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Review

Hereditary motor and sensory neuropathies: Understanding molecular pathogenesis could lead to future treatment strategies ☆

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ABSTRACT

Inherited peripheral neuropathies, like many other degenerative disorders, have been challenging to treat. At this point, there is little specific therapy for the inherited neuropathies other than genetic counseling as well as symptomatic treatment and rehabilitation. In the past, ascorbic acid, progesterone antagonists, and subcutaneous neurotrophin-3 (NT3) injections have demonstrated improvement in animal models of CMT 1A, the most common inherited neuropathy, but have failed to translate any effect in humans. Given the difficulty in treatment, it is important to understand the molecular pathogenesis of hereditary neuropathies in order to strategize potential future therapies. The hereditary neuropathies are in an era of molecular insight and over the past 20 years, more than 78 subtypes of Charcot Marie Tooth disease (CMT) have been identified and extensively studied to understand the biological pathways in greater detail. Next generation molecular sequencing has also improved the diagnosis as well as the understanding of CMT. A greater understanding of the molecular pathways will help pave the way to future therapeutics of CMT. This article is part of a Special Issue entitled: Neuromuscular Diseases: Pathology and Molecular Pathogenesis.

1. Introduction

Inherited peripheral neuropathies, like many other degenerative disorders, have been challenging to treat. At this point, there is little specific therapy for the inherited neuropathies other than genetic counseling as well as symptomatic treatment and rehabilitation. In the past, ascorbic acid, progesterone antagonists, and subcutaneous neurotrophin-3 (NT3) injections have demonstrated improvement in animal models of CMT 1A, the most common inherited neuropathy, but have failed to translate any effect in humans. Given the difficulty in treatment, it is important to understand the molecular pathogenesis of hereditary neuropathies in order to strategize potential future therapies. The hereditary neuropathies are in an era of molecular insight and over the past 20 years, more than 78 subtypes of CMT have been identified and extensively studied to understand the biological pathways in greater detail. Next generation molecular sequencing has also improved the diagnosis as well as the understanding of CMT. A greater understanding of the molecular pathways will help pave the way to future therapeutics of CMT.

2. Background

Inherited neuropathies are some of the most common inherited neurological disorders [1]. Inherited neuropathies not part of another syndrome are named hereditary motor and sensory neuropathy (HMSN) or Charcot Marie Tooth disease (CMT). CMT stands for Charcot Marie Tooth, named after three neurologists who described the condition in 1886 [2]. CMT is the most common inherited disorder of the human peripheral nerve with a prevalence of 1 in 2500 [3]. While CMT is used as a term for hereditary motor and sensory neuropathies, it may also be viewed as a spectrum ranging from the pure motor neuropathies (HMNs) to the predominantly pure sensory neuropathies (HSNs); the following review will focus on hereditary motor and sensory neuropathies.

Over the past 25 years, a dramatic revolution in molecular genetics of inherited neuropathies has occurred. More than 40 genes causing CMT have been identified with many different types of mutations. These mutations provide clues into the cellular pathways of inherited neuropathies and knowledge of cellular pathways can help provide

Abbreviations: CMT, Charcot Marie Tooth disease; AD, Autosomal dominant; AR, Autosomal recessive; MNCV, Motor nerve conduction velocity; CMAP, Compound muscle action potential; PMP22, Peripheral myelin protein 22; HMSN, Hereditary motor sensory neuropathy; LITAF, lipopolysaccharide-induced tumor-necrosis factor (TNF)-alpha factor; HMN, Hereditary motor neuropathy; HSN, Hereditary sensory neuropathy; Cx32, Connexin 32; MPZ, Myelin protein zero; NT3, Neurotrophin-3; INF2, Inverted formin 2; PRX, Periaxin; FGD4, Frabin; LITAF, Lipopolysaccharide-induced tumor necrosis factor-alpha factor; MTMR2, Myotubularin-related protein-2; SBF2, SET binding factor 2; SBF1, SET binding factor 1; SH3TC2, SH3 domain and tetratricopeptide repeat domain 2; NDRG1, N-myc downstream-regulated gene 1; DNM2, Dynamin 2; GJB1, Gap junction beta-1; EGR21, Early growth response-2; HK1, Hexokinase 1; HSPB1, Heat-shock protein beta-1.

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information for therapeutic targets [4]. Although four genes account for the majority (over 90%) of all CMT molecular diagnoses: peripheral myelin protein 22 (PMP22), gap junction β-1 (GJB1), myelin protein zero (MPZ), and mitofusin 2 (MFN2) [5], new genes have recently been found to be associated with CMT including PDK3 [6], GNB4 [7], INF2 [8], and FBLN5 [9,10].

Current classification of CMT depends on electrophysiological studies and patterns of inheritance. Subtypes include autosomal dominant demyelinating (CMT 1), autosomal dominant axonal (CMT 2), autosomal recessive (CMT 4) and X-linked (CMTX). Demyelinating CMT (CMT 1) is characterized by motor nerve conduction velocity (MNCV) less than 38 m/s in the forearm [11]. Dominant intermediate CMT

Table 1
CMT subtypes, genes, and protein product: function.

Disrupted process	Disease	Gene	Protein product: function
Schwann cell			
Myelin assembly	CMT1A, CMT1E, HNPP	PMP22	*Peripheral myelin protein 22*: myelin assembly
	CMT1B, CMT2I/2J, CMTDID	MPZ	*Myelin P_0 protein*: myelin assembly
Cytoskeleton	CMTDIE	INF2	*Inverted formin 2*: actin polymerization and filament severing
	CMT 4F	PRX	*Periaxin*: membrane–protein interactions stabilizing myelin sheath
	CMT 4H	FGD4	*Frabin protein*: regulates cell signaling involved in myelin production and involved in actin cytoskeleton
Channel	CMT X1	GJB1 or Cx-32	*Gap junction beta-1 or connexin-32*: gap junction formation + myelin assembly and transport
Transcription, mRNA processing	CMT 4E, CMT 1D	EGR2	*Early growth response-2*: transcription regulation
Endosomal sorting and cell signaling	CMT1C	LITAF	*Lipopolysaccharide-induced tumor necrosis factor-alpha factor*: regulation of endosome to lysosome trafficking and cell signaling
	CMT 4B1	MTMR2	*Myotubularin-related protein-2*: modifies chemical messengers, which are involved in signal transduction
	CMT4B2	SBF2	*SET binding factor 2*: development of Schwann cells
	CMT4B3	SBF1	*SET binding factor 1*: endosomal trafficking [13]
	CMT 4C	SH3TC2	*SH3 domain and tetratricopeptide repeat domain 2*: targets to intracellular endosome recycling
	CMT 4D	NDRG1	*N-myc downstream-regulated gene 1*: signaling protein shuttling between cytoplasm and nucleus
	CMT 4J	FIG4	*FIG4 protein*: abnormal transport of intracellular organelles
	CMTDIB, CMT 2M	DNM2	*Dynamin 2*: family of large GTPases and part of cell fusion–fission apparatus
Mitochondria	CMT 4G	HK1	*Hexokinase 1*: glucose metabolism
Neuron cell body and axon			
Proteasome and protein aggregation	CMT 2F	HSPB1	*Heat-shock protein beta-1*: microtubule regulator
	CMT 2L	HSPB8	*Heat-shock protein beta-8*: microtubule regulator
	CMT 2P	LRSAM1	*Leucine-rich repeat and sterile alpha motif-containing 1*: E3 ubiquitin ligase, regulates cell adhesion molecules
	CMT 2R	TRIM2	*Tripartate motif-containing protein 2*: E3 ubiquitin ligase
Cytoskeleton, axonal transport	CMT 1F, CMT 2E	NEFL2	*Neurofilament light chain*: intermediate filaments in neurons
	CMT 2O	DYNC1H1	*Dynein, cytoplasmic 1 heavy chain 1*: retrograde axonal transport
Channel	CMT 2C	TRPV4	*Transient receptor potential cation channel subfamily V member 4*: calcium homeostasis, cytoskeleton remodeling
Nuclear envelope, mRNA processing	CMTDIC	YARS	*Tyrosyl-tRNA synthetase*: aminoacyl tRNA synthetase
	CMT 2B1	LMNA	*Lamin A/C*: intermediate filament protein of nuclear envelope
	CMT 2B2	MED25	*Mediator complex subunit 25*: regulated transcription of RNA polymerase II-dependent genes
	CMT 2D	GARS	*Glycyl-tRNA synthetase*: aminoacyl tRNA synthetase
	CMT 2N	AARS	*Alanyl-tRNA synthetase*: aminoacyl tRNA synthetase
	CMT 2	MARS	*Methionyl-tRNA synthetase*: aminoacyl tRNA synthetase
	CMT 2	HINT1	*Histidine triad nucleotide binding protein 1*: modulates transcriptional activity
	CMT X5	PRPS1	*Phosphoribosyl pyrophosphate synthetase 1*: purine and pyrimidine biosynthesis
	CMTRIB	KARS	*Lysyl-tRNA synthetase*: aminoacyl tRNA synthetase
	CMT RIC	PLEKHG5	*Pleckstrin homology domain-containing protein, Family G, member 5*: nuclear factor kB-Activator
Endosomal sorting and cell signaling	CMTDIF	GNB4	*Guanine nucleotide-binding protein B4*: signal transduction
	CMT 2B	RAB7A	*Ras-related protein Rab-7*: vesicular transport and membrane traffic
	CMT 2G	TFG	*Trk-fused gene*: endoplasmic reticulum morphology
Mitochondria	CMT 2A	MFN2	*Mitofusin-2*:mitochondrial fusion
	CMT 4A, CMT2K, CMT RIA	GDAP1	*Ganglioside-induced differentiation-associated protein 1*: mitochondria fission
	CMT2Q	DHTKD1	*2-Oxoglutarate dehydrogenase E1 component*: degradation of amino acids
	CMT X4	AIFM1	*Apoptosis-inducing factor mitochondrion associated 1*: oxidative phosphorylation; apoptosis
	CMT X6	PDK3	*Pyruvate dehydrogenase kinase, isoenzyme 3*: regulates pyruvate dehydrogenase complex

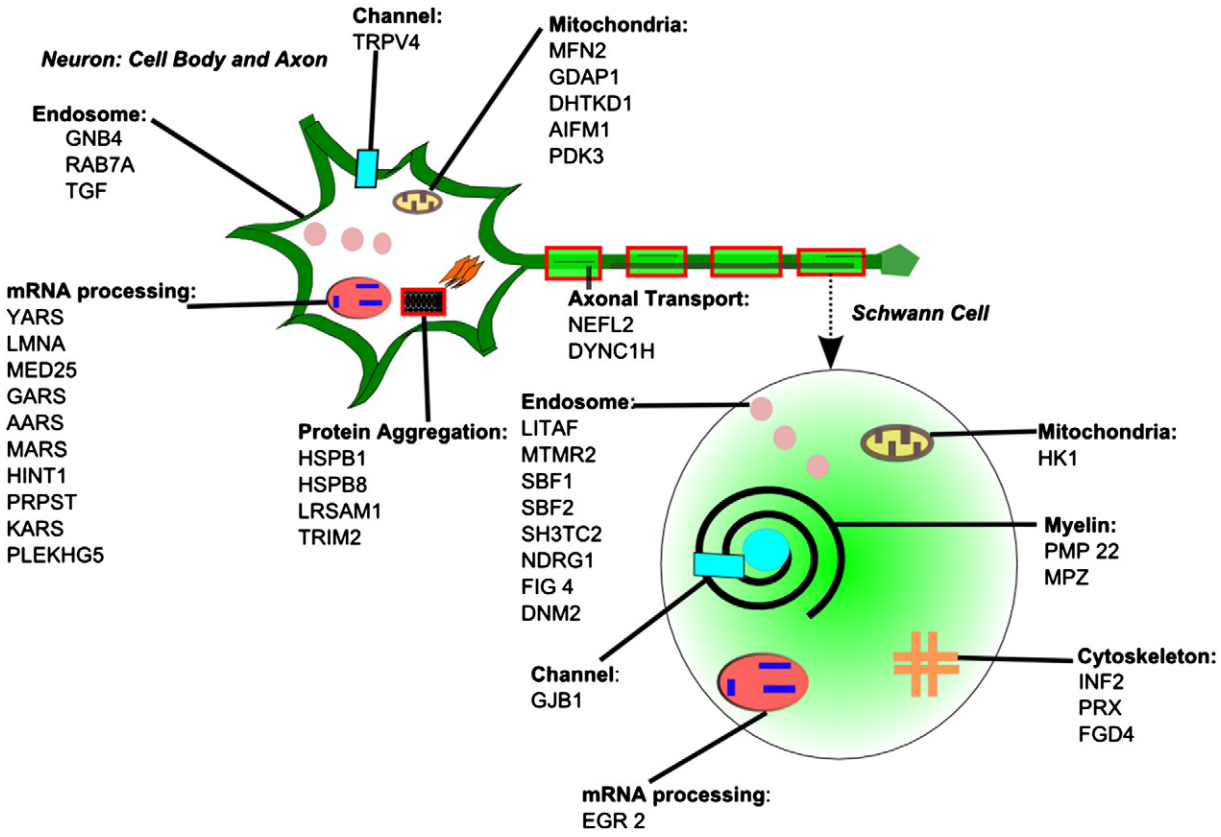

Fig. 1. Genes causing CMT associated with various neuronal and Schwann cell structures: localizations of genes associated with neuron cell body, axon, and Schwann cell are listed.

(CMTDI) and recessive intermediate CMT (CMTRI) range from 25 to 45 m/s on MNCVs [2]. Axonal CMT (CMT 2) is characterized by MNCVs > 45 m/s as well as a compound muscle action potential (CMAP) amplitude decrease. CMT can be divided based on the disrupted process of the mutated gene (Table 1). The following review will utilize the molecular function at the cellular level as the basis of classification and will be divided based on function and protein produced. Each CMT subtype is named based on molecular findings in myelinated axons [12].

This review will also address the molecular processes involved in different types of CMT (Fig. 1). Genes encode for proteins in the Schwann cell and neuron (Table 1). These proteins are involved in the following processes: myelin assembly, cytoskeleton/axonal transport, protein aggregation, endosomal sorting, mitochondrial functions, mRNA processing/transcription, and channel abnormalities. When these proteins are mutated, various cellular processes are disrupted, thus resulting in CMT.

3. Myelin assembly: PMP22, MPZ

A distinguishing feature of axons in the central and peripheral nervous system is the myelin sheath, which increases electrical impulse propagation speed [13]. Myelin is a multi-layer spiral structure ensheathing axons that are larger than 1 μm thick; Schwann cells form the myelin sheath in the peripheral nervous system and the oligodendrocytes do the same in the central nervous system. One Schwann cell myelinates a single axon unlike oligodendrocytes, which myelinate multiple axons. Myelin allows for an increase in axonal conduction velocity without an increase in axonal diameter through the process of salutatory conduction in which nerve impulses jump between nodes of Ranvier, which are gaps between two myelin segments. Although myelinating Schwann cells are responsible for a demyelinating neuropathy, neurons that express a mutant protein can also contribute in a demyelinating neuropathy.

The myelin sheath has two areas, compact and non-compact, which contain unique proteins (Fig. 2) [14]. The compact region contains the myelin structural proteins PMP22, P0 (MPZ encodes it), and myelin basic protein [14]. Most of the myelin is compact, containing cholesterol, sphingolipids, galactocerebroside, and sulfatide. The non-compact region is formed in part because of the long distances between the cell nucleus and Schwann cells; the non-compact myelin contains gap junctions that provide a radial pathway to the myelin sheath for the passage of water, Schmidt–Lanterman incisures, ions, and small molecules. The non-compact myelin can be divided into the paranode, directly adjacent to the node of Ranvier and the juxtaparanode region. The paranodal region contains loops of Schwann cell membrane which contains Schwann cell proteins such as Cx32, the major gap-junction protein in myelin. It also contains myelin associated glycoprotein (MAG), neurofascin 155, and axonal proteins Caspr and Contactin. The juxtaparanodal region contains potassium channels and Caspr2, both expressed by axons.

Schwann cells and axons also interact along the peripheral nerve internodes, which provide mutual benefits to both cells including trophic support. Disorders that occur in Schwann cells, like in CMT 1, result in axonal loss, the final pathway of all CMT disease. Thus Schwann cells serve as a source of neuroprotection [15]. Schwann cell and axonal interactions are disrupted with virtually all demyelinating inherited neuropathies as there are significant changes in axonal physiology. This mutual relationship is evidenced by secondary axonal degeneration in all forms of demyelinating CMT that are observable clinically as muscle atrophy and physiologically, by reduced amplitudes on nerve conduction testing.

Mutations in Schwann cells can cause disease both by interfering or reducing the normal function of the specific molecule (loss of function) or by introducing abnormalities not related to the normal function

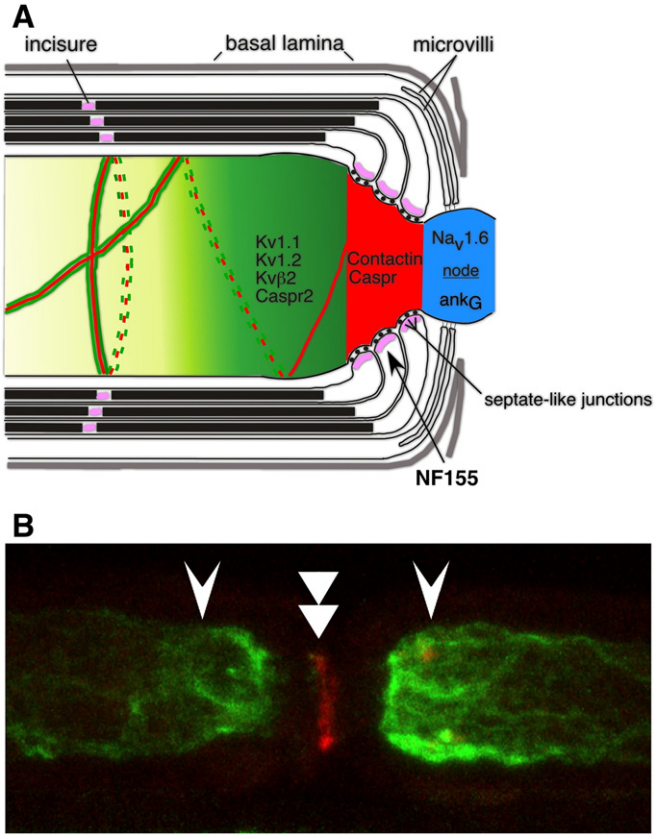

Fig. 2. A. Myelinated axon. A schematic depiction of the node, paranode, and juxtaparanode. B. Laser scanning confocal micrograph of Na^+ and K^+ channels. A myelinated fiber teased from rat sciatic nerve was labeled with a rabbit antiserum against voltage-gated Na^+ channels (rhodamine) and a monoclonal antibody against Kv1.2 (fluorescein). Na^+ channels are restricted to the node (double arrowheads), where Kv1.2 channels are found in the juxtaparanodal region (arrowheads).
Courtesy of Dr. Steven Scherer, University of Pennsylvania.

(gain of function). In heterozygous disorders, the gain of function abnormalities often cause more severe disease than heterozygous loss of function. One example is when the mutation interferes with normal folding and processing of the protein in the endoplasmic reticulum. This example has been investigated in particular with certain PMP22 and MPZ point mutations [14,16,17]. When the misfolded PMP22 or MPZ accumulate in the Schwann cell ER they activate a process known as the unfolded protein response (UPR) which can globally downregulate myelination. Manipulating the UPR is a current therapeutic strategy in these disorders [18].

3.1. Peripheral myelin protein 22 (PMP22) mutations: CMT 1A, HNPP, CMT 1E (17p12)

Peripheral myelin protein 22 (PMP22) causes CMT 1A, the most common form of CMT, when one of its alleles is duplicated and hereditary neuropathy with liability to pressure palsy (HNPP) when an allele is deleted. Thus CMT 1A and HNPP are caused by dosage abnormalities of PMP22. Missense mutations in PMP22 are said to cause CMT 1E to distinguish them from CMT 1A. The PMP22 gene encodes a 22-kDa protein that makes up 2–5% of peripheral nervous system myelin proteins. It is produced by Schwann cells and expressed in the compact myelin in all myelinated fibers of the peripheral nervous system [19]. Its functional role is still debated. It was first identified as a growth arrest gene (Gas 3) in fibroblasts. However whether it plays a role in the cell cycle of Schwann cells is uncertain. CMT 1A is caused by a duplication (overexpression) of PMP22, a 1.4 mega base (Mb) duplication on chromosome 17p11.2–p12; it is one of the most common inherited neuropathies and is usually caused by the heterozygous inheritance of two homologous DNA sequences of PMP22. HNPP is caused by a deletion (under expression) of PMP22, resulting in loss-of-function alleles. Pathologically, biopsies of those with HNPP will show nerves forming tomacula (folding of myelin sheath, demyelination, and remyelinating). Tomacula are unstable structures that are prone to degeneration, thus resulting in disease manifestations [20]. Point mutations causing CMT 1E are relatively rare, causing around 1% of cases of CMT.

Peripheral myelin protein 22 (PMP22) is a molecule that can form aggresomes when mutated, thus forming PMP22 aggregates. Some experimental studies have shown that autophagy enhancement can reduce accumulated PMP22 protein aggregates [21]. PMP22 may also interact with P0, so that an abnormal PMP22/P0 ratio could make the myelin sheath unstable. Mutant PMP22 could have additional effects, which include blocking wild-type PMP22 transportation to the cell membrane by a dominant negative effect [22].

3.2. Myelin protein zero (MPZ) mutation: CMT 1B, CMT 2I/J, CMT DID (1q23.3)

The major and most abundant myelin protein produced by myelinating Schwann cells is myelin protein zero or P0 [23]. The gene for myelin protein zero, MPZ (aka P0) has been mapped to chromosome 1q22. Virtually all MPZ mutations that alter the coding sequence cause a dominantly inherited neuropathy with varying phenotypes [1].

Myelin protein zero accounts for more than 25% of the myelin protein in peripheral nerve sheaths. MPZ links adjacent lamellae and stabilizes myelin assembly [24,25]. MPZ makes up most of compact myelin. It contains a high positively charged intracellular domain with an extracellular single immunoglobulin, which is important for myelin compaction [1]. As in PMP22, the dose of P0 in myelin is important with reduced MPZ causing myelin instability and overexpression causing severe dysmyelination. MPZ is a transmembrane protein that is made in the endoplasmic reticulum where it undergoes N-glycosylation and forms an intramolecular disulfide bond.

If MPZ is misfolded, MPZ proteins can collect in the endoplasmic reticulum causing an unfolded protein response leading to Schwann cell apoptosis and subsequent demyelination [14]. Toxicity of misfolded protein or reduced amounts of P0 could be the etiology behind the phenotypic manifestations of many patients with MPZ mutations [14].

More than 200 different disease-causing mutations in *MPZ* have been identified (http://www.molgen.ua.ac.be/CMTMutations/default.cfm). Many patients with CMT 1B present in two phenotypic groups: one with extremely slow nerve conduction velocities and onset of symptoms during the period of motor development; and a second with normal or near normal nerve conduction velocities and the onset of symptoms as adults [26]. Why particular mutations cause early or late onset neuropathy is not understood [1].

4. Myelin assembly: future therapeutic strategies

4.1. Curcumin: reduction of neurotoxic aggregates/misfolded proteins

Khajavi et al. (2005) found that frameshift mutations of MPZ associated with severe disease result in intercellular accumulations of mutant proteins in tissue culture especially in the endoplasmic reticulum [27]. In mouse models of early-onset CMT 1B, specific mutations in the MPZ gene (S63del and R98C) were seen; mutant S63del and R98C MPZ accumulate in the endoplasmic reticulum of Schwann cells, triggering an unfolded protein response and dedifferentiation of these cells, with consequent demyelination. Curcumin, a chemical compound derived from the curry spice turmeric relieves the endoplasmic reticulum stress as it has been shown to mitigate the phenotype in mice models.

Curcumin derivatives could have the potential to treat selected inherited peripheral neuropathies.

4.2. Geldanamycin: prevention of PMP22 aggregates

Inhibition of heat shock protein 90 by geldanamycin treatment can also prevent PMP22 protein aggregates [21]. Geldenamycin enhanced cytosolic chaperone levels and improved myelination, along with the trafficking of PMP22 in mice [28]. Preclinical therapeutic studies have not been performed yet.

4.3. Nutrient deprivation or rapamycin: autophagy to remove aggresomes

In Schwann cells of *Trembler-J* mice, the protein degrading pathways of the lysosome and proteasome are full and abnormal cytosolic aggregates containing mutant misfolded PMP22 and ubiquitin are formed. Induction of autophagy (which removes aggresomes) via nutrient deprivation or rapamycin resulted in aggresome degradation in Schwann cells of PMP22 mutant and overproducing mice [21,29].

4.4. Onapristone: progesterone receptor antagonist

Progesterone encourages myelination in the peripheral nervous system as well as stimulating PMP22 and MPZ expression [30]. Experiments have shown that daily administration of progesterone to CMT 1A rats increased levels of PMP22 and MPZ mRNA in sciatic nerves, resulting in a more severe neuropathy. Administration of a progesterone receptor antagonist (onapristone), reduced overexpression of PMP22 mRNA and thus improved the CMT phenotype (specifically improving the axonal loss seen during disease progression) [31,32]. At this point, onapristone has been toxic, so currently a safer progesterone antagonist is being developed [31].

4.5. Ascorbic acid: normalization of gene dosage

Ascorbic acid is critical in myelination; it promotes the formation of collagen and laminin containing extracellular matrix and seems to promote myelinating Schwann cells. A mouse model of CMT 1A was treated with high doses of ascorbic acid and myelination was improved; there was also a reduction in PMP22 mRNA levels to those below what was necessary to induce the disease [33]. Although ascorbic acid reduces the severity of neuropathy in transgenic mice overexpressing PMP22 compared to untreated mice, it has no significant effect in humans with CMT 1A testing with different doses of vitamin C (1–4 g/d) for over 2 years [34]. Both ascorbic acid and progesterone antagonists are nonspecific therapies and do not target expression of a single myelin gene, which may be appropriate for CMT 1A but unknown for other types of CMT [14].

4.6. Small molecule screens and normalization of gene dosage

The Charcot Marie Tooth Association has spearheaded an ongoing effort to use high throughput screen (HTS) technology to identify potential existing compounds that have the potential to lower the dosage of PMP22 in patients with CMT 1A. A study in 2012 describes compounds from a collection of 3000 approved drugs which were tested at multiple titration points to achieve a pharmacological end point in an HTS format. In conjunction with an independent counter-screen for cytotoxicity, an orthogonal screen platform designed was effective to select and prioritize active compounds, among which three drugs (fenretinide, olvanil, and bortezomib) were shown to reduce endogenous Pmp22 mRNA and protein [35]. These studies are currently in progress although no current compounds have yet been investigated in human trials.

4.7. siRNA and antisense oligonucleotides: normalization of gene dosage

Small double stranded RNAs (dsRNA) of about 21 nucleotides are used in plants and animals to degrade mRNA and genetically engineered small inhibitory RNAs (siRNAs) can be engineered to reduce the expression of target mRNAs such as PMP22 in patients with CMT 1A; other molecules such as ribozymes and antisense oligonucleotides can downregulate levels of mRNAs as well.

5. Cytoskeleton: INF2, PRX, FGD4, FBLN5 (Schwann cell) and NEFL2, DYNC1H1 (neuron)

The cytoskeleton, nucleus, and mitochondria are several biological elements that define eukaryotic cells. The cytoskeleton is composed of three distinct structures: microtubules, intermediate filaments, and microfilaments [36].

Microtubules form a hollow tube of about 13 protofilaments, which form an outer diameter of 25 nm; they serve as tracks for organelle traffic. Microtubules of neurons are unique as axonal and dendritic microtubules have uniform polarity with plus ends that are distal to the cell body; the microtubules are not continuous back to the cell body and they do not have a microtubule organizing center [37]. Neuronal microtubules are more diverse with a variety of composition according to location (i.e. axon vs. dendrite). Axonal microtubules, for instance, are so stable that they resist treatments that could depolymerize microtubules of other cells [38]. Microtubule cytoskeletal proteins of the nervous system include alpha and beta-tubulin. Microtubules also have microtubules associated proteins, which interact with microtubules; microtubule associated proteins include those that have a high molecular weight, such as tau proteins, and those that have an intermediate molecular weight, such as kinesin, and dynein. In neurons, microtubules assist in the transport of membrane-bound organelles, extend neurites during development, provide scaffolding for neuritis, and maintain intracellular compartments.

Intermediate filaments can be divided into five subtypes: keratin filaments in epithelial cells, vimentin filaments in mesenchymal cells, desmin in muscle cells, glial filaments in astrocytes, and neurofilaments in neurons. Neurofilaments form 8 to 10 nm rope-like filaments that support neuronal and glial cells. Neurofilaments unlike other intermediate filaments have side arms that project outward; instead of being densely packed like other intermediate filaments, neurofilaments are widely spread out. Mutations of neurofilaments may result in abnormal neurofilaments and thus reduce the axon diameter and caliber [39].

Actin microfilaments are critical in neuronal grown and secretion; they are formed into 4–6 nm diameter fibrils like an intertwined string of pearls [40].

5.1. Inverted formin 2 (INF2): CMTDIE (14q32.33)

INF2 protein is a member of the formin family that binds to growing ends of actin filaments, and protects them from being cross-linked into bundles [41,42]. INF2 interacts with actin and essentially accelerates both polymerization and depolymerization of actin filaments [41]. INF2 is expressed in peripheral nerve Schwann cells, some axons, and podocytes of the kidney [8]. When INF2 is mutated, Schwann cell actin is disrupted, leading to abnormal myelin formation and CMT.

Individuals with this mutation not only had CMT, but they also had sensorineural hearing loss as well as focal segmental glomerulosclerosis [8].

5.2. Periaxin (PRX): CMT 4F (19q13.2)

Mutations in periaxin cause the autosomal recessive CMT 4F [43]. Periaxin is a cytoskeletal component and membrane associated protein of Schwann cells located in non-compact myelin [43]. Periaxin is

required for cytoplasmic compartment formation in the Schwann cell and the bands of Cajal (which are important for proper elongation during development) [44]. Its location changes during myelination from near the axon to near the basal lamina in mature myelin sheaths. Periaxin interacts with dystroglycan through dystrophin related protein-2, linking laminin-2 in the basal lamina to the actin cytoskeleton, making a complex, which if disrupted will result in demyelinating neuropathies [1]. Periaxin-deficient mice develop focal myelin outfoldings (tomacula), infoldings, and progressive demyelination. They will not display Cajal bands and will show a decreased intermodal length [44]. Periaxin is involved in membrane–protein interactions that provide myelin stabilization. It also forms tight junctions between the myelin loop and axon. In 2010, Marchesi et al. discovered that all patients with PRX mutations and a neuropathy have truncating mutations in homozygous or compound heterozygous states, suggesting a loss of protein function as a disease [45]. Animal models of periaxin mutations show that periaxin deficient mice will myelinate their peripheral axons normally, but then develop a severe demyelinating neuropathy with allodynia and hyperalgesia [46].

5.3. Frabin protein (FGD4): CMT 4H (12p11.21)

FGD4 protein is a cytoplasmic guanine nucleotide exchange factor for cell division cycle 42 and binds along sides of actin fibers [47]. It alters Schwann cell shape and results in a severe demyelinating neuropathy. Mouse frabin induced the formation of filopodia in fibroblasts [48].

5.4. Neurofilament light chain (NEFL): CMT 1F/2E (8p21.2)

NEFL is one of the three members of the neurofilament family and is seen only in neurons [49]. Neurofilaments are important and prominent structures of the axonal cytoskeleton [49]. Mutations in NEFL cause abnormalities in the neurofilaments, which subsequently lead to a disruption of neurofilament networks and aggregation and result in CMT 4H [50]. Neurofilaments have three subunits: light, medium, and heavy, which become neuronal intermediate filaments that form the cytoskeletons of large axons. Brownless et al. in 2002 demonstrated that abnormal neurofilament disrupted neurofilament assembly and axonal transport as well as disturbing the localization of mitochondria in neurons [51]. Neurofilaments are also pathologic in diseases such as motor neuron disease, Alzheimer's disease, Parkinson's disease, dementia with Lewy body, and diabetic neuropathy [51].

5.5. Dynein, cytoplasmic 1 heavy chain 1 (DYNC1H1): CMT 20.LED-SMA (14q32.31)

Mutations in the heavy-chain of the dynein motor protein (encoded by DNC1H1) result in CMT 20 [52]. Dynein is a motor protein crucial for neuronal axonal retrograde transport. MicaudalD homolog 2 (*Drosophila*) encodes an adaptor protein that interacts with DYNC1H1 and also plays a role in facilitating retrograde axonal transport. Recently dominant mutations in BICD2 have been shown to cause a similar phenotype to those caused by DYNC1H1 [53].

6. Cytoskeleton: future therapeutic strategies

6.1. Celastrol: a chaperone inducer

Celastrol is an inducer of chaperone proteins, and induces HSPA1 (stress inducible heat shock protein 7) expression in motor neurons; it prevents the formation of neurofilament inclusions and mitochondrial shortening induced by expression of NEFL in motor neurons [54]. It had a protective effect against the toxicity of NEFL in large sized sensory neurons, but not motor neurons [54].

6.2. HDAC-6 inhibitors: targeting transport defects

Histone deacetylase-6 inhibitors have been shown to correct axonal transport defects in a mouse model of CMT 2F, improving the axonal loss and clinical phenotype of mice. This is a result of an increased acetylated tubulin in peripheral nerve of mutant HSPB1 expressing mice [55]. HDAC6 helps regulate axonal transport and thus could be a target to treat axonal neuropathies [56].

7. Proteasome and protein aggregation

7.1. Proteasome and protein aggregation: HSPB1/HSPB8, LRSAM1, TRIM2

Ubiquitin is crucial in protein degradation in eukaryotic cells [57]. An increase in protein aggregates because of decreased degradation results in the neuropathology of many neurodegenerative disorders such as Parkinson's disease, dementia with Lewy body, prion diseases, and hereditary neuropathies. The neuron is sensitive to protein aggregates as they are sophisticated post-mitotic cells that need to remain plastic and renew internal components properly; they are thus dependent on efficient protein degradation [57].

7.2. Heat shock protein beta 1/8 (HSPB1/HSPB8): CMT 2F/2L (7q11.23/12q24.3)

Mutations in HSPB1 (also called HSP 27), and HSPB8 (heat shock proteins) cause CMT 2F and CMT 2L respectively. HSPB1 and HSPB8 are members of the heat shock protein superfamily and are protective stress proteins [58]. Heat shock proteins maintain neuronal integrity and function. They regulate and maintain the cytoskeleton as well as microtubules. They interact with intermediate filament proteins. Heat shock proteins appear also to play a role in muscle contraction. When mutated, they are not transported and form protein aggregates [58]. These aggregates are thought to interfere with axon function and disrupt neurofilament assembly, affecting axon diameter and nerve impulse transmission.

7.3. Leucine-rich repeat and sterile alpha motif-containing 1 (LRSAM1): CMT 2P (9q33.3)

Leucine-rich repeat and sterile alpha motif-containing 1 is an E3 ubiquitin ligase that regulates cell adhesion molecules and plays a role in receptor endocytosis and viral budding.

7.4. Tripartate motif-containing protein 2 (TRIM 2): CMT 2R (4q31)

Mutations in the TRIM gene results in an inability to degrade unwanted proteins via ubiquitin, which moves unwanted protein into proteasomes. TRIM2 is an E3 ubiquitin ligase that ubiquitinates neurofilament light chain.

8. Proteasome: future therapeutic strategies

8.1. Arimoclomol: coinducer of heat shock protein

A promoter polymorphism has been found in HSPB1 that impairs the stress response of this protein and may be a risk factor for motor neuron disease [59]. Arimoclomol, a coinducer of heat shock proteins, delays disease progression in *SOD1* mutant ALS mice, implying that these proteins are important to motor neuron survival [60]. This suggests that molecules that upregulate heat shock proteins can be beneficial for motor neuron disease and other neurodegenerative diseases such as distal HMN [61]; this could include CMT also.

9. Endosomal sorting and cell signaling

9.1. Endocytic cycling/membrane trafficking: CMT 1C, CMT 4B1, CMT 4B2, CMT 4B3, CMT 4C, CMT 4J (Schwann cell) CMTDIF, CMT 2B, CMT 2G (neuron)

Defects in membrane trafficking and degradation are common themes of many neurodegenerative disorders (Alzheimer's disease, Parkinson's disease, trinucleotide repeat expansion diseases, peripheral neuropathies, and lysosomal storage disorders); these defects result in undegraded proteins due to abnormal endosomal, lysosomal, or autophagic activity [62].

9.2. Lipopolysaccharide-induced tumor-necrosis factor: LITAF/SIMPLE: CMT 1C (16p13)

SIMPLE mutations result in dominantly inherited CMT 1C. The SIMPLE gene is expressed widely and encodes a 161-amino acid protein that plays a role in protein degradation [63]. Although the exact function is unknown, it is essentially involved in facilitating membrane proteins to the lysosome for degradation [14,64]. SIMPLE mutations cause a predominantly demyelinating neuropathy event though it is expressed in many cells types. Because the phenotype appears very similar to CMT 1A it has been suggested that impaired degradation of Schwann cell proteins such as PMP22 may be involved [14].

9.3. Myotubularin-related protein-2 (MTMR2): CMT 4B1 (11q22)

Mutations in MTMR2 cause CMT 4B1. The pathological characteristics of CMT 4B1 demonstrate damage in myelinating Schwann cells which demonstrate a characteristic myelin outfolding. MTMR2 is a member of the myotubularin related dual specific phosphatases, whose substrates are phosphorylated inositol phospholipids [65]. The MTMR mutations result in reduced or absent 3′ phosphatase activity. Phosphoinositides control intracellular membrane trafficking, and thus mutations may result in altered endocytic or exocytotic process or abnormal membrane transport pathways. Membrane trafficking is critical for myelin membrane biogenesis and homeostasis [66]. MTMR2 interacts with neurofilament light chain, which has an important role in axon development [67]. The phenotype is identical to that caused by mutations in MTMR13/SBF2, described below. This is presumably because MTMR2 and MTMR13 interact with each other in carrying out their phosphatase activity.

9.4. Set binding factor 2 (SBF2/MTMR13): CMT 4B2 (22q13)

Similar to MTMR2, SPF2/MTMR13 belongs to a family of proteins that regulate vesicular trafficking in Schwann cells and their loss leads to uncontrolled folding of myelin [68]. A mouse model of abnormal SPF2 developed a demyelinating peripheral neuropathy, myelin outfoldings and infoldings seen on nerve biopsies, and progressive demyelination similar to CMT 4B2 [68]. Furthermore, MTMR2 levels were also decreased in 50% of SPF2 deficient sciatic nerves, suggesting a relationship between the two proteins [68]. SPF2 and MTMR2 play an important role in the sorting and modulating of cellular signaling.

9.5. Set binding factor 1 (SBF1/MTMR5): CMT 4B3 (11p15)

Even though SBF1/MTMR5 molecular pathways have not been fully understood, it is similar in structure to MTMR2 and SBF2. SBG1 has functions similar to other myotubularin-related proteins. The current hypothesis is that SBF1 may play a role in motor cells. SBF1 interacts with MTMR2 via a coiled-coil domain and SBF1 increases the enzymatic activity of MTMR2 commanding its subcellular localization [13].

9.6. SH3 domain and tetratricopeptide repeat domain 2 (SH3TC2): CMT 4C (5q32)

CMT 4C is the most common recessive CMT in North America and Northern Europe. SH3TC2 has been found to participate in the endocytic pathway of cell trafficking and is anchored to the plasma membrane [69]. SH3TC2 is located to structures of the endocytic pathway, the clathrin coated vesicles including the trans-Golgi network, early endosomes, late endosomes, and other domains of the plasma membrane [69]. SH3TC2 is thought to be involved maintaining myelin formation via trafficking membrane components from the trans-Golgi network to the endocytic compartments [69]. Early and pronounced scoliosis is a characteristic feature of patients with CMT 4C [70].

9.7. N-myc downstream-regulated gene 1 (NDRG1): CMT 4D (8q24.3)

Mutation in NDRG1 results in a “Lom type” of CMT as Lom is the Bulgarian City where it was first described [68]. There is Schwann cell dysfunction and early axonal loss suggesting impairment of axon–glial interaction. NDRG1 plays a role in growth arrest and cell differentiation as a signaling protein shuttling between the cytoplasm and nucleus [71]. Studies have suggested that failed endosomal transport processes from abnormal Schwann cell trafficking failed to meet the demands of nerve growth, which was the likely pathogenetic mechanism in NDRG1 deficiency [68].

9.8. FIG4: CMT 4J (6q21)

FIG4 is a lipid phosphatase involved in vesicle trafficking; it removes the 5-phosphate from phosphatidylinositol-3,5-bisphosphate located on the cytoplasmic surface of vesicles of the endosome/lysosome pathway [68]. MTMR2 and FIG4 have shown to interact together in neurons and Schwann cells to control phospholipid metabolism [68]. The CMT 4J phenotype has been unusual in that some patients have had a slowly developing demyelinating neuropathy during childhood that has rapidly accelerated and resulted in death as adults. It may be that there are independent pathogenic processes occurring in Schwann cells and neurons in this unique disease [72,73].

9.9. Trk-fused gene (TFG): CMT 2G (3q12.2)

TFG was identified as part of the neurotrophic tyrosine kinase receptor type 1 in human papillary carcinoma [74]. TFG inhibition in cell lines slows protein secretion from the endoplasmic reticulum and alters endoplasmic reticulum morphology, disrupting organization of the peripheral tubules of the endoplasmic reticulum, and collapsing the endoplasmic reticulum onto the microtubule cytoskeleton. TFG forms a matrix between the endoplasmic reticulum sites and the ER-Golgi area and serves as a sorting hub for secretory material. It also provides long-term axonal maintenance [74].

9.10. Ras-related protein Rab-7 (RAB7A): CMT 2B (3q13–q22(61))

CMT 2B is caused by mutations in *RAB7* gene. Rab7 encodes Ras-associated GTP-binding protein 7, which regulates intracellular membrane vesicle led transport. If mutated, it disrupts the vesicular transport to late endosomes and lysosomes in the endocytic pathway. It causes a primarily sensory neuropathy that clinically resembles hereditary sensory neuropathy type 1 (HSN1). Mutations in Rab7 has made GTPase activity abnormal and slowed nucleotide dissociation [49]. Rab7 controls endocytic sorting by axonal retrograde transport. Rab proteins are involved in trafficking of motor proteins and vesicular trafficking as well as transportation of proteins to the cytoskeleton [75]. Rab7 is involved in lysosomal transport. Rab 7 mediates microtubule based transport of early melanosomes suggesting a link between Rab7 and cytoskeleton [49]. Rab7 and Rab5 control endocytic sorting by

axonal retrograde transport [49]. Rab7 interacting lysosomal protein (RILP) controls lysosomal transport by recruiting the dynein–dynactin motor system [76].

9.11. Dynamin 2 (DNMT2): CMTDIB/CMT 2M (19p13.2)

Mutations in DNM2, which encodes Dynamin-2 result in dominant intermediate CMT 2D and CMT 2M. Dynamin-2 helps change the cell membrane and cytoskeleton to form vesicles. Mutations in DNM2 result in altered microtubule dynamics, which is important in correcting axonal transport [4]. DNM2 seems to help assist the separation of newly formed endosomes from the cell membrane [14,77].

9.12. G protein beta-4 subunit (GNB4): CMTDIF (3q26.33)

Heterotrimeric G proteins are important for signal transduction from an activated receptor to effectors. GNB4 is widely expressed in all cell types and GB4 is thought to modulate important effector systems like N-type calcium channels, GIRK potassium channels, and phospholipase isoforms [78].

10. Mitochondria

10.1. Mitochondrial role in inherited neuropathies: MFN2, GDAP1, PDK3, AIFM1, DHTKD1, HK1

Neurons contain hundreds of mitochondria that help with a neuron's large energy demands. Mitochondria form cable-like structures along neuronal projections and have a dynamic structure and function. Mitochondria are transported up and down axons by anterograde and retrograde transport systems. They are strategically positioned in sites where ATP supply and calcium handling are required in neurons [79]. Neurons need energy for the transport of organelles and cargo along microtubules or actin; energy is also needed for maintaining ion gradients, the membrane potential with ATP-dependent calcium, ion channels, neurotransmitter-vesicle loading at presynaptic terminals, calcium mediated neurotransmitter release, and sodium–potassium pumps. Neurons depend on mitochondrial function to maintain membrane excitability and to process neurotransmission and plasticity [80].

10.2. Mitofusin-2 (MFN2): CMT 2A (1p36.22)

Mutations in MFN2 account for 20% of CMT 2 [2]. MFN2 is located in the outer membrane of mitochondria. MFN2 depletion causes CMT by impairing oxidative phosphorylation and cell bioenergetics [2]. Mitofusin 2 is necessary for mitochondria to fuse, as mitochondria need to be fused into chains before being transported by kinesin KIF1B [14,81]. MFN2 is a GTPase that is located in the outer mitochondrial membrane, and helps mediate mitochondrial fusion. MFN2 mediates the tethering of the endoplasmic reticulum and mitochondrial membranes [82]. MFN2 mutations not only cause mitochondrial fusion disruption but also severe axonal transport defects [83,84]. Disrupted axonal transport may cause CMT 2A, which ultimately results in length-dependent axonal degeneration as mitochondria need to be transported to distal axons and dendrites. Repairing the axonal transport is one therapeutic approach to treating many disorders including CMT 2A. Another approach is to try and upregulate another mitofusin, MFN1, in neurons. MFN1 is also located on the outer mitochondrial membrane, promotes fusion to other mitochondria, and has been shown to complement (overcome) MFN2 induced abnormalities in culture [85] and in mice [86].

10.3. Ganglioside induced differentiation-associated protein-1 (GDAP1): CMT 4A, CMT 2K, CMT RIA (8q21.11)

GDAP1 mutations cause CMT 4A. GDAP1 protein is expressed by neurons and Schwann cells, and the encoded protein is predicted to have two transmembrane domains and a glutathione S-transferase domain, suggesting a role in antioxidant pathways [87]. GDAP1 is a nuclear encoded gene expressed by both neurons and Schwann cells, which has a protein product localized to the mitochondrial outer membrane; it affects mitochondrial fission (rather than fusion, which is mediated by MFN2). GDAP1 activity is dependent on fission factors Drp1 and Fis1 and promotes fission without increasing the risk of apoptosis. Mutant GDAP1 can cause mitochondrial fragmentation when mutated [88]. Mutant GDAP, like MFN2, might be connected to the microtubular network in peripheral axons, which could lead to transport defects of the mitochondria [49]. GDAP1 is expressed by both neurons and Schwann cells, and it has been hypothesized that they can cause either a demyelinating or axonal early onset severe sensorimotor neuropathy [2]. Most GDAP1 mutations cause recessively inherited neuropathy but a few specific mutations can cause dominantly inherited CMT. The inheritance pattern could affect the underlying mitochondrial cellular mechanism that is impaired [88].

10.4. Pyruvate dehydrogenase kinase, isoenzyme 3 (PDK3): CMT X6 (Xp22.11)

PDK3 is an isozyme for pyruvate dehydrogenase kinase, which is located in the mitochondrial matrix and regulates the pyruvate dehydrogenase complex. It converts pyruvate to acetyl-coA, which is oxidized in the mitochondria to produce energy in the citric acid cycle. A mutation in PDK3 results in the PDK3 to be “open”, leading to increased activity that locks the pyruvate dehydrogenase complex in an inactive state building up to impaired ATP production and lactate accumulation [6].

10.5. Apoptosis-inducing factor mitochondrion-associated 1 (AIFM1): CMT X4 (Xq26.1)

The AIFM1 gene encodes for an apoptosis-induced factor that results in oxidative phosphorylation in normal healthy cells. AIFM1 is released from mitochondria and moves to the nucleus, where nuclear features of apoptosis (chromatin condensation and large-scale DNA degradation) are mediated [89]. Mutations result in increased apoptotic cells.

10.6. 2-Oxoglutarate dehydrogenase E1 component (DHTKD1): CMT 2Q (10p14)

DHTKD1 has an important role in mitochondrial energy production and nerve development. DHTKD1 encodes a mitochondrial 2-oxoglutarate-dehydrogenase-complex-like protein involved in amino acid degradation. Mutations in DHTKD1 lead to abnormal energy production (as can be seen by decreased ATP and decreased total NAD and NADH levels) [90].

10.7. Hexokinase 1 (Hk1): CMT 4G (10q22.1)

Hexokinase 1 catalyzes the first step in glucose metabolism via ATP for phosphorylating glucose to glucose-6-phosphate. It is hypothesized that mutated Hk1 alters myelin protein synthesis regulation, axonal transport, and axon–Schwann cell interactions [91].

11. Therapeutic strategies for abnormal mitochondrial function

Focusing on mitochondrial function could be an area of potential therapeutic research (given the involvement of CMT 2A with MFN2, GDAP's role with CMT 4A, and HSP27's role in CMT 2F).

11.1. Increase in MFN1: a potential therapeutic approach for MFN2

Studies have shown that both Mfn1 and Mfn2 are involved in mitochondrial fusion. Neural tissues have low Mfn1 expression and are vulnerable when Mfn2 is mutated and neither Mfn1 nor Mfn2 exist. Studies have attempted to increase Mfn1 expression in the peripheral nervous system, which would benefit CMT 2A patients. Wild-type Mfn2 can form heterooligomeric complexes to complement mutant Mfn2; thus, methods that would increase Mfn1 can be beneficial in those with CMT 2A [85].

12. mRNA processing

12.1. mRNA processing and transcription factors: GARS/YARS/MARS/AARS/KARS, MED 25, HINT1, PRPS1, PLEKHG5

RNA processing occurs before the formation of proteins during translation. Mutations in genes involving RNA processing appear to cause primarily CMT rather than a disorder of all cell processes.

12.2. Glycyl/tyrosyl/methionyl/alanyl/lysyl-tRNA synthetases: (GARS/YARS/MARS/AARS/KARS): CMT 2D (7p14)/CMTDIC (1p35)/CMT 2 (12q13.3)/CMT2N (16q22)/CMTRIB (16q23)

Aminoacyl-tRNA synthetases are vital in protein synthesis as they attach the proper amino acid to its appropriate tRNA in the cytoplasm and mitochondria. These enzymes are necessary for each cell to undergo translation and for the proper protein to be formed; CMT is caused by mutations in a number of these enzymes, which thus link protein-synthesizing complexes with neurodegeneration [92].

GARS is a glycyl-tRNA transferase that is responsible for placing glycine on the appropriate tRNA.

Missense mutations in GARS cause CMT 2D [93].

Similarly, YARS [92], MARS [32], AARS, and KARS are responsible for placing tyrosyl, methionyl, alanyl, and lysyl on the appropriate tRNA respectively. Mutations in YARS cause CMTDIC; mutations in MARS cause CMT 2. Interestingly, a *Drosophila* model of CMTDI has been created with a YARS mutation; this induced a progressive deficit of motor function with axonal degeneration [94].

AARS is involved in aminoacylation and editing function; mutations could lead to qualitative errors in neurodegeneration causing CMT 2N [95].

KARS is associated with recessive intermediate CMT (CMT RIB), and in patients with this mutation, not only do they have CMT, but they also have developmental delay, self-abusive behavior, dysmorphic features, and vestibular Schwannoma [96].

12.3. Mediator complex subunit 25 (MED 25): CMT 2B2 (19q13.33)

MED 25 is a transcriptional co-activator involved in transcriptional regulation; it is a subunit of the human activator-recruited cofactor (ARC), a family of large transcriptional coactivators related to the yeast mediator. Mutation of MED25 causes a decreased binding specificity for SH3 domain proteins, which are found in proteins of signaling pathways regulating the cytoskeleton, Ras protein, and many others [97]. MED25 gene is expressed with the PMP22 gene dosage and expression in transgenic mice and rats, which suggests the role of the protein in CMT 2B2 as well as a role in peripheral neuropathy pathogenesis [97].

12.4. Histidine triad nucleotide-binding protein 1 (HINT 1): CMT 2 (5q23.3)

The HINT1 gene encodes a purine phosphoamidase, and has been implicated as a tumor suppressor protein that is involved in apoptotic pathways [98]. In vitro, HINT1 hydrolyzes lysyl-AMP that is generated by lysyl-tRNA synthetase [98]. Maintenance and function of HINT1 is important for peripheral nerve physiology but the exact mechanism is still in question; there is some suggestion that HINT 1 is linked with transcriptional regulation and RNA metabolism [97]. HINT1 also could modulate the B-catenin transcriptional activity, and HINT1 mutations could also lead to toxic metabolites [98]. Patients with HINT mutations appear to originate largely from eastern European populations [68].

12.5. Phosphoribosyl pyrophosphate synthetase 1 (PRPS1): CMT X5 (Xq22.3)

PRPS1 is a member of the PRPS gene family; it is expressed in all human cells including the cochlea. It mediated the critical biochemical step for purine metabolism and nucleotide biosynthesis [99]. Mutations result in CMTX5, which includes a severe axonal neuropathy, peripheral neuropathy, hearing loss, and optic neuropathy [99].

12.6. Pleckstrin homology domain-containing protein, family G, member 5 (PLEKHG5): CMT RIC (1p36.31)

PLEKHG5 mutations cause RICMTC as it was found that if mutated it had a defect in activating the NF-κB signaling pathway, which is involved in DNA transcription. NF-κB responds to multiple stresses (cytokines, free radicals, bacterial or viral antigens) and plays a key role in regulating the immune response to viral infection. Incorrect regulation of NF-κB can lead to peripheral neuropathy as well as cancer, inflammation, autoimmune disease, and improper immune development. PLEKHG5 is expressed in the peripheral nervous system, and contains a motif known as the minimal unit for the nucleotide exchange-promoting function of guanine nucleotide exchange factors [100]. It is involved in neuronal cell differentiation [100]. Neurotoxicity could be due to the loss of function in the NF-κB transduction pathway and aggregate formation of mutant PLEKHG5 [100].

12.7. Early growth response 2 (EGR2): CMT 4E, CMT 1D (10q21.3)

EGR2 (historically termed KROX-20) is a zinc finger transcription factor, which is a regulator of Schwann cell differentiation and myelin gene expression. EGR2 transcription factor is critical for peripheral nerve myelination. Expression of EGR2 in Schwann cells increases the expression of myelin related genes such as MPZ, PMP22, GJB1, and PRX [101]. EGR2 mutants dominant-negatively inhibit wild-type EGR2 mediated expression of essential myelin genes to levels low enough that result in abnormal myelination and result in CMT 4E and CMT 1D [101].

13. Channel

13.1. Neuronal channel: TRPV4A, GJB1

Transmembrane ion channels allow ions to move into or out of cells. Neuronal channels allow for neurotransmission by changing the polarization of the neuronal membrane. Neuronal ion channel activation can occur in a voltage- or ligand-gated manner. Each channel is usually selective for one ion type (sodium, calcium, potassium, chloride).

13.2. Gap junction beta-1 protein or connexin 32 (GJB1 or Cx32): CMT 1X (Xq13)

Cx32 is encoded by GJB1; mutations of GJB1 can result in loss of function mutations and dysfunctional gap junctions that disrupt communication between Schwann cells and neurons, all leading to CMT 1X [102]. CMT 1X is the second most frequent form of CMT, affecting about 15% of all patients with CMT [103]. Cx32 is located in non-compact myelin and forms functional channels, allowing for rapid transport of ions between two cells via radial migration of the myelin layers [2,104]. Six connexins form a hemichannel and two

hemichannels form a gap junction. Cx32 is seen in paranodal loops of myelinating Schwann cells and helps form gap junctions between the myelin sheath; the gap junctions are aligned radially, thus making a shorter pathway than a circumferential route [105]. A disruption of the radial pathway of the gap junctions could result in demyelination. Mutant Cx32 in the endoplasmic reticulum or Golgi could affect intracellular trafficking and trigger cellular clearance responses [106]. Recent studies have found that intracellular trafficking, abnormal synthesis, and loss of gap junction function are the main alterations of the majority of CMT 1X mutations [107].

There are more than 400 different CMT 1X GJB1 mutations, which affect different aspects of the Cx32. The clinical manifestations, however, are more homogenous with late childhood onset and more axonal loss than CMT 1A. The clinical manifestations in women vary depending on the number of myelinating Schwann cells that make the X chromosome with the mutant allele inactive [108].

13.3. Transient receptor potential cation channel of the vanilloid-type member 4 (TRPV4A): CMT 2C (12q 23–24)

TRPV4 is responsible for encoding a calcium permeable nonselective channel that plays a role in regulating systemic osmotic pressure. Mutant TRPV4 could cause increased calcium influx and calcium overload, which could be deleterious to axonal transport [109,110].

14. Therapeutic approaches (other)

14.1. Trophic factors and CMT

Neurotrophins are growth factors; they have the ability to promote development of both neurons and glia; many of the trophic factors are expressed in Schwann cells and levels are altered in nerve injury [14]. The trophic factors include NGF (nerve growth factor), BDNF (brain derived neurotrophic factor), neurotrophin-3 (NT3) and neurotrophin-4/5. Each is secreted as a homodimer, and then cleaved by a protease. Neurotrophins bind to a protein Trk receptor that activates downstream signaling pathways in the target cell. Some studies have shown that trophic factors such as NT3 may be beneficial in the future; NT3 has been reported to promote nerve regeneration after injury and the survival of Schwann cells [111]. Therapeutic application of NT3 in immune incompetent mice of sural nerve biopsies from patients with CMT 1A promoted axonal regeneration [112]. In eight patients with CMT 1A, NT3 treatment was tolerated three times a week over six months. Patients showed an increase in myelinated fiber density, a reduction in the neurological impairment score and improved sensory modalities when compared with the controls [112]. Future larger studies need to be performed to confirm results. Overall results, however, have been disappointing as attempts to halt diseases such as familial ALS have been unsuccessful. This may be due to methods of delivery and short half-lives of trophic factors (only several minutes) [14].

14.2. Schwann cell and axonal interaction treatment strategies

Strategies to treat the Schwann cell and axonal interaction abnormality include providing trophic factor support, the use of potassium channel blockers (3,4 diaminopyridine) to prevent potassium ions from leaking out of the axon during depolarization, sodium channel blockers (which would prevent the neuron from overworking by inhibiting the sodium channels to depolarize the axon in the generation of the axon potential), and manipulating signaling pathways between the Schwann cell and axon. The latter could be done using the adaxonal internode and the paranodal region of the myelinating Schwann cell. Furthermore, Nrg1, which belongs to the epidermal growth factor family regulates myelin thickness, and could potentially be used to manipulate myelin thickness in the future [14]. Treatment strategies are being targeted to develop a method to regulate PMP22 mRNA levels [14].

14.3. Stem cells

Stem cells could potentially develop into Schwann cells or neurons, but it would be difficult to have stem cells differentiate into neurons, generate into axons and then travel down a limb. Stem cells, however, could be used as a trophic support for inherited neuropathies (like NT3) [14]. In certain studies of motor neuron disease and elsewhere, stem cells have the potential to provide trophic support of mesenchymal stromal cells. Stems cells can also result in transdifferentiation; a study has shown that mouse fibroblasts and hepatocytes can transdifferentiate into neurons [113]. Studies taking advantage of this approach are currently ongoing.

14.4. Gene replacement

Gene replacement could be done with mutant genes causing neuropathy by a simple loss of function of the normal gene. Autosomal recessive forms of CMT such as CMT 4, for example, could be cured with simple gene replacement as there is a simple loss of function of the normal gene. A major challenge for this type of approach is being able to specifically target adequate numbers of Schwann cells or neurons to make this approach feasible. This approach could also be considered for dominant CMT caused by clear loss of function mutations such as in HNPP. Other targets for gene replacement are nonsense mutations that can result in a premature termination of the mutant protein in which mRNAs are degraded [14,114].

14.5. Cellular reprogramming and high-throughput drug screening

Cellular reprogramming can be used to search for compounds to treat CMT; cellular reprogramming generates specific cell types (such as stem cells, neurons, or glia) by genetically modifying somatic cells such as fibroblasts or lymphocytes [115]. Using this technique, research can generate supplies of patient specific cell lines used for mechanistic studies and drug development [116]. These cell lines will be useful when combined with high-throughput screening of drug libraries containing thousands of these compounds. The process of identifying compounds that are capable of correcting certain disease related cell phenotypes is created in a way for a faster target selection of compounds to be tested in phase 1 animal studies.

15. Conclusion

Although neuromuscular disorders such as CMT have been challenging to treat, the abundance of knowledge regarding genes, proteins associated with genetic mutations, and specific cellular functions of these proteins will further the advancement of therapeutic strategies and drug trials in the future.

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