger RNAs are high. Furthermore, as in CMT1A nerves, Schwann cells co-express P0 and p75^{NTR}. So, in the CMT rat, myelin gene transcription seems to be uncoupled from myelin assembly, and the normal reciprocal expression of markers of myelinating (P0) versus non-myelinating (p75^{NTR}) Schwann cells is altered as described for CMT1A.

Recent studies highlight the possibility that Schwann cell-extracellular matrix interactions contribute to the pathogenesis of PMP22-based neuropathies. First, some TETRASPAN proteins functionally interact with INTEGRINS. In particular, epithelial membrane protein-2, a close homologue of PMP22 (REF. 35), interacts with $\beta 1$ integrins and regulates adhesion³⁶. Second, PLP1 (the intrinsic membrane protein of CNS myelin that is often considered to be the counterpart of PMP22 owing to its role in inherited dysmyelinating CNS diseases³⁷) also forms a complex with integrins and might regulate myelination by oligodendrocytes³⁸. Third, integrin signalling seems to be crucial for the proper development of myelinated axons³⁹. So, it is possible that the effect of PMP22 overexpression on cell spreading²⁸ is mediated by integrins, so that altered levels of PMP22 might change the function or even the repertoire of integrins in Schwann cells. Further, dysmyelinated nerves have an altered extracellular matrix⁴⁰, so, altered Schwann cell-matrix interactions might contribute to early and late phases of the disease.

Besides gene duplications and deletions, more than 40 different *PMP22* mutations cause amino-acid substitutions (missense mutations), premature stops (nonsense mutations) or frameshifts (FIG. 4). Except for patients with the HNPP phenotype, most patients with *PMP22* point mutations — including those with a CMT1 phenotype — are more severely affected than those carrying the duplication or deletion¹. The inference that these mutations cause a gain-of-abnormal function is supported by the finding that the neuropathy in heterozygous Trembler (*Tr*^{r/+}) and Trembler-J (*Tr*-J^{r/+}) mice is more severe than that in *Pmp22*^{r/+} mice⁴¹. The gain-of-abnormal function could be a DOMINANT-NEGATIVE effect on the wild-type PMP22 product from the other allele, or a 'toxic' effect. The intracellular location of mutant PMP22 provides the clue. In cultured cells, dominant PMP22 mutant proteins are retained intracellularly in the endoplasmic reticulum and/or the intermediate compartment^{16,42-47}. In addition, the mutant Tr and Tr-J proteins aggregate abnormally in transfected cells¹⁸. However, heterologous cells or even cultured Schwann cells can differ from myelinating Schwann cells, which are highly specialized and require axonal interactions for efficient insertion of PMP22 into their cell membrane⁴⁸. Expression of EPITOPE-TAGGED versions of Tr and Tr-J in developing rat nerves through adenoviral gene transfer has confirmed that these mutant proteins do not reach the myelin sheath⁴⁵.

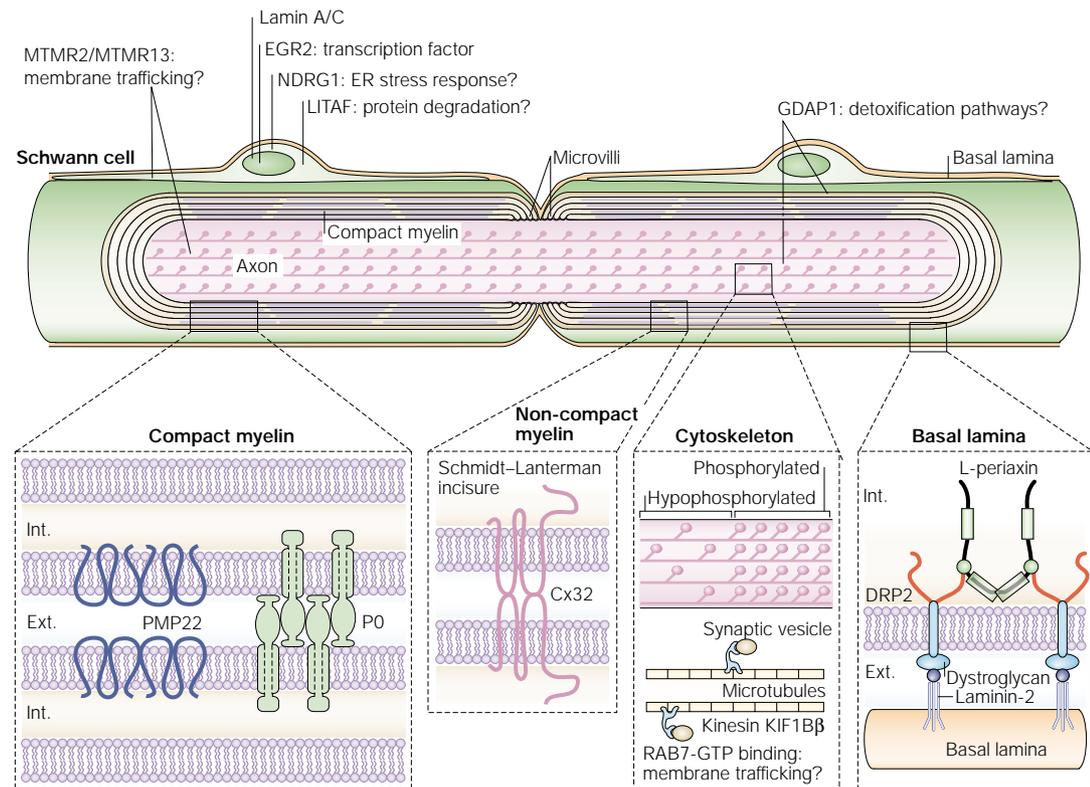


Figure 3 | **Schematic overview of the molecular organization of myelinated axons highlighting the proteins affected in Charcot-Marie-Tooth disease (CMT).** The figure depicts the locations of the wild-type proteins encoded by the genes that are mutated in CMT. Cx, connexin; EGR, early growth response; ER, endoplasmic reticulum; Ext., extracellular; GDAP, ganglioside-induced differentiation-associated protein; Int., intracellular; KIF, kinesin family member; LITAF, lipopolysaccharide-induced tumour-necrosis factor (TNF)- α factor; MTMR, myotubularin-related protein; NDRG, N-myc downstream-regulated gene; PMP, peripheral myelin protein.

Mutant PMP22 could have several effects. Because PMP22 forms dimers/multimers, retained mutant PMP22 could block a proportion of wild-type PMP22 from being transported to the cell membrane by a dominant-negative effect^{16,18,43}. This should result in a phenotype between a heterozygous and a homozygous *Pmp22*-null mouse, but the phenotype of *Tr* and *Tr-J* mice indicates that the mutated protein has an additional autonomous, toxic gain-of-function effect^{14,41}. Because PMP22 and P0 might physically interact, a trans-dominant effect of PMP22 mutants on the trafficking of P0 is yet another possibility, but experimental evidence for such a mechanism is lacking. Accumulation of mutant PMP22 could trigger the unfolded protein response (UPR) in the endoplasmic reticulum, as for PLP mutants⁴⁹, but markers of the UPR are not grossly altered in *Tr-J* nerves⁴⁶. Instead, wild-type PMP22 and mutant *Tr-J* proteins form a complex with the chaperone protein CALNEXIN in the endoplasmic reticulum. *Tr-J* has an increased association time with calnexin, indicating that the sequestration of calnexin might contribute to the disease mechanism by affecting the pathway that controls protein folding⁴⁶. In support of this hypothesis, reduced calnexin function in transgenic mice leads to signs of a motor disorder that is associated with a loss of large myelinated axons⁵⁰.

CALNEXIN
A calcium-binding protein of the endoplasmic reticulum that processes and monitors endoplasmic reticulum proteins, retaining those that are unassembled or incorrectly folded.

Upregulation of both the ubiquitin-proteasomal and lysosomal pathways has been described in PMP22 mutant nerves, indicating that two pathways of PMP22 degradation are present⁵¹⁻⁵³. Whether these degradation pathways contribute to the disease is unclear. Overexpression of wild-type PMP22 leads to the formation of intracellular ubiquitinated PMP22 aggregates or 'aggresomes'. Furthermore, inefficient proteasome function can lead to the formation of aggresomes of wild-type PMP22, *Tr* or *Tr-J* protein⁵³. Detrimental effects of aggresome formation might include alteration of cell differentiation and induction of cell death, but aggregation might also confer protection through sequestration of toxic proteins⁴⁷. The role of the lysosomal pathway is less well defined, but it is known that mutations affecting lysosomal proteins can lead to demyelinating neuropathies, both in humans (in *CMT1C*, see later in text) and in mice (lysosomal membrane protein *Limp2/Lgp85* (REF 54)).

Effects of *MPZ* mutations

Approximately 80 different *MPZ* mutations have been identified, including missense, nonsense and frameshift mutations (FIG. 4). Almost all cause a dominantly inherited neuropathy, but the phenotypes are remarkably variable, including *CMT1B*, 'severe CMT', DSS/CHN,

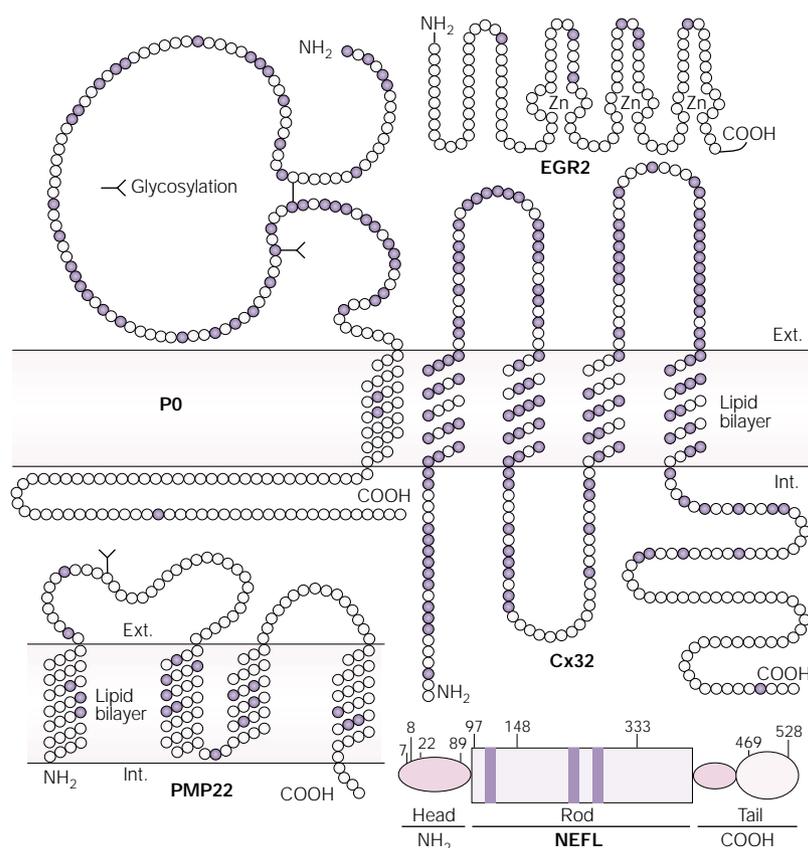


Figure 4 | Locations of mutations in the Cx32, P0, PMP22, EGR2 and NEFL proteins. For connexin 32 (Cx32), P0, peripheral myelin protein 22 kDa (PMP22) and early growth response 2 (EGR2), the proteins are indicated schematically. The amino-acid residues that are affected by missense mutations and that cause a disease phenotype are shown as solid purple circles. Only part of the EGR2 protein is shown. For neurofilament light (NEFL), the domain structure of the protein and the approximate locations of disease-related mutations are indicated (based on an original figure provided by R. Perez-Olle). Ext., extracellular; Int., intracellular.

'CMT2-LIKE' or HNPP-like³. At present, for almost any known *MPZ* (or *PMP22*) mutation, the clinical phenotype cannot be predicted on the basis of the mutation, reflecting our limited knowledge of how mutant myelin proteins affect myelinated axons.

P0 is the most abundant protein in compact PNS myelin (FIG. 3). As a type I transmembrane protein, it contains a single immunoglobulin-like motif in its extracellular domain and a highly positively charged intracellular domain⁵⁵ — both are important for myelin compaction. The extracellular domain forms adhesive tetramers that interact homophilically both in *cis* (in the plane of the membrane) and in *trans* (on the apposed membrane)⁵⁶. The intracellular domain helps to hold together the apposed cytoplasmic surfaces of the membranes⁵⁷. These functions have been confirmed in transfection experiments^{58,59}, and in mice lacking P0 (*Mpz*^{-/-} mice), which have uncompacted myelin and severe demyelination³². Aggregation assays of transfected cells have also delineated important functional P0 domains, including a protein kinase C phosphorylation site, mutations of which diminish P0-mediated adhesion and cause CMT1B⁶⁰. These findings provide evidence

that some aspects of myelination can be regulated post-translationally by signalling pathways. Like PMP22, the 'dose' of P0 in myelin is crucial; reduced P0 causes myelin instability¹⁹, and overexpression of P0 causes severe dysmyelination that is correlated with the level of expression^{61,62}.

What accounts for the wide range of phenotypes observed in patients with *MPZ* mutations? Heterozygous deletion of *Mpz* causes a late onset demyelinating neuropathy in mice¹⁹; the corresponding loss-of-function mutations in humans probably cause mild cases of CMT1B. More severe demyelinating phenotypes are probably caused by mutations that act by a dominant-negative or a gain-of-function mechanism; the former might be explained by aberrant interactions within the P0 tetramer itself or between P0 and PMP22 (FIG. 3). The discovery of mutation-specific phenotypes in transgenic mice carrying different *Mpz* mutations indicates that there are additional gain-of-function effects that originate either from defects of the myelin sheath itself or from another cellular location^{61–63}. Determining how some *MPZ* mutations (particularly the T124M mutation) cause a 'CMT2-like' phenotype might provide key insights into the molecular basis of axon–Schwann cell interactions (FIG. 2).

Effects of *GJB1* mutations

Mutations in *GJB1*, which encodes the GAP JUNCTION protein connexin 32 (Cx32), cause CMT1X. Cx32 is one of about 20 connexins in mammals⁶⁴. All connexins are highly homologous — their general structure is shown in FIG. 4. Six connexins oligomerize to form a hemichannel (or connexon); two hemichannels from apposing membranes interact to form a gap junction⁶⁵. Cx32 is localized to incisures and paranodal loops of myelinating Schwann cells⁶⁶, and probably forms gap junctions between adjacent layers of the myelin sheath (FIG. 3). Such a radial pathway — directly across the layers of the myelin sheath — is advantageous, as it provides a shorter pathway (by up to 1000-fold) than a circumferential route. Disruption of this radial pathway might be the reason that *GJB1* mutations cause demyelination in humans and mice^{67,68}. However, the pathway and the rate of 5,6-carboxyfluorescein diffusion in *Gjb1*^{-/-} mice are similar to those in wild-type mice⁶⁹, implying that another connexin forms functional gap junctions in PNS myelin sheaths. This connexin could be Cx29 (human homologue, Cx31.3), which is also localized to the incisures and paranodes^{70,71}, although additional connexins might also contribute. It remains to be determined whether Cx29 forms gap junctions, and what functions of Cx32 cannot be substituted in the myelinated peripheral nerves of CMT1X patients and *Gjb1*-null mice.

There are more than 240 different CMT1X-associated *GJB1* mutations. These affect every domain of the Cx32 protein (FIG. 4), as well as the promoter and 3' untranslated region, and include deletion of the entire gene. By comparison with *PMP22* and *MPZ* mutations, the clinical manifestations of *GJB1* mutations are more homogeneous; affected males have a 'CMT phenotype'

CMT2-LIKE PHENOTYPE
A late-onset neuropathy with pronounced axonal loss.

GAP JUNCTION
A junction between two cells consisting of pores that allow passage of molecules (up to 1 kDa).

with onset in late childhood or adolescence, but as the disease progresses, axonal loss is more pronounced than in CMT1A^{72,73}. Clinical involvement in affected women varies, probably in proportion to the number of myelinating Schwann cells that inactivate the X chromosome carrying the mutant *GJB1* allele⁶⁸. One mutation (F235C) seems to cause DSS-like neuropathy, and a few mutations are associated with hearing loss or clinical involvement of the CNS. These additional phenotypes might result from dominant effects of Cx32 mutants on co-expressed connexins. Hearing loss is associated with Cx43, Cx31, Cx30 and especially Cx26 mutations, but it is yet to be determined whether Cx32 is co-expressed with one of these connexins in the cochlea. The CNS manifestations might reflect abnormal interactions between certain Cx32 mutants and other connexins that are expressed by oligodendrocytes (Cx29/Cx31.3 and Cx47) (REFS 70,74,75). The Cx32 mutants associated with these additional phenotypes seem to be retained in the endoplasmic reticulum or Golgi^{76,77}, and could have dominant-negative effects or result in aberrant trafficking of co-expressed connexins^{78,79}. Furthermore, trafficking of Cx32 mutants in mammalian cells is often disrupted⁷⁷, although the actual process could be more complex than is indicated by the apparent patterns of retention⁸⁰.

The similar clinical phenotypes of most CMT1X patients indicate that most *GJB1* mutations cause a loss of function⁸¹. Many mutants do not form functional channels in *Xenopus* oocytes or mammalian cells, and others form functional channels with altered biophysical characteristics. Several disease-related mutants form fully functional channels⁸², raising the question of how these mutants cause demyelination. In this regard, transfected cells might not recapitulate the complex interactions and functions of Cx32 in myelinating Schwann cells, so it will be important to evaluate these mutants in an appropriate setting.

LITAF/SIMPLE mutations cause CMT1C
Mutations in *LITAF* (lipopolysaccharide-induced tumour-necrosis factor (TNF)- α factor, also referred to as *SIMPLE*) are associated with dominantly inherited CMT1C. *LITAF* encodes a widely expressed transcript that is translated into a 161-amino-acid protein. *LITAF* mRNA is expressed in sciatic nerve, but, in contrast to other genes that are known to cause CMT1, its level of expression is not altered in response to nerve injury. The *LITAF* gene has been described as a transcription factor that regulates TNF- α expression⁸³, but other reports indicate that the gene encodes a lysosomal protein⁸⁴. These findings are yet to be reconciled. Initial analysis indicates that the mutations associated with CMT1C (G112S, T115N and W116G) cluster, potentially defining a putative domain of the *LITAF* protein that might have a crucial role in peripheral nerve function.

Loss-of-function mutations cause CMT4
Recessive forms of demyelinating CMT are collectively designated CMT4 (TABLE 1). Each kind is rare, and most are associated with severe neuropathy with onset before

birth (CHN) or during infancy (DSS). However, the phenotypes associated with each of these diseases have some distinctive characteristics that provide insights into the molecular mechanisms that are essential for the formation of myelinated axons.

Recessive mutations in ganglioside-induced differentiation-associated protein-1 (*GDAP1*) cause CMT4A, initially characterized as a severe demyelinating neuropathy. However, recessive *GDAP1* mutations have also been described in severe axonal neuropathy (AR-CMT2; TABLE 1). With the exception of the relative preservation of NCV, some affected individuals might be categorized as having DSS. Further studies confirmed that *GDAP1* mutations cause a range of phenotypes: a demyelinating or an axonal peripheral neuropathy, or an intermediate peripheral neuropathy with features of both. This variability raises the possibility that *GDAP1* mutations affect both neurons and Schwann cells, but it remains to be determined whether these effects are cell-type autonomous or caused by altered axon-Schwann cell interactions. *GDAP1* is probably expressed by neurons and Schwann cells^{85,86}, but this has not been decisively demonstrated (FIG. 3). The encoded protein is predicted to have two transmembrane domains and a glutathione *S*-transferase domain, indicating a role in antioxidant pathways or detoxification⁸⁵⁻⁸⁷. If such a function can be experimentally confirmed, damage that is attributable to reactive oxygen species would be an attractive hypothesis for the underlying disease mechanism.

Recessive mutations in myotubularin-related protein-2 (*MTMR2*) cause CMT4B1. Weakness begins between two and four years of age, and might progress to wheelchair dependency by adulthood. Motor NCVs are uniformly slow, and nerve biopsies show distinctive irregular folding and redundant loops of myelin. The pathological characteristics of CMT4B1 indicate primary damage to myelinating Schwann cells, but because *MTMR2* is expressed at high levels by peripheral neurons^{88,89} there is also a potential neuronal contribution (FIG. 3). Cell-type specific gene ablation in mice is expected to resolve this issue.

MTMR2 is one of about a dozen members of the highly conserved family of myotubularin-related dual specific phosphatases, whose principal physiological substrates are phosphorylated inositol phospholipids^{90,91}. *MTMR2* contains a PLECKSTRIN HOMOLOGY-GRAM (glucosyltransferase, Rab-like GTPase activator and myotubularin) domain, a phosphatase domain, a COILED-COIL DOMAIN and a PDZ-BINDING MOTIF⁹¹. The *MTMR2* mutations affect different parts of the protein, but reduced or absent phosphatase activity seems to be a common feature⁸⁸. The membrane phospholipids phosphatidylinositol-3-phosphate (PtdIns3P) and phosphatidylinositol-3,5-phosphate (PtdIns(3,5)P) are dephosphorylated by *MTMR1*, *MTMR2*, *MTMR3* and *MTMR6* at the D3 position. All myotubularin family members with phosphatase activity might have this substrate specificity^{88,92}. Because phosphoinositides regulate intracellular membrane trafficking, CMT4B1 might be caused by several processes, including neural

PLECKSTRIN HOMOLOGY DOMAIN

A sequence of about 100 amino acids that is present in many signalling molecules. Pleckstrin is a protein of unknown function that was originally identified in platelets. It is a principal substrate of protein kinase C.

COILED-COIL DOMAIN

A protein domain that forms a bundle of two or three α -helices. Whereas short coiled-coil domains are involved in protein interactions, long coiled-coil domains, which form long rods, occur in structural or motor proteins.

PDZ-BINDING MOTIF

A peptide-binding domain that is important for the organization of membrane proteins, particularly at cell-cell junctions, including synapses. PDZ-domain-containing proteins bind to the PDZ-binding motifs that are located at the carboxyl termini of proteins or can form dimers with other PDZ domains. PDZ domains are named after the proteins in which these sequence motifs were originally identified (PSD95, Discs large, zona occludens 1).

membrane recycling, endocytic or exocytotic processes and disturbed membrane-mediated transport pathways. The disease mechanism might also involve altered cargo delivery to endosomes and the function of lysosomes — processes that are implicated in other demyelinating peripheral neuropathies.

As *MTMR2* mutations cause CMT4B1, it was not surprising that the related gene *MTMR13/SBF2* was found to cause CMT4B2, which has similar pathological features to CMT4B1. Some mutations cause neuropathy alone, whereas other mutations also cause GLAUCOMA. *MTMR13* encodes a protein without a functional phosphatase domain. However, this protein might interact directly with *MTMR2*, just as *MTMR1* and *MTMR2* can interact with the pseudophosphatases *MTMR12* and *MTMR5*, respectively^{91,93,94}. By analogy, *MTMR13* might regulate the phosphatase activity and/or alter the subcellular localization of *MTMR2*. *MTMR13* mRNA is expressed in a broad range of tissues, including brain, spinal cord and sciatic nerve (FIG. 3). Given their possible interactions, it will be imperative to determine the cellular/subcellular locations and activities of *MTMR2*, *MTMR5* and *MTMR13*, and to correlate these findings with the various potential disease mechanisms outlined above.

Recessive mutations in N-myc downstream-regulated gene-1 (*NDRG1*) cause CMT4D. CMT4D is a syndromic neuropathy, as affected individuals also have hearing loss and dysmorphic features. It was initially described in gypsies from Lom, Bulgaria, but has been subsequently found in gypsies from other countries. The disease has a clinical onset in childhood and progresses to severe disability by the fifth decade of life. Biopsies show demyelination, onion bulbs and cytoplasmic inclusions in Schwann cells. Many cell types, including Schwann cells, express *NDRG1* as an intracellular protein, but its function is unknown⁹⁵ (FIG. 3).

Recessive mutations in the periaxin gene (*PRX*) cause CMT4F; the mutations are expected to cause loss of function. Affected individuals usually have a DSS phenotype, with delayed motor milestones, progressing to severe distal weakness, reflex loss, sensory loss and even sensory ataxia, but milder CMT-like cases have also been described. Sensory loss is more prominent in CMT4F than in other inherited demyelinating neuropathies, consistent with the phenotype of *Prx*^{-/-}; myelin sheaths are initially formed, then develop outfoldings and extensive demyelination⁹⁶. Periaxin is a membrane-associated protein with a PDZ domain that is exclusively expressed in myelinating Schwann cells⁹⁷ (FIG. 3). Its location changes during myelination from the adaxonal membrane (nearest the axon) to the abaxonal membrane (nearest the basal lamina) in mature myelin sheaths⁹⁸. Abaxonal periaxin interacts with the dystroglycan complex through dystrophin-related protein-2 (DRP2), thereby linking laminin-2 in the basal lamina to the actin cytoskeleton and, possibly, to other components in the cytoplasm⁹⁹. It is surprising that disrupting this complex results in a severe

demyelinating neuropathy, as Schwann cells also express two other dystrophin complexes: one containing utrophin and another containing an isoform of dystrophin. The indispensable nature of the dystroglycan–DRP2–periaxin complex might be related to the peripheral neuropathy of leprosy, as the binding of *Mycobacterium leprae* to α -dystroglycan can cause demyelination¹⁰⁰.

Transcription factors that affect Schwann cells
The discovery that Schwann cells fail to make myelin sheaths in mice that lack the zinc-finger transcription factor *Egr2* (also called *Krox20*) catapulted *Egr2* into the spotlight as a potential regulator of Schwann cell differentiation¹⁰¹ (FIG. 3). The pathology of these mice resembles that of CHN. It was subsequently shown that mutations in zinc-finger domains (FIG. 4) cause a range of dominantly inherited demyelinating neuropathies, from CHN/DSS to CMT1 (REF. 102). Overexpression of *EGR2* in cultured Schwann cells increases the expression of a number of myelin-related genes, including *MPZ*, *PMP22*, *GJB1* and *PRX*¹⁰³. For some of these genes, their induction is probably direct. *EGR2* activates the *MPZ* promoter in co-transfected cultured Schwann cells¹⁰⁴, and some *EGR2* mutants have reduced affinity for an *EGR2*-binding site in the *GJB1* promoter¹⁰⁵. All of the mutations in the zinc-finger domains probably act through gain-of-function or dominant-negative effects, as heterozygous *Egr2*^{-/-} mice appear normal. Three of the mutant proteins reduce DNA binding and transactivation *in vitro*. The mechanisms behind this remain unclear, but might involve an additional adaptor or co-activators¹⁰⁶, or inappropriate activation or repression of incorrect recognition sites¹⁰⁵. One recessive *EGR2* mutation (I268N) is located in the R1 repressor domain, and prevents interactions with NAB co-repressors and can activate a synthetic *EGR2* target promoter¹⁰⁶. So, the resulting disease might be attributable to overexpression of myelin genes that are sensitive to increased dosage, with *PMP22* and *MPZ* as possible candidates.

The transcription factor *SOX10* can directly activate *MPZ* and *GJB1* (in synergy with *EGR2*)^{107,108}. In line with these observations, CMT1-like and DSS/CHN-like neuropathies, as well as CNS dysmyelination, are associated with dominant *SOX10* mutations. The syndromic nature of *SOX10* mutations is attributable to the various functions of this important regulator in maintaining multipotency of neural crest stem cells^{109,110}, and in the development of oligodendrocytes and Schwann cells^{111,112}. Heterozygous *SOX10* loss-of-function mutations seem to cause Waardenburg-Shah/Waardenburg type IV syndrome, while mutations associated with dysmyelination seem to act through a dominant-negative mechanism¹⁰⁸. Yet another disease mechanism has been revealed by a CMT1X-associated mutation of a *SOX10*-binding site in the *GJB1* promoter, impairing activation by *SOX10* (REF. 108). This is an elegant example of how the evaluation of the cellular and molecular networks of genes involved in hereditary neuropathies can lead to exciting new findings.

GLAUCOMA

A group of eye diseases characterized by an increase in intraocular pressure which causes pathological changes in the optic disk and typical defects in visual fields.

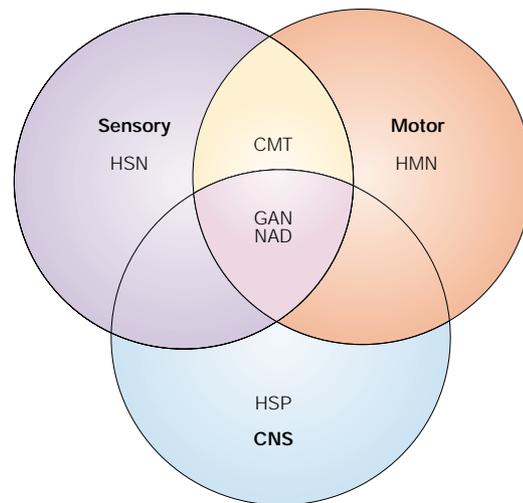


Figure 5 | Relationships between inherited neuropathies and other diseases. The relationships between length-dependent axonal diseases in the central nervous system (CNS) and peripheral nervous system (PNS); the latter is subdivided into motor and sensory neurons. CMT, Charcot–Marie–Tooth disease; GAN, giant axonal neuropathy; HMN, hereditary motor neuropathy; HSN, hereditary sensory neuropathy; HSP, hereditary spastic paraparesis; NAD, neuroaxonal dystrophy.

Axonal proteins and axonal neuropathies
Dominant mutations in two genes that encode proteins of the axonal cytoskeleton — kinesin family member 1B (KIF1B) and NEFL — cause axonal neuropathies (FIG. 3). A dominant missense mutation affecting the highly conserved ATP-binding region of KIF1B, probably generating a loss-of-function allele and HAPLOINSUFFICIENCY, causes CMT2A¹¹³. Kinesins are a large family of microtubule-activated ATPases that transport proteins and organelles along microtubules¹¹⁴. The isoform KIF1B β binds to synaptic vesicles. *Kit1B*^{-/-} mice develop a peripheral neuropathy, and peripheral axons have decreased levels of synaptic vesicle proteins and other proteins^{113,115}. This genetic evidence provides support for the long-standing doctrine that neuropathies are length-dependent, because the longest axons are most vulnerable to defects in axonal transport¹¹⁶.

Dominant mutations in *NEFL* cause CMT2E. The resulting disease state is heterogeneous and probably depends on the effects of the individual mutation (FIG. 4). Features range from a slowly progressive neuropathy with onset in the second or third decade of life and normal to mildly decreased motor NCVs, to a DSS-like phenotype with distinct demyelinating features. Neurofilaments consist of three subunits: neurofilament light, medium and heavy chains. These assemble into the neuronal intermediate filaments that form the most conspicuous cytoskeletal components of large axons (FIGS 2 and 3). *Neff*^{-/-} mice do not develop a CMT2-like neuropathy¹¹⁷, indicating that the dominant *NEFL* mutations are not simple loss-of-function alleles. Introduction of a L394P mutation

disrupts neurofilament assembly, causing a severe peripheral neuropathy and neuronopathy in transgenic mice¹¹⁸. Similarly, quail with a naturally occurring *Neff* mutation develop a peripheral neuropathy with profoundly reduced axonal calibre¹¹⁹. So, missense mutations might disorganize neurofilament assembly, causing neuropathy and even altered axonal calibre; the latter accounting for the decreased NCVs in CMT2E patients. In agreement with this, gene transfer experiments in cultured neurons indicate that at least some CMT2E-associated mutations disrupt the assembly and axonal transport of neurofilaments^{120,121}. Defining the molecular mechanisms of these relatively rare *NEFL* mutations might shed light on the neurofilament abnormalities that are associated with more common diseases, such as Parkinson's disease and AMYOTROPHIC LATERAL SCLEROSIS¹²².

In addition to these 'pure' neuropathies, defects in axonal transport have been implicated in a host of other inherited neurological diseases (FIG. 5). In particular, mutations in some of the genes that cause inherited spastic paraplegia (HSP) probably disrupt axonal transport¹²³. HSPs are analogous to inherited neuropathies, but principally affect CNS axons in a length-dependent pattern, although PNS axons are sometimes also affected. Mutations in *KIF5A* cause a dominantly inherited form of HSP¹²⁴, possibly by disrupting microtubule-dependent slow axonal transport, including the transport of neurofilaments¹²⁵. Retrograde axonal transport is mediated by the DYNEIN–DYNACTIN complex. If this complex is disrupted in transgenic mice, a progressive motor neuron disease is observed that is caused by deficient retrograde transport and neurofilament accumulation¹²⁶. In line with these findings, a dominant mutation in the p150 subunit of dynactin has been found in a family affected by an inherited motor neuron disease¹²⁷. Finally, mutations in gigaxonin cause giant axonal neuropathy¹²⁸, a syndromic condition in which CNS and PNS neurons are affected, possibly in a length-dependent pattern. Gigaxonin binds to microtubule-associated protein 1B (MAP1B), and enhances microtubule stability¹²⁹.

Dominant *RAB7* mutations cause CMT2B. Length-dependent weakness and severe sensory loss, leading to distal ulcerations of the feet and even amputations, are the hallmarks of CMT2B¹³⁰. Two missense mutations in *RAB7*, which encodes a member of the Rab family of Ras-related GTPases, cause CMT2B. Despite the underlying disease mechanisms being unknown, there is particular interest in the mechanism by which the mutant protein exerts a dominant effect. Rab proteins are involved in intracellular membrane trafficking, and have special roles in vesicular trafficking of motor proteins and targeting proteins to the cytoskeleton¹³¹ (FIG. 3). In particular, Rab7 controls lysosomal transport through the effector protein RILP by inducing the recruitment of dynein–dynactin motors^{132,133}. Motor and sensory neurons express *RAB7* (REF. 134), indicating a potential connection between

HAPLOINSUFFICIENCY
 Loss of one copy (one allele) of a gene is sufficient to give rise to disease. Haploinsufficiency implies that no dominant-negative effect of the mutated gene product has to be invoked.

AMYOTROPHIC LATERAL SCLEROSIS
 A progressive neurological disease that is associated with the degeneration of central and spinal motor neurons. This neuron loss causes muscles to weaken and atrophy.

DYNEIN–DYNACTIN
 Dynein is a motor protein complex involved in minus-end directed microtubule transport. Dynactin is a biochemically separable complex that links dynein to target organelles.

CMT2B and other forms of CMT2 with altered axonal transport (see earlier in text). Rab7 is also involved in Golgi targeting of glycosphingolipids¹³⁵. This is of particular interest, as patients who have CMT2B and hereditary sensory neuropathy type 1 (HSN1) patients share many clinical features¹³⁰. HSN1 is caused by mutations in *SPTLC1*, a key enzyme in the biosynthesis of sphingolipids.

Mutations in *LMNA* cause CMT2B1/AR-CMT2A. Several neuromuscular diseases are caused by mutations in proteins of the nuclear envelope¹³⁶. Among these, homozygous missense mutations of *LMNA*, which encodes A-type lamins, cause an autosomal recessive axonal neuropathy, AR-CMT2A. In one large family, the onset of symptoms occurred during the second decade of life, with subsequent progression to severe weakness and atrophy of distal limb muscles. Lamins A/C are components of the intermediate filaments that coat and organize the interior surface of the nuclear envelope (FIG. 3), and they are expressed in all cells. However, most of the diseases are tissue-specific, such as limb-girdle muscular dystrophy 1B, autosomal dominant Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy type 1A and autosomal dominant partial lipodystrophy, but Hutchison-Gilford progeria syndrome is pervasive¹³⁷. Like CMT2A patients, *Lmna*^{-/-} mice develop a neuropathy, but have additional defects that lead to premature death¹³⁸. Genetic models recreating the different mutations that are found in humans, in conjunction with a more detailed understanding of the nuclear envelope at the cellular and molecular level, are needed to define the different *LMNA* mutations and their disease-causing mechanisms.

GARS is responsible for CMT2D and dSMAV. Dominant missense mutations affecting the glycyl transfer RNA synthetase gene (*GARS*) are responsible for CMT2D and the related allelic disorder distal spinal muscular atrophy type V (dSMAV). Both diseases have the hallmarks of axonal neuropathies, with an unusually severe phenotype in the upper extremities and distal sensory loss confined to CMT2D. *GARS* is expressed ubiquitously¹³⁹, as expected for a member of the aminoacyl tRNA synthetase family, as these proteins are responsible for placing the correct amino acid on each tRNA. The four known disease-causing mutations are located throughout the *GARS* protein without a specific pattern, but affect amino acids that are highly conserved through evolution. Their dominant nature might be explained by the fact that the functional holoenzyme is a homodimer¹⁴⁰. Why these mutations seem to exclusively affect peripheral nerves, with a prominent phenotype in specific muscle groups of the hands, is unclear. It will be particularly interesting to determine whether the defect reflects a specific effect related to requirements of, or dependence on, glycine. Depending on the disease mechanism, other aminoacyl tRNA synthetases might be candidates for involvement in diseases that affect peripheral nerves.

Wrapping up

The inherited neuropathies were first described by Charcot, Marie, Tooth and Herringham in the 1880s, before Mendelian inheritance was fully understood. As the genetics and molecular causes have been unravelled over the last twenty years, the complexity of these diseases has become apparent. We are just starting to understand the complicated relationships between genotypes and phenotypes, and how disease mechanisms can converge. Investigating how the different disease-causing genes cause neuropathy provides a direct path to a better understanding of how axon-Schwann cell interactions control the development and maintenance of myelinated nerves, as well as important clues to the pathogenesis of acquired neuropathies.

For demyelinating neuropathies, defects originating in Schwann cells seem to result in demyelination, which in turn leads to length-dependent axonal loss (FIG. 2). There is a correlation between the lack of myelin and the degree of axonal loss (FIG. 1), and this has been convincingly elucidated in genetically authentic CMT animal models that recapitulate key aspects of the corresponding human diseases¹⁴¹. Regardless of the cause of demyelination, the lack of the myelin sheath has pronounced effects on axonal calibre, axonal transport, the phosphorylation and packing of neurofilaments, and the organization of ion channels in the axonal membrane^{141,142}. However, at least in the CNS, pronounced axonal loss has been observed even in genetic models in which axons are associated with seemingly normal myelin sheaths¹⁴³. The crucial questions are: how do myelinating glia communicate with axons (and vice versa), and how is this altered by demyelination? Possibilities include increased energetic costs of propagating action potentials, decreased trophic support from Schwann cells, abnormal signalling emanating from the altered myelin sheath itself, and the loss of signals from the adaxonal glial membrane and/or cytoplasm, especially in the paranodal region where axons and glial cells are intimately connected¹⁴². Other contributing factors to demyelination and/or axonal loss include inflammatory changes initiated by demyelination¹⁴⁴.

Determining the causes of axonal loss in hereditary neuropathies is an important objective. Existing animal models should be key to this endeavour¹⁴⁵. Such studies will determine whether the mechanisms are the same in distinct demyelinating and axonal neuropathies, and reveal why axonal loss is length-dependent. These issues are of general importance, since axonal loss is a primary feature of many inherited and acquired neurological diseases and probably causes the clinical disabilities. These diseases include HSP and more pervasive neurodegenerative disorders such as neuroaxonal dystrophy and giant axonal neuropathy, as well as disorders that are often considered to be neuronopathies, such as HSN1 and hereditary motor neuropathies/distal spinal muscular atrophy (FIG. 5). The differences in their names and classification reflect the clinical manifestations of the affected neuronal populations; fundamentally they might all be axonopathies.

The most suitable hypothesis to account for the observation that axonal degeneration is maximal at the distal ends is altered axonal transport. Axonal transport is crucial, owing to the extreme polarity and size of neurons. In humans, motor and sensory axons extend for one metre or more. Most axonal proteins, including the cytoskeletal components, organelles, synaptic vesicle precursors and mitochondria, must be transported along the axon. This system must function efficiently without perturbation by mutated proteins or the deleterious influence of demyelination, as observed in the neuropathies discussed previously. Defects in axonal transport have also been observed in other neurodegenerative diseases, including amyotrophic lateral sclerosis and Alzheimer's disease. However, it is not clear in these disorders whether defective axonal transport is a trigger or a consequence of the disease.

Such considerations have an impact on how we might conceptualize therapeutic interventions. For demyelinating neuropathies that are caused by altered gene dosage, equilibrating the levels of expression might be feasible. A transgenic mouse provides proof-of-concept for the recovery of demyelinating Schwann cells¹⁴⁶. However, axonal damage might not be reversible, and transfer to the clinic is still an important challenge. Given the widely distributed arrangement of our peripheral nerves, somatic replacement of defective genes is not a feasible strategy. However, if preventing or slowing axonal loss is sufficient to arrest disease progression — as demonstrated by the partial 'rescue' of axons in *Mpz*^{-/-} mice with the expression of the *Wld^s* gene¹⁴⁷ — then systemic administration of neuronal trophic factors might be effective. Finally, discovering the epigenetic factors that influence the severity of neuropathy might provide new therapeutic opportunities.

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FURTHER INFORMATION

Charcot–Marie–Tooth Association: <http://www.charcot-marie-tooth.org/>
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