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REVIEW

Axonal Charcot-Marie-Tooth Disease: from Common Pathogenic Mechanisms to Emerging Treatment Opportunities

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Abstract

Inherited peripheral neuropathies are a genetically and phenotypically diverse group of disorders that lead to degeneration of peripheral neurons with resulting sensory and motor dysfunction. Genetic neuropathies that primarily cause axonal degeneration, as opposed to demyelination, are most often classified as Charcot-Marie-Tooth disease type 2 (CMT2) and are the focus of this review. Gene identification efforts over the past three decades have dramatically expanded the genetic landscape of CMT and revealed several common pathological mechanisms among various forms of the disease. In some cases, identification of the precise genetic defect and/or the downstream pathological consequences of disease mutations have yielded promising therapeutic opportunities. In this review, we discuss evidence for pathogenic overlap among multiple forms of inherited neuropathy, highlighting genetic defects in axonal transport, mitochondrial dynamics, organelle-organelle contacts, and local axonal protein translation as recurrent pathological processes in inherited axonal neuropathies. We also discuss how these insights have informed emerging treatment strategies, including specific approaches for single forms of neuropathy, as well as more general approaches that have the potential to treat multiple types of neuropathy. Such therapeutic opportunities, made possible by improved understanding of molecular and cellular pathogenesis and advances in gene therapy technologies, herald a new and exciting phase in inherited peripheral neuropathy.

Keywords Charcot-Marie-Tooth disease · Axonal neuropathy · Peripheral neuropathy · Pathogenesis · Treatment

Introduction

Inherited peripheral neuropathies are a diverse group of disorders caused by dominant, X-linked, recessive, or maternal (mitochondrial) mutations in hundreds of different genes. In addition to their mode of inheritance, each kind of inherited neuropathy also differs according to whether demyelination or axonal loss is the primary pathogenic event, and whether the neuropathy is the principal manifestation of the disorder. There are more than 200 inherited syndromes in which a neuropathy is typically present but overshadowed by other aspects of the disorder; these have been given many different names [1, 2]. When neuropathy is the singular manifestation of the disease, it usually called Charcot-Marie-Tooth disease (CMT). Dominant and recessive demyelinating forms are classified as CMT type 1 (CMT1) and type 4 (CMT4), respectively; dominant and recessive axonal forms are classified as CMT type 2 (CMT2) and autosomal recessive CMT2 (AR-CMT2), respectively [3]. Sensory (with variable autonomic involvement) and motor inherited axonal neuropathies also occur and are usually grouped into hereditary sensory and autonomic neuropathy (HSAN) and hereditary motor neuropathy (HMN), respectively. Finally, mutations in many different genes can cause similar phenotypes, and different mutations in the same gene can cause different phenotypes on an apparent continuum with different patients manifesting variable degrees of sensory and/or motor dysfunction. Together, CMT (including HMN and HSAN) comprises the most common inherited neurological disease [4, 5].

Length-dependent, progressive, sensory and/or motor dysfunction, beginning first in the distal lower limbs and later involving the distal upper limbs is the usual manifestation of CMT and is thought to result from progressive,

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length-dependent loss of axons [6]. The loss of motor axons results in foot drop and weakness of the intrinsic hand muscles; the loss of sensory axons results in sensory loss in the feet and lower legs and impaired balance. The severity of these impairments can be highly variable, even among patients with the same pathogenic variant in a given gene. The heterogeneity of clinical presentation is not well understood — it may owe to the underlying genetic defect along with genetic susceptibility factors [7] and possibly environmental contributors.

Over the past two to three decades, the genetic landscape of CMT has exploded, with identification of more than 100 different genes, and a steady discovery of new ones [8]. The expansion of genetic defects has been accompanied by experimental evidence illuminating the pathological consequences of these mutations. In CMT1 and CMT4, the causative genes are expressed by myelinating Schwann cells, and their mutations result in dysmyelination or demyelination [9]. The pathogenesis and treatment opportunities for CMT1 are discussed in a separate article in this special issue (Volume 18, issue 4). In CMT2, AR-CMT2, HSAN, and HMN, the causative genes are expressed by PNS neurons, and their mutations lead to axonal degeneration. The relevant functions of these genes are diverse, but at least some genes that cause CMT2 share common pathophysiological mechanisms, which, along with their therapeutic implications, are the subject in this review.

Pathogenesis

In this section, we highlight pathways that are affected by genes whose mutations result in axonal neuropathy. While most of the causative genes in CMT2 encode proteins expressed widely (beyond peripheral sensory and motor neurons), manifestations in other organ systems and even other parts of the nervous system, such as brain and spinal cord, are almost uniformly absent. The basis for this striking peripheral nervous system specificity is not yet understood for the majority of CMT2 forms but likely hints at unique vulnerabilities of peripheral neurons to specific molecular and cellular insults. There also exist numerous examples of hereditary multisystem diseases in which peripheral neuropathy is one of many manifestations, and many of the causative mutations in these multisystem conditions disrupt pathways that are commonly involved in CMT2. These examples further highlight some of the specific metabolic and molecular vulnerabilities of neurons of the peripheral nervous system.

Axonal and Endosomal Trafficking Defects

Axons present unique challenges to the preservation of homeostasis. The volume of a neuronal cell body is dwarfed by the mass of its axon, which can be as long as 1 m. Moreover, the metabolic and energetic support required to maintain the electrochemical gradients that underlie neurotransmission is substantial. Due to the unique demands of axonal physiology, neuronal homeostasis and survival are critically dependent on long-range transport of various cargoes between the cell body and most distal axonal segments. Thus, it is not surprising that many forms of inherited neuropathy are caused by mutations in genes that are involved in axonal transport. Axonal transport is an energy-dependent and highly regulated process of directed movement of various organelles and essential cargo, including mRNA transcripts, translation machinery, and trophic factor signaling complexes, along axonal microtubules [10]. Microtubules are highly dynamic polymers of α and β tubulin dimers that run the length of the axon and serve as tracks for molecular motor protein complexes of the dynein and kinesin families. Transport toward the distal end of the axon is termed "anterograde" and is accomplished by binding of cargo to kinesin motors, while transport toward the cell body is termed "retrograde" and involves dynein motors and the cytoplasmic dynein adaptor protein complex. Diversity of molecular motors as well as motor adaptor proteins imparts specificity to the axonal transport of specific cargoes [11]. In contrast to other cell types, axons contain a large population of long-lived and stable microtubules, and their stability can be regulated by various post-translational modifications, such as reversible acetylation of α tubulin [12].

Genes involved directly with microtubule transport include *BICD2* (which binds directly to the retrograde microtubule motor protein complex cytoplasmic dynein and is mutated spinal muscular atrophy lower extremity-predominant; SMALED) [13–15], *RAB7* (which regulates microtubule trafficking of lysosomes, late endosomes, and autophagosomes within axons and is mutated in CMT2B) [16], *DCTN1* (which is incorporated into the cytoplasmic dynein motor complex and is mutated in distal HMN7B) [17], *DYNC1H1* (which is also part of the cytoplasmic dynein complex and is mutated in CMT2O, hereditary spastic paraplegia (HSP), and SMALED) [18, 19], *KIF1A* (which is an anterograde kinesin motor protein and is mutated in HSAN2C) [20], and *KIF5A* (another anterograde kinesin motor mutated in CMT2, HSP, and amyotrophic lateral sclerosis, ALS) [21–23] among others. While often not directly demonstrated experimentally, mutations in these genes are assumed to result in some measure of axonal transport defects. As an example, human *DYNC1H1* mutations as well as mutations found in so-called *Loa* and *Cra* mice (legs at odd angles and cramping), which harbor distinct missense mutations in *Dync1h1*, all show reduced motor processivity and slowed retrograde axonal transport [18, 24], directly linking

defective transport with peripheral nerve degeneration. In addition, mutations in mitofusin-2 (*MFN2*), which cause CMT2A, likely impact mitochondrial trafficking within axons, among other aspects of mitochondrial biology, as discussed in more depth below. Moreover, mutations in axonal trafficking proteins not only cause forms of CMT, but are found in some genetic syndromes in which peripheral nerve degeneration is a feature. For example, mutations in the microtubule-severing protein spastin (*SPAST*) cause HSP, and mutations in the tubulin chaperone *TBCE* and tubulin isoforms *TUBB2A* and *TUBB3* are mutated in complex syndromic conditions that included axonal neuropathy [2].

Notably, many acquired forms of peripheral neuropathy are also associated with axonal transport defects. Chemotherapy-induced peripheral neuropathy (CIPN) is a prime example, as multiple causative agents either directly or indirectly impact microtubule transport [25, 26]. The chemotherapeutic taxanes, such as paclitaxel and docetaxel, and vinca alkyloids, such as vincristine and vinblastine, interfere with microtubule assembly or disassembly and frequently cause dose-related sensory-predominant axonal neuropathy. These agents directly impair either anterograde and/or retrograde microtubule transport within peripheral sensory neurons [27, 28], which is likely central to the pathogenesis of CIPN [25]. Notably, patients with CMT1A appear to be particularly vulnerable to chemotherapy-induced neuropathy, with descriptions of severe and rapid quadriplegia due to vincristine [29, 30].

Mitochondrial Dynamics

One vitally important cargo transported through axons are mitochondria, which are normally maintained throughout the axonal compartment but are enriched at highly metabolically active sites, including at the axon initiation segment, nodes of Ranvier, and distal terminals such as sensory nerve endings and motor neuron neuromuscular junctions [31]. Proper mitochondrial function requires precise regulation of mitochondrial dynamics—the complex and dynamic processes of mitochondrial fission and fusion as well as trafficking along microtubules. In addition, mitochondria undergo transient interactions with adjacent organelles, including endoplasmic reticulum (ER), late endosomes, and lysosomes, and these contacts are important for regulation of mitochondria function. Thus, mitochondrial homeostasis is dependent on properly regulated transport, fusion, fission, and contacts with other organelles, and genetic and biochemical evidence suggests that perturbation of each of these components of mitochondrial dynamics can contribute to axonal dysfunction in neuropathy.

Mutations in *MFN2*, a mitochondria-associated dynamin-like GTPase involved in multiple aspects of mitochondrial dynamics, are the best example of how mitochondrial dysfunction can lead to axonal pathology. Dominant *MFN2* mutations cause CMT2A, which is the most common form of CMT2, accounting for ~ 20% of all cases and 90% of severe cases [32–35]. The clinical manifestations of different *MFN2* mutations range from severe, infantile-onset to mild, adult-onset neuropathy. Recessive *MFN2* mutations also occur, and these typically cause a severe, early-onset axonal neuropathy [35]. MFN2 resides within the mitochondrial outer membrane via two transmembrane domains and contains a GTPase domain as well as two coiled-coil heptad repeat regions, designated HR1 and HR2. The HR2 domains from MFN2 proteins on neighboring mitochondria undergo antiparallel interactions in *trans* that serve to tether adjacent mitochondria, thereby promoting mitochondrial fusion [36]. MFN2 also supports mitochondrial trafficking through interactions with Miro1, which connects mitochondrial membrane-bound MFN2 to Trak1 and microtubule motor complexes [37].

In addition to MFN2, mutations in other genes involved in mitochondrial dynamics are linked with inherited neuropathies, including ganglioside-induced differentiation-associated protein-1 (*GDAP1*) and optic atrophy-1 (*OPA1*) [38–41]. Like MFN2, GDAP1 localizes to the outer mitochondrial membrane, but unlike MFN2, it interacts with mitochondrial fission machinery. Most *GDAP1*-associated neuropathy is due to biallelic mutations in *GDAP1*, causing forms of AR-CMT2, but dominant mutations also occur (designated CMT2K) and generally cause less severe disease [38]. OPA1 is likewise involved in mitochondrial fission as well as other aspects of mitochondrial biology, and dominant mutations appear to lead to loss of function. The clinical presentation in patients with *OPA1* mutations is dominated by progressive optic atrophy and is thus not classified as CMT, but about 20% of patients display additional clinical features including hearing loss and peripheral neuropathy [42]. Notably, mutations in the inner mitochondrial membrane protein *OPA3* also cause optic atrophy and neuropathy [43], and some patients with *MFN2* mutations also develop optic atrophy [35].

While mutations affecting mitochondrial dynamics most often cause forms of CMT2, mutations that impair mitochondrial energy production or disrupt mitochondrial DNA stability cause multisystem disease in which peripheral neuropathy is one of many manifestations [44]. Such mitochondrial diseases can be caused by dominant, recessive, or X-linked mutations of nuclear genes, or through mutations in mitochondrial DNA, resulting in matrilineal inheritance. Additional clinical features in mitochondrial diseases are variable, but often include visual disturbances (as in optic atrophy plus due to mutations in *OPA1* and *OPA3*, and NARP (neuropathy, ataxia, and retinitis pigmentosa) due to mutations in *MTATP6*), gastrointestinal disturbances (as

in MNGIE (mitochondrial neurogastrointestinal encephalopathy) due to *TYMP* mutations), ataxia (as in SANDO (sensory ataxic neuropathy, dysarthria, and ophthalmoparesis) due to mutations in *POLG*), myopathy (as in MERRF (myoclonic epilepsy with ragged red fibers) due to mutations in MT-TK and MELAS (mitochondrial encephalopathy with lactic acidosis and stroke-like episodes) due to mutations in MT-TL1), and other CNS manifestations (seizures in MERRF, encephalopathy in MNGIE and MELAS) [44, 45]. Thus, peripheral nerves appear to be particularly vulnerable to defects in mitochondrial dynamics and mitochondrial energetics.

The study of defects due to pathogenic *MFN2* mutations provides a window into the myriad mechanisms by which mitochondrial dynamics can contribute to axonal pathology. The majority of pathogenic *MFN2* mutations affect amino acid residues within or near the N-terminal GTPase domain, with few mutations affecting the C-terminal region of the protein [46]. In vitro, most mutations lead to dominant-negative effects that impair mitochondrial fusion, thereby leading to mitochondrial clustering [47] and ultimately, defective mitochondrial quality control [48]. Disease mutations also impair long-range axonal transport and distribution of mitochondria [49–51], which could explain the particular vulnerability of the most distal portions of peripheral axons to degeneration in CMT2A. In addition, MFN2 function in facilitating mitochondrial-ER contacts may also be altered by neuropathy mutations [52]. This molecular understanding of CMT2A pathogenesis presents multiple opportunities for treatment as discussed below.

Messenger RNA Processing and Protein Translation

The discovery of multiple aminoacyl-tRNA synthetase (ARS) genes as causes of dominantly inherited axonal neuropathies was completely unexpected. The original identification of dominant mutations in glycyl-ARS (*GARS*) as the cause of CMT2D [53] was followed by reports of dominant mutations in *AARS*, *HARS*, *KARS, MARS*, *WARS*, and *YARS* [54–58]. ARS genes encode enzymes that catalyze the charging of tRNAs with the appropriate amino acid, thus playing a critical role in protein translation in all tissues [59]. Inherited neuropathies caused by mutations in ARS genes are classified as HMN or CMT2 and generally present with typical length-dependent sensory and/or motor axonal neuropathy.

The pathogenesis of ARS-related CMT2 remains unresolved, with multiple potential pathological mechanisms. Initial suppositions placed impairment of enzymatic activity as the most likely causative disruption in ARS mutations. Indeed, many disease mutations have been shown to disrupt tRNA charging function in vitro and in yeast complementation experiments [56, 60, 61]. However, impairment of enzymatic function is not universal among disease mutations, suggesting that other mutation-dependent effects contribute to pathology [54, 59, 60, 62, 63]. There is also evidence for disrupted dimerization and neomorphic binding activities of disease-causing ARS mutant proteins [62, 64, 65]. An alternative mechanism of pathogenesis was suggested by elegant studies in a *Drosophila* model of disease-causing ARS mutations [66]. Expression of mutant GARS or YARS caused motor and sensory degeneration that was associated with global reductions in protein translation rates. The impairment of global translation by mutant GARS could not be rescued by overexpression of wild-type GARS, indicating that the mutant protein had a toxic gain of function. Furthermore, genetic inhibition of translation could recapitulate some of the degenerative phenotypes due to expression of GARS or YARS mutants, demonstrating that impaired protein translation is sufficient to cause neuronal degeneration in flies [66]. Together, these studies hint at the possibility that disruption of translation and activation of downstream cellular stress pathways may provide a unifying explanation for how various ARS mutations lead to axonal degeneration. As discussed below, impairment of intra-axonal protein synthesis may also be relevant in other forms of acquired and inherited neuropathy.

Organelle-Organelle Contacts

With advancements in high-resolution microscopy has come an increasing recognition of the highly dynamic and interconnected nature of intracellular organelles. Rather than existing as discrete and disconnected biological compartments, organelles undergo extensive physical and functional interactions [67, 68]. Organelle-organelle contacts have been described to occur between mitochondria, lysosomes/late endosomes, and ER, among others. These contacts occur both within non-neuronal cells and within peripheral nerve axons. Furthermore, new evidence continues to demonstrate important functional consequences for these highly fluid and often transient interactions [69]. CMT2 disease genes have emerged as critical regulators of organelle-organelle contacts, potentially providing a previously unrecognized pathophysiologic connection among disease-associated pathways thought to be distinct and unrelated.

Studies of organelle-organelle contacts have revealed important roles for both MFN2 and the small GTPase Rab7 in regulating these dynamic processes. MFN2 plays a critical role in mediating contacts between ER and mitochondria, which occur at mitochondria-associated membrane regions and may facilitate transfer of Ca^{2+} from ER to mitochondria [70]. The formation and dissolution of ER-mitochondria contacts are regulated by MFN2 homotypic and heterotypic binding events, and CMT2A mutations alter the dynamics of these contacts with resultant ER stress [71].

Interestingly, the small GTPase Rab7, which is mutated in CMT2B, has also been implicated in regulating organelle-organelle contacts between mitochondria and lysosomes [72]. Dominant *RAB7* mutations cause a severe sensory-predominant neuropathy with ulcero-mutilating features that are phenotypically similar to patients with congenital insensitivity to pain [16]. Rab7 localizes to late endosomes, lysosomes, and autophagosomes, where it recruits various effector proteins, including the retrograde microtubule motor cytoplasmic dynein [73–75]. Rab7 has been shown to support long-range axonal trafficking of its target organelles, and disease mutations appear to confer dysregulation of Rab7 activity and resultant gain of function [76, 77]. Recent work has shown that Rab7 plays a critical role in lysosome-mitochondria contacts, with Rab7 GTPase activity being particularly important for promoting untethering of lysosomes from mitochondria [72]. Surprisingly, lysosomes can serve as important platforms for the transport of RNA granules along axons, a process that is required to support delivery of mRNA for local axonal protein translation [78]. In addition, lysosome-mitochondria tethers are sites of local protein translation, and disruption of these contacts impairs local translation as well as mitochondrial morphology. Strikingly, Rab7 disease mutations impair axonal protein translation on late endosomes and lysosomes and disrupt mitochondrial morphology and trafficking [78]. Thus, Rab7 and MFN2 have been unexpectedly linked to common pathways involving axonal transport of RNA granules and intra-axonal protein translation.

In addition to CMT, the underlying genetics in HSP provide further evidence for the importance of organelle-organelle contacts in axonal biology. HSP is primarily a distal axonopathy of CNS spinal cord projecting neurons that results in progressive lower extremity spasticity and weakness [79]. Variable peripheral sensory and motor involvement can also occur, resulting in phenotypic overlap with CMT2. Much like CMT, HSP is genetically diverse, with over 80 genetic loci resulting in either dominant, recessive, or X-linked disease. However, a substantial fraction of HSP genes are directly implicated in various aspects of ER membrane dynamics, including ER-organelle contacts and ER membrane bending and tubulation [80, 81]. Mutations in ER-shaping proteins are particularly common, with mutations in spastin (*SPAST*), atlastin-1 (*ATL1*), reticulon 2 (*RTN2*), and *REEP1*, accounting for around half of all HSP cases [82]. The mechanisms by which HSP-causing mutations alter ER membrane dynamics and function are diverse, but serve to further highlight the vulnerability of long axons to disruption of organelle membrane formation and maintenance [81].

With the finding that CMT2-causing mutations in various amino-acyl-tRNA synthetases lead to suppression of global translation that is particularly detrimental to neuronal homeostasis, a pattern emerges suggesting that many distinct forms of CMT may share the common pathological insult of impaired axonal protein translation. Disruption of local translation within axons can result from a range of primary pathological insults in various forms of CMT2, such as impaired axonal trafficking, disrupted organelle tethering, or failure of RNA granule delivery (Fig. 1). Further highlighting these pathways, there is a growing body of evidence linking impaired local protein translation to the pathogenesis of spinal muscular atrophy and ALS [83]. Furthermore, the microtubule-disrupting chemotherapeutic agent paclitaxel may lead to axonal neuropathy in part through impaired delivery of mRNA to distal axonal compartments [84]. This confluence of neuropathy-related genes onto pathways involved in microtubule transport, mitochondrial energetics, organelle-organelle interactions, and local protein translation underscores the delicate nature of axonal physiology, particularly in the longest peripheral nerves, and suggests that multiple perturbations of metabolic processes can expose the unique vulnerability of this highly specialized portion of the nervous system. However, from an optimistic perspective, these pathophysiological connections may provide the potential to uncover therapeutic targets that could be relevant for multiple forms of CMT, and perhaps other forms of neuromuscular degenerative diseases.

Treatment

General Considerations

Treatment strategies fall broadly into two categories — "specific" therapies that target a unique aspect in the pathogenesis of a subtype of neuropathy and "general" therapies that utilize an approach that can be readily applied and/or adapted to multiple neuropathy subtypes. In either case, treatment efforts rely on the underlying assumption that the pathogenic mechanisms of axonal CMT of varied causes are related to cell-autonomous dysfunction conferred by a specific genetic defect in peripheral motor and/or sensory neurons. The nature of the genetic defect largely dictates the therapeutic possibilities for a given form of axonal CMT. For instance, dominant mutations that cause disease either through a gain-of-function or dominant-negative effect can be targeted by therapeutic strategies that silence the mutant allele, inhibit the mutant protein function, or suppress downstream consequences of the mutant protein activity. For dominant-negative mutations, an additional possibility is "overwhelming" the mutant protein through supplementation of expression of the wild-type protein. On the other hand, recessive/loss-of-function forms of CMT should be amenable to gene replacement strategies.

Some specific CMT subtypes have received particular attention with respect to clinical and translational efforts

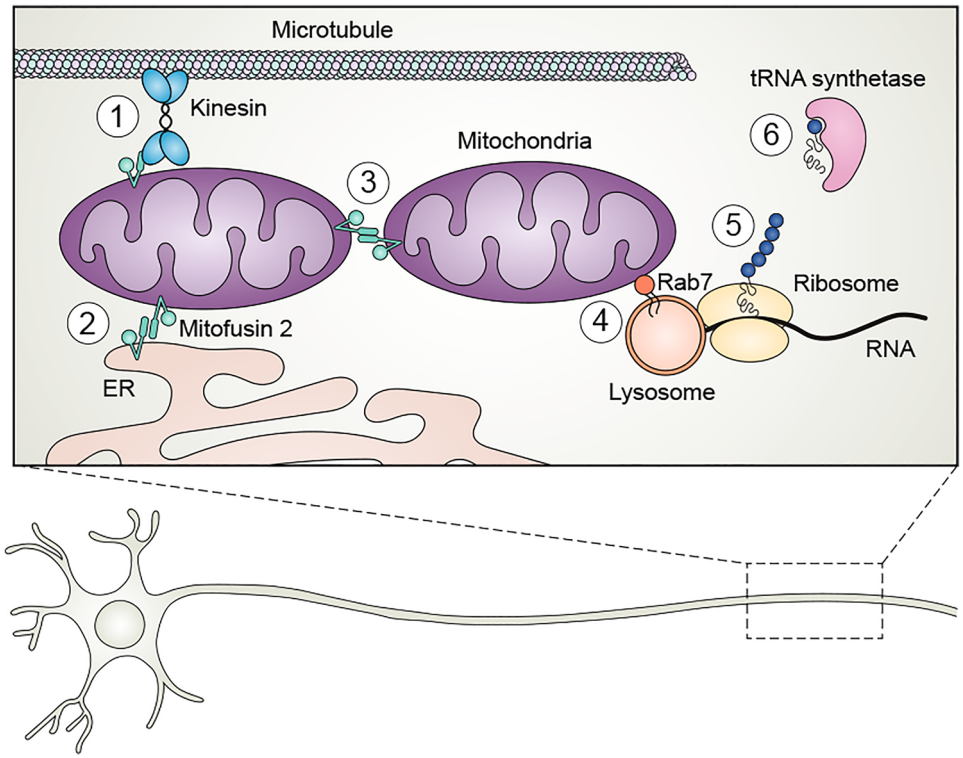

Fig. 1 The confluence of axonal neuropathy genes in the regulation of axonal transport, organelle-organelle contacts, and local protein translation. Schematic representation of multiple gene products and associated pathways implicated in inherited axonal neuropathy. (1) Mitofusin 2 (MFN2) regulates mitochondrial axonal transport through recruitment of adaptor proteins and microtubule transport machinery such as kinesin motors. (2) MFN2 regulates mitochondrial-ER contacts. (3) MFN2 regulates mitochondrial tethering and fusion. (4) Rab7 regulates late endosome and lysosome axonal transport and contacts with mitochondria. (5) Rab7-positive late endosomes and lysosomes promote long-range axonal transport of RNA granules and protein translation machinery to support local axonal protein translation. (6) Aminoacyl-tRNA synthetases charge tRNAs to support axonal protein synthesis

for several reasons. First, CMT subtypes that are more common and thereby affect a larger fraction of the patient population, such as CMT2A, have received more consideration. Second, increased disease prevalence also impacts an often overlooked but critical facet of the therapeutic development pipeline: the identification of sufficient numbers of patients for detailed natural history studies. These studies provide longitudinal clinical information that is fundamental to multiple aspects of clinical trial design, including the identification of relevant endpoints and biomarkers, calculation of statistical power, and choice of patient subgroups most likely to demonstrate therapeutic efficacy. Third, both basic and translational researches have been dramatically aided in CMT types for which validated and robust animal models have been developed. While generation of an animal model that recapitulates important features of dominant, monogenic human disease may seem straightforward, the realities of modeling length-dependent, slowly progressive axonal neuropathies in small rodents have frequently proved challenging. Still, many robust CMT2 mouse models have now become available [85], and rat models are increasingly being generated, sometimes with improved recapitulation of the human disease phenotypic features [86, 87]. Fourth, with the gradual progression of most forms of CMT, the identification of biomarkers of disease activity and therapeutic target engagement can greatly enhance the power to detect a biological effect at an early stage. In the sections below, we discuss specific examples of genetic axonal neuropathies that are likely to be on the leading edge of therapeutic development and clinical trial initiation.

Specific Approaches for Individual Neuropathies

For many forms of axonal CMT, understanding of the specific genetic defects and molecular pathogenesis make treatments directed at the underlying pathology at least theoretically possible. Likely the most straightforward pathway for translation from molecular pathogenesis to therapeutics relates to inherited neuropathies due to metabolic defects. In these forms of neuropathy, knowledge of the affected metabolic pathway suggests therapeutic opportunities to reduce accumulation of toxic intermediates or to otherwise

address enzymatic deficiencies. HSAN1 and SORD-related neuropathy are illustrative cases of metabolic neuropathies that are potentially treatable. In other specific CMT2 forms, understanding of the distinct mechanisms of pathogenesis has led to identification of small molecules that may have therapeutic benefit; CMT2A and TRPV4 neuropathy are examples. In addition to these specific examples, there are several genetic axonal neuropathies, many of which are not classically defined as CMT, which can be viewed as potentially treatable. These inherited neuropathies, which have either some experimental evidence for potential efficacy of correcting a metabolic defect, or an animal model demonstrating proof of principle for a specific therapeutic intervention, are presented briefly in Table 1.

HSAN1

HSAN1 is caused by dominant missense mutations in either *SPTLC1* or *SPTLC2*, which encode subunits of the serine palmitoyl transferase (SPT) enzyme that functions in production of sphingolipids [110, 111]. Patients develop a sensory and motor neuropathy with predominantly axonal features, and also develop autonomic dysfunction. Sensory loss can be profound, leading to recurrent painless injuries that can be complicated by ulceration and osteomyelitis [112]. Disease-causing mutations decrease the affinity of SPT for its normal substrate, L-serine, and increased affinity for alanine and glycine, leading to production and accumulation of atypical, toxic sphingolipids [113]. Administration of L-serine in a mouse model of HSAN1 led to reduction in toxic sphingolipids as well as behavioral and histological improvements [114], and a small human clinical trial also showed reduction in toxic sphingolipid accumulation and a hint of clinical benefit [115]. While the trial size (18 patients) and short follow-up (2 years) limited the power to detect significant clinical impact, these results demonstrate successful targeting of sphingolipid accumulation and warrant further study.

SORD Neuropathy

The recent identification of recessive mutations in *SORD* represents a promising therapeutic opportunity [116]. SORD neuropathy is a motor-predominant, axonal neuropathy, [116], and may be the most common cause of recessively inherited peripheral neuropathy. *SORD* encodes sorbitol dehydrogenase, the second step in the polyol pathway that enzymatically converts glucose to fructose. In this pathway, aldose reductase first converts glucose to the poorly metabolized sugar sorbitol, and then SORD catalyzes the conversion of sorbitol to fructose. Loss of SORD activity results in accumulation of sorbitol, which has previously been implicated as a potentially neurotoxic species in diabetic neuropathy [117]. *SORD* mutations that cause neuropathy produce loss of function, either by introducing premature stop codons or by disrupting amino acid residues critical for enzymatic activity. Accumulation of sorbitol was demonstrated in patient serum and patient-derived cells as well as in a *Drosophila* model. Deletion of the fly SORD ortholog resulted in degenerative phenotypes that could be reversed by FDA-approved medications that inhibit aldose reductase (epalrestat and ranirestat) and thereby reduce sorbitol accumulation. These results have led to ongoing efforts to identify additional patients and better define the natural history of SORD neuropathy in preparation for a human clinical trial.

CMT2A

In contrast to SORD neuropathy, in which gene discovery suggested an obvious therapeutic approach, the path toward a rational therapeutic for CMT2A required hard-earned insights into molecular pathogenesis of disease-causing mutations. As described above, MFN2 has several functions — mitochondrial transport, tethering to organelles, fusion, and homeostasis. Pathogenic mutations likely act in a dominant-negative fashion, thereby impairing one or more of these normal functions [47, 50, 118]. Importantly, MFN2 is highly homologous to MFN1, and studies from various experimental systems have demonstrated that MFN1 and MFN2 can directly interact, can support mitochondrial fusion in *trans*, and that MFN1 can compensate for MFN2 deficiency [47, 50]. The relative paucity of expression of MFN1 within axons may explain the selective vulnerability of motor and sensory neurons to *MFN2* mutations [47]. In non-neuronal tissues, there may be enough MFN1 expression to counteract the effects of mutant MFN2, whereas low levels of MFN1 in neurons make them almost solely reliant on MFN2.

Recent studies have highlighted the functional importance of conformational changes within the heptad repeat regions of MFN2 for its function in facilitating intermolecular MFN2 interactions and mitochondrial tethering [119]. Dimerization of mitofusin molecules present on adjacent mitochondria is facilitated by switching of the HR2 domain from a "closed" (HR2 binding to HR1 of the same MFN2 molecule) to an "open" conformation (HR2 binding to the HR2 of an opposing MFN2). Small molecules targeting HR1/HR2 of MFN2 can function as "mitofusin agonists" by shifting the equilibrium of mitofusins toward the open/active state and can restore mitochondrial axon trafficking defects in cultured motor neurons from MFN2 T105M mutant mice [51]. Subsequently, an orally available "mitofusin agonist" (MiM111) was shown to ameliorate disease phenotypes in the T105M mutant mice [120, 121]. As there are both mouse and rat animal models of CMT2A that recapitulate core features of the human disease, these models could be readily utilized for further pre-clinical trials of mitofusin conformation-altering drugs.

Table 1 Potentially treatable inherited neuropathies

Condition	OMIM disease ID	Gene	Inheritance	Clinical features in addition to peripheral neuropathy	Treatment
Cerebrotendinous xanthomatosis	213700	CYP27A1	Recessive	Neonatal jaundice, ataxia, myelopathy, dementia, cataracts, low cholesterol, xanthomas, atherosclerosis	Cholic acid, chenodeoxycholic acid, and pravastatin[88,89]
Methylmalonic aciduria and homocystinuria type cblC (MAHCC)	277400	MMACHC	Recessive	Retinitis pigmentosa, myelopathy, thrombotic thrombocytopenia, methylmalonic aciduria, homocystinuria	Hydroxocobalamin (parenteral)[90–92]
Glutaric acidemia IIC	231680	ETFDH	Recessive	Variable depending on age of onset; glutaric aciduria, hypoglycemia, nausea, vomiting, myopathy	Coenzyme-Q and/or riboflavin[93,94]
Mitochondrial trifunctional protein deficiency (MTPD)	609015	HADHA/HADHB	Recessive	Cardiac and hepatic disease, myopathy, recurrent rhabdomyolysis	Medium-chain triglycerides, creatine, avoidance of fasting, frequent meals[95]
Hereditary motor and sensory neuropathy type VIC with optic atrophy (HMSN6C), aka CMT6C	618511	PDXK	Recessive	CMT plus optic atrophy	Pyridoxal 5′ phosphate (PLP)[96]
Brown-Vialetto-Van Laere syndrome-1 (BVVLS1)	211530	SLC52A3	Recessive	Hearing loss, bulbar dysfunction	Riboflavin[94]
Brown-Vialetto-Van Laere syndrome-2 (BVVLS2)	614707	SLC52A2	Recessive	Hearing loss, bulbar dysfunction	Riboflavin[94]
X-linked recessive Charcot-Marie-Tooth disease-4 (CMTX4)/Cowchock syndrome (COWCK)	310490	AIFM1	X-linked	Deafness and mental retardation	Riboflavin[94]
Fabry disease	301500	GLA	X-linked	Cardiac and renal disease, strokes, corneal lesions, skin lesions	Enzyme replacement, migalastat[97,98]
Refsum disease	266500	PHYH	Recessive	Deafness, cardiomyopathy	Dietary restriction of foods that contain chlorophyll, phytol, phytanic acid, or their precursors; plasmapheresis[99,100]
Maple syrup urine disease*	248600	CKDHB	Recessive	Progressive encephalopathy	Dietary restriction of protein intake, thiamine[101]
Thiamine metabolism dysfunction syndrome 4	613710	SLC25A19	Recessive	Episodic encephalopathy, striatal necrosis	Thiamine (unproven)[102]
Hyperoxaluria, primary, type 1	259900	AGXT	Recessive	Renal failure, calcium oxalate deposition in body tissues	Pyridoxine (vitamin B6)[103]

Table 1 (continued)

Condition	OMIM disease ID	Gene	Inheritance	Clinical features in addition to peripheral neuropathy	Treatment
Porphyria, acute intermittent	176000	HMBS	Dominant	Recurrent episodes of abdominal pain, gastrointestinal dysfunction, and neurological symptoms	Hematin, givosiran (RNAi targeting delta-aminolevulinic acid)[104,105]
Coproporphyria	121300	CPOX	Dominant	Skin photosensitivity	Hematin[104]
Porphyria, variegata	176200	PPOX	Dominant	Skin photosensitivity	Hematin[104]
Tyrosinemia, type 1	276700	FAH	Recessive	Hepatic and renal disease, rickets	(2nitro4trifluoromethylbenzoyl) 1,3cyclohexanedione (NTBC, nitisinone)[106]
Abetalipoproteinemia	200100	MTTP	Recessive	Retinitis pigmentosa, acanthocytosis, fat malabsorption, vitamin E deficiency	Low fat diet, fat-soluble vitamin supplementation (A, D, E, K)[107]
Mental retardation, enteropathy, deafness, peripheral neuropathy, ichthyosis, and keratoderma (MEDNIK)	609313	AP1S1	Recessive	Mental retardation, enteropathy, deafness, ichthyosis, keratoderma	Zinc[108]
X-linked spinal and bulbar muscular atrophy (SBMA, SMAX1)/Kennedy disease	313200	AR	X-linked	Motor neuron disease, androgen insensitivity	Anti-androgens (unproven)[109]

*Reported as a cause of acute axonal neuropathy mimicking Guillain-Barré syndrome

TRPV4-Related Neuropathies

Neuropathy due to mutations in *TRPV4* (transient potential vanilloid 4), which encodes a calcium permeable ion channel, represents another form of axonal neuropathy that is potentially treatable. While manifestations of TRPV4 mutations result in heterogeneous forms of motor predominant or motor exclusive neuropathy, studies in cultured cells and in a *Drosophila* model suggest that gain of ion channel function, leading to increased Ca^{2+} influx, is likely the most proximal event in the pathogenic cascade [122, 123]. Mutations that cause neuropathy largely localize to a single intracellular domain, the ankyrin repeat domain, and predominantly affect conserved arginine residues [124]. Neuropathogenic mutations also disrupt protein–protein interactions, including with the small GTPase RhoA [122]. Expression of human TRPV4 with neuropathy-causing mutations results in multiple neurodegenerative phenotypes in flies, which can be rescued with genetic or pharmacologic inhibition of ion channel activity [123]. Importantly, many highly specific TRPV4 antagonists have been generated, including an orally available drug (GSK2798745) that showed good tolerability in a clinical trial for cardiogenic pulmonary edema [125]. This TRPV4 antagonist, or similar drugs, could potentially be repurposed for use in patients with TRPV4 neuropathy. Current barriers to a clinical trial include the rarity of patients with TRPV4-related neuropathy, their heterogeneous clinical manifestations, and an incomplete understanding of the natural history.

General Pharmacologic Approaches

Given the convergence of pathological pathways among multiple forms of CMT, there is great interest in identifying therapeutic targets that might be broadly applicable. The landmark identification of *Sarm1* as a critical gene in axonal degeneration currently represents the most promising discovery that has emerged from these endeavors [126]. Sarm1 is an NADase, which depletes NAD^+, thereby activating metabolic signaling events that culminate in axonal degeneration [127]. Inhibiting Sarm1 activation is an attractive therapeutic target, and is reviewed in another article in this issue (Volume 18, issue 4).

Abnormal tubulin acetylation has also emerged as a potentially unifying downstream pathological abnormality in multiple forms of CMT2. Acetylated tubulin is highly abundant within axons, and acetylation increases the stability of microtubules and may also facilitate transport of cargoes along microtubules, particularly in the anterograde direction, through increased affinity for kinesin motors [128]. The first description of altered tubulin acetylation in CMT came from work on a mouse model of CMT2F (related to *HSPB1* mutations) [129]. Acetylated tubulin was reduced in primary neurons and sciatic nerve from these mice, but not in the spinal cord. Similar deficiencies of acetylated tubulin have subsequently been demonstrated in mutant CMT2A/*Mfn2* and CMT2D/*Gars* mouse models, suggesting that dysregulation of tubulin acetylation within axons might be a common feature across genetically diverse forms of CMT2 [130].

Tubulin acetylation is principally regulated by the opposing activities of the deacetylase HDAC6 and the acetylase α-tubulin acetyltransferase 1. HDAC6 is a unique member of the HDAC family — its expression is restricted to the cytosol, its primary enzymatic targets are not histones, it contains a ubiquitin binding domain, and it interacts with microtubule transport machinery [131, 132]. In the same mouse models mentioned above, inhibition of HDAC6, either pharmacologically or through genetic deletion, restored levels of acetylated tubulin within the peripheral nerve and partially restored sensory and motor function. In the HSPB1 mutant mouse model, HDAC6 inhibition improved mitochondrial trafficking, rotarod performance, and the amplitudes of motor and sensory nerve responses [129]. Similar but modest improvements in sensory and motor function were seen with pharmacologic HDAC6 inhibition in a mouse model of CMT2A, but the effects were more pronounced with genetic deletion of HDAC6 in these mice [133]. Finally, in a mutant mouse model of CMT2D, pharmacological inhibition of HDAC6 also led to modest improvements in several motor and sensory parameters [130].

In addition to its function in regulating tubulin acetylation, HDAC6 serves other important roles, including facilitating protein turnover by linking proteasome-dependent and autophagy-dependent protein degradation pathways [134]. In this latter context, HDAC6 activity plays a protective role through promoting clearance of misfolded and potentially proteotoxic species. Thus, clinical use of HDAC6 inhibition to correct defects in peripheral nerve tubulin acetylation could potentially lead to important off-target effects that could be detrimental to the nervous system in other ways.

Genetic Approaches

Gene Replacement

The success of adeno-associated virus (AAV)-mediated gene delivery for spinal muscular atrophy demonstrates its feasibility for inherited axonal neuropathies [135], as current vectors can deliver the missing gene to both sensory and motor neurons [136, 137]. In addition to

recessive axonal neuropathies caused by loss-of-function mutations, some dominant mutations could also be treated by overexpression of wild-type protein [47, 138], or by incorporating a small interfering RNA (siRNA) against the endogenous gene into the viral vector. In the future, gene-editing strategies, such as deleting dominant alleles, could become feasible.

The first foray into gene therapy for inherited axonal neuropathy is underway. Giant axonal neuropathy (GAN) is a recessive, multisystem neurodegenerative disease caused by loss of function mutations in *gigaxonin*. GAN is extremely rare and presents in early childhood with a range of signs and symptoms, including sensory and motor neuropathy, ataxia, and gait difficulty, as well as characteristic, kinky hair [139]. The disease is relentlessly progressive, with brainstem pathology leading to dysphagia, dysarthria, and ultimately death due to respiratory failure. Loss of gigaxonin, an E3 ubiquitin ligase involved in intermediate filament organization and turnover, leads to massively enlarged axons that are filled with disorganized intermediate filaments. *Gan* knockout mice have abnormal accumulation of intermediate filaments within cells, with mild axonal degeneration and functional motor impairment. Intrathecal delivery of *GAN* with AAV9 into these mice reduced intermediate filament accumulations in sensory neurons and peripheral nerve, and modestly improved rotarod performance [140]. In light of the fatal nature of GAN, these promising preclinical proof-of-principle results helped launch the first intrathecal AAV gene transfer clinical trial in humans (NCT02362438), which is currently underway. While the primary goal of the study is to evaluate the safety of this treatment approach, additional clinical endpoints will be concurrently assessed.

The overexpression of MFN1 or MFN2 is a promising approach for CMT2A. Transgenic mice that express the disease-causing R94Q mutation (a common mutation that causes severe neuropathy) of *MFN2* in PNS and CNS neurons develop axonal degeneration, reduced strength, poor motor performance, vision loss, and modestly reduced survival [138]. In line with prior studies, the R94Q MFN2 functions as a dominant negative, leading to impaired mitochondrial fusion. Importantly, over-expression of human MFN1 or MFN2 within the nervous system using the prion promotor reversed mitochondrial clustering, axonal degeneration, and gliosis. Together, these results provide a proof-of-concept that gene therapy to increase expression of functional, fusion-competent mitofusins may at least partially overcome the effects of dominant negative *MFN2* alleles and provide therapeutic benefit in CMT2A patients. With the recent description of the natural history of CMT2A in a large patient cohort, these pre-clinical animal studies could help pave the way for future gene therapy human trials in CMT2A [35].

Gene Knockdown

The successful employment of antisense oligonucleotides (ASOs) and siRNA in familial amyloid polyneuropathy is truly a remarkable achievement in neuromuscular medicine, and is reviewed in another article in this issue (Volume 18, issue 4). However, these strategies are not currently viewed as generally applicable for treatment in inherited axonal neuropathies. Most of the genes that cause inherited axonal neuropathies are probably essential for neuronal health and are thus poor candidates for the global knockdown strategy that was used for treating familial amyloid polyneuropathy.

Selective knockdown or removal of the disease-causing allele, sparing the wild-type allele, is not yet feasible, but some pioneering work underscores the potential of mutant allele–specific genetic silencing. Two distinct *GARS* mutations that cause CMT2D have been successfully targeted by allele-specific siRNA in mouse models [141]. Both alleles had multiple nucleotide substitutions, increasing the ability of ASOs to specifically target the mutant allele. In the mouse models, the mutant *GARS* alleles were successfully knocked down using AAV9-mediated delivery of siRNA, and the degree of phenotypic rescue was dramatic. Whether disease mutations arising from a single nucleotide change, which are by far the most common variants responsible for disease, could be efficiently and specifically targeted while sparing the wild-type allele remains to be determined. In addition, employing allele-specific knockdown strategies in human patients poses a substantial regulatory challenge in that each ASO or siRNA sequence for an individual patient would be unique and might have to undergo a separate safety and efficacy evaluation prior to approval. If these technical and regulatory hurdles can be overcome, strategies to specifically silence (or correct with gene editing) dominant missense mutant alleles would revolutionize the treatment of diseases caused by dominant alleles.

Conclusions

Research on inherited neuropathies is entering an exciting new phase — clinical and translational directions into the pathogenesis and treatment of CMT. Recent work has added granularity to the understanding of long-held notions of how disrupted axonal biology leads to neuronal degeneration and has begun to provide some unexpected pathophysiological connections that may ultimately reveal therapeutic targets shared across unrelated forms of CMT. Available drugs could very likely impact underlying pathological mechanisms of HSAN1, SORD neuropathy, and TRPV4 neuropathy. CMT2A has emerged at the leading edge of pre-clinical therapeutic development, and rational clinical trials for this form of axonal CMT can be envisioned. In addition, the field of gene therapy has made tremendous advances, with

identification of viral vector-based methods capable of robust transduction of sensory motor and sensory neurons. Much work remains to be done to facilitate clinical trials for various forms of CMT, including natural history studies and biomarker identification and validation. However, with the confluence of efforts across these varied disciplines and approaches, we foresee a day when clinicians can tell some patients that their form of CMT is a treatable condition.

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Declarations

Competing Interests S.S.S. has been compensated for serving on the scientific advisory boards of Mitochondria in Motion, which is developing drugs to treat CMT2A, and Disarm Therapeutics, which is developing drugs to block SARM1 as a treatment of peripheral neuropathies.

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