NeuroMolecular Medicine

ISSN0895-8696/06/08:255–278/$30.00 (Online) 1559-1174
doi: 10.1385/NMM:8:1–2:255

REVIEW ARTICLE

Therapeutic Strategies for the Inherited Neuropathies

Michael E. Shy*

Department of Neurology and Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit MI 48201, USA

Received August 29, 2005; Revised November 21, 2005; Accepted November 30, 2005

Abstract

More than 30 genetic causes have been identified for the inherited neuropathies collectively referred to as Charcot-Marie-Tooth (CMT) disease. Previous therapies for CMT were limited to traditional approaches such as rehabilitation medicine, ambulation aids, and pain management. Identification of the genes causing CMT has led to improved genetic counseling and assistance in family planning. Identification of these genes is beginning to delineate common molecular pathways in multiple forms of CMT that can be exploited in future molecular therapies. Scientifically based clinical trials for CMT are currently being implemented. Techniques of gene therapy are advancing to the point that they may become feasible options for patients with CMT and other neurodegenerative diseases.

doi: 10.1385/NMM:8:1–2:255

Index Entries: Charcot-Marie-Tooth disease; neuropathy; genetics; therapy; disease mechanisms; gene therapy.

Introduction

Inherited neuropathies, often known as Charcot-Marie-Tooth (CMT) diseases, were described by Charcot and Marie in France and, independently, by Tooth in England in the 1886 (Charcot and Marie, 1886; Tooth, 1886). Early investigators recognized the weakness and atrophy of muscles innervated by the peroneal nerve, the characteristic foot abnormalities, and the familial nature of the disease. However, advances in understanding causes and disease mechanisms for the inherited neuropathies did not come rapidly. Dejerine and Sottas (1893) described cases that were more severe and had an onset in infancy; Roussy and Levy (1926) described cases associated with tremor, ataxia, areflexia, and *pes cavus*. Studies beginning in the 1960s suggested that most CMT patients had autosomal-dominant disorders that could be divided into one group with slow nerve conduction velocities and pathological evidence of a hypertrophic demyelinating neuropathy (CMT type 1), and a second group with relatively normal nerve conduction velocities and axonal degeneration (CMT type 2) (Dyck and

*Author to whom all correspondence and reprint requests should be addressed. E-mail: m.shy@wayne.edu

Lambert, 1968; Harding and Thomas, 1980). Most patients were found to have weakness and sensory loss predominantly in the distal legs, and muscle wasting in the distribution of weakened muscles; most of them had also developed clinical evidence of disease within the first two decades of life. Linkage studies designed to identify the causal genes in CMT also began during this period (Bird et al., 1982).

However, it was not until 1991 that two groups independently identified the first genetic cause of CMT (Lupski et al., 1991; Raeymaekers et al., 1991). As is now well known, both groups demonstrated that CMT1A, the most common form of CMT1, is caused by a 1.4-Mb duplication within chromosome 17p11.2, in the region containing the *PMP22* gene. Reports of other genetic defects associated with hereditary neuropathies soon followed. CMT1B, linked to chromosome 1, was found to be caused by mutations in the gene encoding myelin protein zero *(MPZ)* glycoprotein (Hayasaka et al., 1993), and the X-linked neuropathy, CMTX1, was shown to be caused by mutations in the gap-junction β1 *(GJβ1)* gene that encodes the protein connexin (Cx) 32 (Bergoffen et al., 1993). Deletion, instead of duplication of the CMT1A 1.4 Mb region on chromosome 17, was found to cause hereditary neuropathy with liability to pressure palsies (HNPP) (Chance et al., 1993). At present, mutations in more than 30 genes have been identified that cause inherited neuropathies (http://www.molgen.ua.ac.be/ CMTMutations/).

The dramatic progress in CMT-related research over the past 15 yr is a result of the recent revolution in the field of *molecular genetics*. Fortunately, we are also in the midst of a revolution in *molecular biology*. Investigators have now generated "knockout mice," in which the causal genes for many forms of CMT have been deleted, to determine the function of the gene in question. Similarly, animal models are being made in which particular mutations have been "knocked in" to provide disease models to investigate. Advances have been made in physiology, tissue culture, and patient evaluations. These recent advances allow us to realistically consider therapeutic strategies to treat and eventually cure patients with CMT. The remainder of this chapter will be directed toward evaluating these strategies. However, to place the strategies in a biological context, we need to briefly review the cellular and molecular biology of the peripheral nervous system (PNS).

Biological Background

Most peripheral nerves are mixed, containing both motor and sensory axons that are ensheathed along their length by Schwann cells. During development, Schwann cell precursors from the neural crest migrate out and contact the developing peripheral axons (Harrison, 1924; Le Douarin and Dupin, 1993). These "immature" Schwann cells then ensheath bundles of developing axons, a process called "radial sorting," and further differentiate into myelinating or nonmyelinating Schwann cells (Webster, 1993). Schwann cells that establish a one-to-one association with an axon, called the "promyelinating stage" of Schwann cell development, initiate the program of myelination and become myelinating Schwann cells (Webster, 1993; Scherer, 1997). In contrast, Schwann cells that do not establish this relationship with an axon do not activate the program of myelin gene expression, and become nonmyelinating Schwann cells (Webster, 1993; Mirsky and Jessen, 1996). Interestingly, this decision process is directed by axons, so that all immature Schwann cells have the potential to become either myelinating or nonmyelinating cells.

The primary function of myelin is to increase axonal conduction velocity without a significant increase in axonal diameter. This is accomplished by the process of saltatory conduction, in which nerve impulses jump between electrically excitable regions of the axon, called nodes of Ranvier, located between the electrically insulated areas ensheathed by myelinating Schwann cells. Because most peripheral nerves are mixed, however, with bundles of both large- and small-diameter myelinated axons, their conduction velocity is determined mainly by the speed of their largest diameter myelinated fibers.

Recent investigations of myelinated axons and their nodes of Ranvier have demonstrated a surprising structural complexity. As can be seen in the cartoon from Suter and Scherer (2003) (Fig. 1), the myelin sheath has two regions, compact and noncompact, each of which contains a unique nonoverlapping set of protein constituents. The compact region contains the myelin structural proteins, PMP22 and MPZ, both of which are associated with

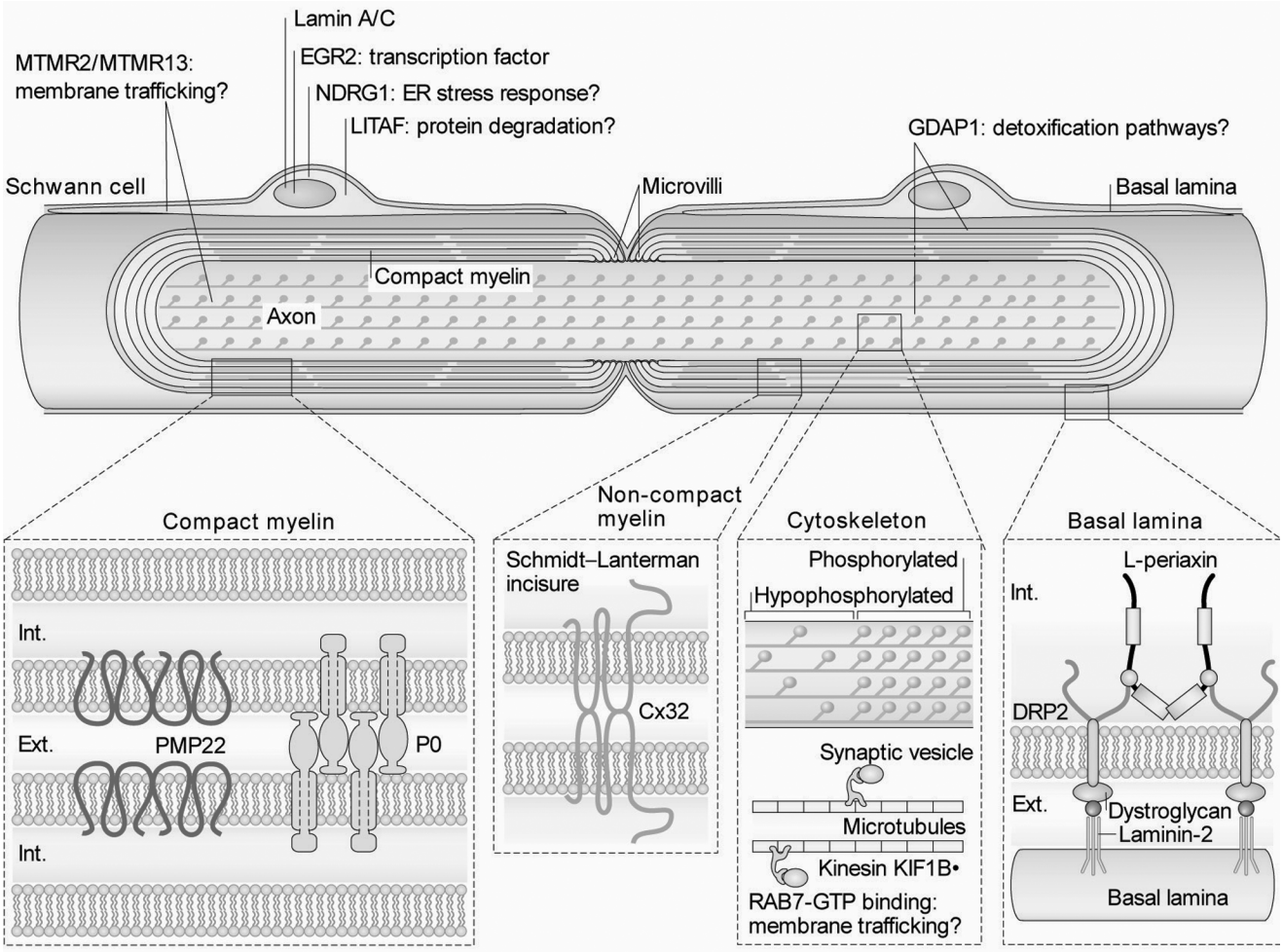

Fig. 1. Schematic overview of the molecular organization of peripheral nervous system-myelinated axons highlighting many of the proteins affected in Charcot-Marie-Tooth disease (CMT). The figure depicts the location of the wild-type proteins accorded by the genes that are mutated *n*CMT. Cx, connexn; EGR, early growth response; ER, endoplasmic reticulum; Ext, extracellular; GDAP, ganglioside-induced differentiation associated protein; Int, intracellular; KF, kinesin family member; LITAF, lipopolyscaccharide-induced tumor necrosis factor (TNF); MTMR, myotubularin-related protein; NDRG, *N*-myc downstream-regulated gene; PMP, peripheral myelin protein. (With permission from Suter and Scherer, 2003.)

CMT1, as well as myelin basic protein. All of these participate in forming the highly organized myelin sheath and in electrically insulating axons. The noncompact region is made up of two subdomains, the paranode and the juxtaparanode. The paranodal region, the loops of Schwann cell membrane and interacting axonal membrane adjacent to the node of Ranvier, contains the Schwann cell proteins Cx 32 (the cause of CMTX1), MAG, Neurofascin 155, and the axonal proteins Caspr and Contactin. These proteins participate in Schwann cell–axonal or Schwann cell–Schwann cell interactions, and act to electrically isolate the nodal region. The juxtaparanodal region, the portion of Schwann cell and interacting axonal membrane adjacent to the paranode, contains potassium channels and Caspr 2, both expressed by axons (*see* also references in Arroyo and Scherer, 2000; Salzer, 2003). The complex cellular structures formed by myelinating Schwann cells and their axons are thus analogous in many respects to the neuromuscular junction formed between motor axons and muscle cells: both are highly

ordered, multicomponent systems formed by the interaction of two distinct cell types in order to carry out a specific biological function related to nerve transmission.

The process of nerve development and associated Schwann cell differentiation, myelination, and establishment of an electrically insulated node of Ranvier capable of saltatory conduction provides the biological framework for understanding the pathogenesis of all types of peripheral neuropathy, including CMT. In fact, one might anticipate that inherited peripheral neuropathies would be caused by mutations that alter crucial aspects of this biological process, such as the critical interactions between Schwann cells and their axons at the paranodal region, or the process of myelin compaction. For the purpose of developing rational treatments, each of these sites are potential targets.

Biologically Based Treatment Strategies

Evaluation of the more than 30 mutant genes and proteins that cause inherited neuropathies has identified a number of molecular processes and pathways that are involved in the pathogenesis of CMT as well as in many other neurodegenerative diseases. Manipulation of these processes and pathways will provide the basis for future therapeutic strategies.

Gene Dosage and Regulating Myelination: Therapy for CMT1A

The most common form of CMT, CMT1A, is caused by a 1.4-Mb duplication on chromosome 17 in the region carrying the gene encoding *PMP22* (Lupski et al., 1991; Raeymaekers et al., 1991). Patients with CMT1A develop a progressive length-dependent sensorimotor neuropathy with an onset usually in the first two decades of life (Thomas et al., 1997). Duplication of the *PMP22* gene within the region is the likely cause of the disease because mice and rats with extra transgenic copies of the *Pmp22* gene develop a similar demyelinating neuropathy (Huxley et al., 1996; Magyar et al., 1996; Sereda et al., 1996), as do some patients with *PMP22* point mutations (Roa et al., 1993; Vallat et al., 1996). In contrast, deletion of the same 1.4 Mb region on chromosome 17 that is duplicated in CMT1A causes HNPP (Chance et al., 1993), a distinct disorder characterized by focal episodes of weakness and/or sensory loss (Li et al., 2004). Decreased expression of PMP22 is the cause of HNPP. (Chance et al., 1993). Thus, alterations in PMP22 dosage cause two distinct disorders depending on whether there is too much or too little PMP22 in myelin. The situation is somewhat more complicated because up to 90% of translated PMP22 is targeted for lysosomal degradation before reaching the myelin sheath (Pareek et al., 1997). Nevertheless, treatment strategies are currently being devised to regulate *PMP22* mRNA levels as a method of treating CMT1A. Some approaches, such as the use of siRNA or antisense oligonucleotides, will be discussed in the section on gene therapy. However, in the current absence of effective gene therapy, investigators are turning toward currently available agents that have demonstrated ability to manipulate *PMP22* mRNA levels. One of these compounds is the hormone, progesterone.

A Progesterone Antagonist Improves Neuropathy in CMT1A Rats

Sereda and his colleagues (2003) in Klaus Nave's laboratory have taken advantage of the known property of progesterone to increase expression of *PMP22* and *MPZ* mRNA levels in cultured Schwann cells. Previously, these same investigators have generated a CMT1A rat by specific overexpression of a *Pmp22* cDNA. Heterozygous animals develop a progressive, demyelinating neuropathy with clinical, neurophysiological, and pathological features that resemble CMT1A (Sereda et al., 1996). Daily administration of progesterone to these CMT1A rats elevated the steady-state levels of *Pmp22* and *Mpz* mRNA in sciatic nerves, resulting in enhanced Schwann cell pathology and a more severe clinical neuropathy. In contrast, administration of the selective progesterone receptor antagonist, onapristone, reduced overexpression of *Pmp22* mRNA, and improved the CMT phenotype, without obvious side effects, in treated rats. Taken together, these data provide proof of principle that the progesterone receptor of myelin-forming Schwann cells is a promising pharmacological target for therapy of CMT1A. Unfortunately, onapristone has been shown to be toxic to humans so that it will probably not be used in clinical trials. However, current research is underway to develop a less toxic

progesterone antagonist that can be used in clinical trials of CMT1A.

Ascorbic Acid and CMT1A

Richard and Mary Bunge utilized rat Schwann cells cocultured with neurons derived from dorsal root ganglia to investigate PNS myelination in a number of classic studies (Bunge et al., 1980; Bunge, 1987). In these cocultures, Schwann cells first ensheath axons when the cultures are maintained in defined media. They then form a myelin sheath around axons when serum and ascorbic acid are added to the culture media. Ascorbic acid is critical to this process, presumably by linking hydroxyproline residues in the extracellular matrix (Bunge et al., 1980). In the absence of ascorbic acid, the cocultures will not myelinate (Bunge et al., 1980). Because of the critical role of ascorbic acid in myelinating cocultures and because ascorbic acid (vitamin C) is routinely ingested by humans without harm, Fontes and his colleagues treated their YAC mouse model of CMT1A with ascorbic acid and demonstrated an improvement in myelination and performance on tasks such as a Rotarod. They also demonstrated a reduction of *Pmp22* mRNA levels to levels below those necessary to induce the disease phenotype (Passage et al., 2004). Planning for clinical trials of ascorbic acid for CMT1A is now underway on several continents.

Both of the above treatment approaches are nonspecific because neither ascorbic acid or progesterone antagonists have been shown to target expression of a single myelin gene. Both therapies are directed toward regulating the overall program of myelination. For CMT1A, this may be appropriate because decreasing overall myelin mRNA levels by progesterone antagonists may reduce *PMP22* mRNA levels without significantly decreasing mRNA levels of other myelin genes in a fashion that would cause disability. Whether progesterone antagonists or ascorbic acid would be candidates for treating additional forms of CMT1 is not currently known and has not yet been investigated in animal models.

Trophic Factors and CMT

Three families of trophic factors, or growth factors, have been extensively used in recent years to treat neurodegenerative diseases, including CMT. (reviewed in Massicotte and Scherer, 2004). The first is the neurotrophin family consisting, in mammals, of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), and neurotrophin-4/5 (NT4/5). Each of these is secreted as a homodimer and is cleaved by a protease to its mature form. All neurotrophins bind to a protein tyrosine kinase (Trk) receptor that in turn activates downstream signaling pathways in the target cell. All neurotrophins also interact with the low affinity p75 receptor that can signal independently of the Trks. The second family of trophic factors is the glial cell line-derived neurotrophic factor (GDNF) family, which is part of the transforming growth factor (TGF)-β superfamily. GDNF family members include GDNF itself as well as neurturin, artemin, and persephin. GDNF family members bind to at least one of a group of glycosyl phosphatidylinositol (GPI)-linked proteins known as GFRα1-4, which then bind to a common signal-transducing subunit, c-ret, which is a protein kinase receptor. The third family is the cililiary neurotrophic factor (CNTF) family of cytokines that includes CNTF, leukemia inhibitory factor (LIF), cardiotrophin 1 (CT-1), cardiotrophin-like cytokine (CLC), and interleukins (IL)-6 and -7. All members share a common set of receptors that have the intrinsic membrane protein, gp130, as a common signal transducing subunit. A review of these families with their neuronal targets is provided in Table 1.

Enthusiasm for the use of trophic factors to treat CMT and other degenerative diseases results from (1) the ability of the factors to promote the development and maintenance of both neurons and glia, (2) the demonstrated ability of several of these factors to rescue or ameliorate animal or tissue culture models of degenerative disease, and (3) axonal degeneration being a common feature of both demyelinating and axonal CMT. Many of the trophic factors cited here are already expressed in Schawnn cells or neurons, and expression levels are often endogenously altered in nerve injury (Massicotte and Scherer, 2004). Exogenous NGF, BDNF, NT-3, and NT-4, a pan engineered neurotrophin, CNTF, IGF-1, and IL-6 have all been shown to promote nerve regeneration (Gravel et al., 1997). There is target specificity for neurotrophic factors based on the localization of their receptors (*see* Table 1). For example, NT-3 has been shown to reduce neuropathy caused

Table 1
Trophic Factors and Their Neuronal Targets

Family	Trophic factor	Receptor	Neuronal target
Neurotrophins	NGF	trk A	Sympathetic, trk A nociceptive
	BDNF	Trk B	Motoneurons
	NT-3	trk C	Motoneurons, muscle spindle afferents, mechanoreceptors
	NT-4	Trk B	Motoneurons, preganglionic autonomic
GDNF	GDNF	GFRα1 + ret	Motoneurons, preganglionic autonomic, IB4 nociceptive, enteric
	Neurturin	GFRα2 + ret	Motoneurons, parasympathetic, enteric
	TGF-β	Types 1 and 2	Autonomic
CNTF	CNTF	CNTFRα_+ gp130	Motoneurons
	LIF	LIFβR + gp130	Motoneurons
	CT-1	LIFβR + gp130	Motoneurons

Table modified from Massicotte and Scherer (2004).

by B6/pyridoxine and cisplatin that effect large sensory neurons that express the trk C receptor, whereas NGF has demonstrated potential benefit in a phase II trial for HIV-related neuropathy (McArthur et al., 2000). Results with NGF in the treatment of painful diabetic neuropathies, however, have not demonstrated such positive results (Apfel, 2002; Pradat, 2003). Trophic factors have also demonstrated effectiveness in several animal models of axonal, and even demyelinating neuropathies. Treatments with GDNF have prolonged survival and function of the superoxide dismutase 1 (SOD1) mutant model of familial amyotrophic lateral sclerosis (FALS) (Acsadi et al., 2002; Manabe et al., 2002) and insulin-like growth factor (IGF)1 (Kaspar et al., 2003).

The use of trophic factors to treat models of neurodegenerative diseases has not been limited to members of the three families discussed earlier. Vascular endothelial growth factor (VEGF), which is essential in angiogenesis, slowed progression and prolonged life in the SOD1 mouse mutant form of FALS (Azzouz et al., 2004). The cytokine hormone, erythropoietin, proved effective in promoting nerve regeneration in animal models of nerve injury (Kretz et al., 2005) and its receptor is expressed by Schwann cells (Li et al., 2005). The potential use of Neuregulin (Nrg) 1 to promote myelin thickness is discussed later in this review.

Despite the therapeutic promise of trophic factors, results of treatment studies in human patients have been disappointing. Attempts to halt the progression of FALS with CNTF, BDNF, IGF-1, and NT-3 have been unsuccessful in phase II or III clinical trials (Apfel, 2002). Trials of GDNF in FALS were halted after adverse reactions in phase I trials (Yuen, 2001). Part of the reasons for disappointing results with these proteins may relate to methods of delivery. For example, the half-lives of many trophic factors are only several minutes and they have frequently been administered to patients by subcutaneous injection. It is debatable whether the trophic factor would have adequate time to reach its target and affect neurons or Schwann cells within this limited period of activity.

Neurotrophin-3 Treatment of CMT1A

In a recent report, Sahenk and colleagues used subcutaneous injections of NT3 to treat sciatic nerves of Trembler J (Tr^J) mice, a naturally occurring mouse-demyelinating mutant caused by a missense mutation in the *Pmp-22* gene. Similar injections were also given to xenografts of CMT1A patient nerves in nude mice. Myelinated fibers and Schwann cell numbers were evaluated. NT3 appeared to augment axonal regeneration in both models. In the same publication, the investigators reported their results from a 6-mo comparison between four CMT1A patients treated with NT3 injections (three times a week) and four CMT1A patients treated with placebo. The results from this pilot study showed an increase in the mean number of small myelinated fibers within nerve regeneration clusters and an increase in solitary myelinated fibers in treated but not placebo groups. Clinically, improvements in the Neuropathy

Table 2
Future Molecular Targets

Disrupted process	Disorder	Gene
Axonal transport	CMT2A	MFN2
	Giant axonal neuropathy	GAN
	HMN dynactin	Dynactin
	SPG10	KIF5A
Mitochondrial function	CMT2A	MFN2
	CMT4A	GDAP1
	Familial ALS	SOD 1
Membrane fusion/fission	CMT1C	LITAF/Simple
	DI-CMT	DNM2
	SMA/ALS	VAPB
Protein misfolding	CMT1A	PMP22
	CMT1B	MPZ
DNA/RNA processing	CMT2D/HMNV	GARS
	ALS4	SETX
	SMARD1	IGHMB2
	SMA	SMN
SC–axonal interactions	Demyelinating CMT	PMP22, MPZ, Cx32, LITAF/Simple, EGR2, MTMR2, MTMR13, GDAP1, and others

Impairment Score (NIS) were obtained in treated patients for pinprick sensation, vibration sensation, and cold temperature sensation in distal limbs. The authors concluded that NT3 improved sensory modalities in CMT1A after 6 mo (Sahenk et al., 2005). Although these results need to be interpreted with caution, given the small sample size and short duration period, they suggest that NT3 may prove beneficial in treating CMT1A in the future and the trophic factor is likely to be investigated in future CMT1A clinical trials with larger numbers of patients.

Future Molecular Targets

The increasing number of genes causing CMT serve as an "in vivo microarray" of genes that are light necessary for the normal functioning of the Schwann cells and neurons. More than 30 of these genes presently identified also provide clues into cellular pathways that contribute to the pathogenesis of CMT. These cellular pathways are shown in Fig. 1 and summarized in Table 2.

Disruption of Axonal Transport

Neurons transport proteins, cytoskeletal elements, synaptic vesicle precursors, mitochondria, and other organelles along axons that can be more than a meter in length. Orthograde transport, from the perikaryon down the axon, is mediated by molecular motors known as kinesins, a gene family, that carry specific cargoes down tracks of microtubules. Fast retrograde transport in motor axons is carried out by dynein–dynactin complexes that serve as motors to return materials to the cell body from nerve terminals, for restoration and reuse. Disruption in both of these systems has caused neurodegenerative diseases including CMT. Kinesin KIF5A mutations cause a dominantly inherited spastic paraparesis (Reid et al., 2002), possibly by disrupting microtubule-dependent axonal transport of neurofilament (Xia et al., 2003). Mutations in the dynactin gene cause a chronic motor neuron disease (Puls et al., 2003). In this latter disorder, a single base-pair change, resulting in an amino acid substitution, is predicted to distort the folding of the dynactin domain responsible for binding to the microtubules. This domain is necessary for retrograde transport. In another disorder, recessive mutations in the gigaxonin gene cause "giant axonal neuropathy" (Bomont et al., 2000). Gigaxonin binds to microtubule-associated protein (MAP) 1B to enhance microtubule stability (Ding et al., 2002).

Presumably, a disruption in microtubule stability in giant axonal neuropathy contributes to the pathogenesis of this unusual disorder. Finally, axonal transport of mitochondrial is probably disrupted in CMT2A, the most common form of CMT2 (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15064763) (Zuchner et al., 2004). CMT2A is caused by mutations in the nuclear encoded mitochondrial gene mitofusin (*MFN*) 2, and is cited in the next section. Mitochondria need to fuse into chains before being transported by kinesin KIF1B. Several *MFN2* mutations appear to prevent mitochondrial fusion (Zuchner et al., 2004). Taken together, these results suggest that disrupted axonal transport of mitochondria may contribute to the pathogenesis of CMT2A. It has been proposed that disruptions in axonal transport are responsible for length-dependent axonal degeneration in many neurodegenerative disorders (Griffin and Watson, 1988). Repairing axonal transport is an attractive therapeutic approach to treating many forms of CMT.

Mitochondrial Function in CMT

Mitochondrial abnormalities have been found in a number of neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and spastic paraplegia (reviewed in Zuchner and Vance, 2005). They have also been identified in an increasing number of inherited neuropathies. MFN2, the cause of CMT2A, is a dynamin-like GTPase that spans the outer mitochondrial membrane where it helps mediate mitochondrial fusion. Mitochondria exist in a dynamic state, alternating between fusion and fission. As noted earlier, mitochondria need to fuse in order to be carried by kinesins in orthograde axonal transport. In addition, MFN2 mutations are likely to disrupt normal mitochondrial functions such as supplying energy to the cell or participating in apoptosis pathways.

Mutations in the putative glutathione transferase protein GDAP1 cause CMT4A. GDAP1, also encoded in the nucleus, is predominantly expressed in neurons where it associates with mitochondria. Mammalian glutathione transferase families are involved in the inactivation of endogenous hydroperoxides formed as secondary metabolites during oxidative stress. GDAP1 has been found to colocalize with mitochondria in a series of in vitro transfection assays (Pedrola et al., 2005). Its function in mitochondria is at present unknown.

Mutations in SOD1 cause approx 20% of cases of FALS. SOD1 is localized to mitochondrial membranes and it has been proposed that alterations in mitochondrial function are involved in the pathogenesis of FALS (Pasinelli et al., 2004). Recently, it has been found that small heat-shock proteins such as HSP27, the cause of CMT2F and a form of distal hereditary motor neuronopathy (dHMN), block the uptake of mutant SOD1 into mitochondria, although not that of wild-type SOD1 (Okado-Matsumoto and Fridovich, 2001). It is hypothesized that this binding would make heat-shock proteins unavailable for their apoptotic function, which would ultimately lead to motor neuron death.

Taken together, these CMT models suggest that manipulating mitochondrial function is an area of potential therapeutic research into at least some forms of CMT.

Membrane Fusion, Fission, and Protein Transport

Fusion and fission are important cellular processes in other areas of the cell besides mitochondria. In addition to MFN2, several forms of CMT and related disorders appear to be caused by abnormalities in the fusion and fission of other cellular membranes. The GTPase dynamin (DNM) 2 mutations cause dominant intermediate CMT (DI-CMT). The role of DNM2 appears to be in aiding the separation of newly formed endosomes from the cell membrane (Gurunathan et al., 2002; Bhattacharya et al., 2005). Additionally, the vesicle-associated protein B (VAPB) participates in membrane fusion and has recently been shown to cause ALS in several Brazilian families (Nishimura et al., 2004). VAPB contains a v-SNARE domain. SNARE refers to soluble NSF-attachment protein receptor proteins. Membrane proteins from vesicles (v-SNARES) and proteins from target membranes (t-SNARES) govern the specificity of vesicle targeting and docking through mutual recognition. However, members of the vesicle-associated protein family also associate with microtubules and function in membrane transport (reviewed in Zuchner and Vance, 2005).

Mutations in the putative protein degradation protein LITAF/SIMPLE cause the demyelinating autosomal-dominant disorder CMT1C (Street et al., 2003). Initial patients with CMT1C have had phenotypes that strongly resemble those with CMT1A. Although the precise function of SIMPLE is unknown, its murine orthologue interacts with Nedd4, an E3 ubiquiton ligase. Monoubiquitination of plasma proteins by Nedd4 family members serve as internalization signals that are recognized by protein TSG101 that facilitate the sorting of membrane proteins to the lysosome for degradation (Bennett et al., 2004). Although SIMPLE is expressed in many cell types, when mutated it seems to cause only a demyelinating neuropathy. This suggests that the disease specificity may come from impaired targeted degradation of specific Schwann cell proteins such as PMP22.

Protein Misfolding and ER Retention

PMP22 missense mutations Leu16Pro (Valentijn et al., 1992) and Leu147Arg (Navon et al., 1996) cause a demyelinating neuropathy in humans and the naturally occurring demyelinating trembler J (Tr^J) (Suter et al., 1992) and Tr (Suter et al., 1992) mouse mutants. Because both of these mutations are more severe in humans than HNPP (also more severe than CMT1A caused by *PMP22* duplication), they are likely to cause disability by causing an abnormal gain of function by the mutant PMP22, rather than by a simple loss of PMP22 function. When epitope-tagged Tr, Tr^J, and wild-type Pmp22 were microinjected into sciatic nerves of rats and analyzed by immunohistochemistry, wild-type Pmp22 was transported to compact myelin, but both Tr and Tr^J and Pmp22 were retained in a cytoplasmic compartment that colocalized with the endoplasmic reticulum (ER) (Colby et al., 2000). Other studies have also shown that mutant Tr and Tr^J proteins aggregate abnormally in transfected cells (Tobler et al., 1999). In fact, aggresome-like structures have been identified in sciatic nerves of Tr^J mice, surrounded by chaperones and lysosomes, suggesting that abnormalities in intracellular degradation of mutant PMP22 contributed to the pathogenesis of the neuropathy (Fortun et al., 2003). More recent studies have shown that there are abnormalities of proteosome function resulting in the accumulation of ubiquitinated substrates in the Tr^J model (Fortun et al., 2005). Recent cell-based studies showed that mutant MPZ could accumulate in the ER and induce apoptosis. This aggregation-induced apoptosis was abrogated by pretreatment with curcumin (Khajavi et al., 2005). Whether curcumin will have similar effects in whole animal studies remains to be determined. Transfection studies have also demonstrated that other PMP22 and MPZ mutations result in mutant proteins being retained in intracellular compartments (Shames et al., 2003). Whether these other mutations also disrupt proteosome activity or cause abnormal gain of function by other mechanisms such as activating the unfolded protein response (UPR) (Southwood et al., 2002) are areas of active investigation that may lead to future treatments.

RNA Processing

Extensive RNA processing occurs following transcription before the formation of proteins in the process of translation. Nascent RNA transcripts undergo splicing, add a cap to their 5′-end and a polyalanine tail to their 3′-end before leaving the nucleus for the ribosome. Transfer RNAs specifically add their cognate amino acid to the developing protein on the ribosome. One might expect that abnormalities in all of these processes would seriously disrupt all cells, not just those of the PNS. Interestingly, however, mutations in a number of genes involved in these processes appear to cause primarily CMT or a similar disorder. GARS is a glycyl-tRNA transferase that is responsible for placing a glycine on the appropriate tRNA in both the cytoplasm and mitochondria. This is an essential process in all cells. However, missense mutations in GARS appear to cause only CMT2D and dHMN type V (Antonellis et al., 2003).

Senataxin contains a DNA/RNA helicase that causes ALS4 and dHMN. It has been proposed to participate in DNA repair (Chen et al., 2004). Mutations in senataxin have also caused ataxia-ocular apraxia type 2 (Moreira et al., 2004). Senataxin also is homologous to immunoglobulin μ-binding protein (IGHMB) 2, another DNA/RNA helicase that has a putative role in transcriptional regulation and splicing. Mutations in IGHMB2 cause a variant of infantile spinal muscular atrophy (SMA) called SMARD1 (Pitt et al., 2003).

Werdnig-Hoffman, Kugelberg-Welander, and the other classic forms (I–IV) of SMA are caused by

mutations in the survival motor neuron (SMN) protein, which is part of a complex of proteins, that participates in the assembly of spliceosomal small nuclear ribonuclear ribonucleoproteins (snRNPs) The SMN complex binds to specific sequences in the snRNAs and facilitates snRNP assembly.

Schwann Cell–Axonal Interactions

Schwann cell–axonal interactions are necessary for normal axonal function and are often disrupted in demyelinating inherited neuropathies causing significant changes in axonal physiology. Consequences of these disruptions include changes in the phosphorylation status and packing density of neurofilaments and abnormal axonal transport (de Waegh and Brady, 1990). Ultimately, axonal degeneration occurs, which may contribute more to disability than the initial demyelination (Krajewski et al., 2000). Therefore strategies directed toward preserving Schwann cell interactions may play an important role in future therapies for the demyelinating forms of CMT. Currently, these strategies are focusing on three areas, one of which is to provide trophic factor support that has been previously discussed.

The second strategy is based on the hypothesis that demyelination places increased energy demands on the neuron to generate action potentials and salutatory conduction velocity. Thinning or absence of myelin reduces its ability to maintain a charge separation that results in a leaking of capacitance. Thinning of the axon, perhaps from decreased neurofilament phosphorylation, leads to increased electrical resistance along the axon. Taken together, these factors make it more difficult for depolarization to occur at nodes of Ranvier. This "impedance mismatch" can even lead to conduction block at individual nodes of Ranvier (Waxman and Bangalore, 2004). It also places increased energy demands on the neuron to propagate action potentials by salutatory conduction. Voltage-gated potassium channels (Kv1.1 and Kv1.2) are exposed on the axolemma as a consequence of paranodal retraction, a common early feature of demyelination. As a result, potassium ions can leak out of the axon, down their concentration gradient, also making it more difficult for depolarization to occur at the node of Ranvier (Waxman and Bangalore, 2004). This has led investigators to consider the use of potassium channel blockers to treat demyelinating neuropathies including CMT1. Preliminary studies with 3,4-diaminopyridine did not demonstrate significant improvement in a population of CMT patients, most of whom had CMT1 (Russell et al., 1995). However, more specific potassium channel blockers are becoming available, including agents that are capable of blocking the channels from inside the axolemma. These may have better access to the channels than agents such as 4-aminopyridine that bind to potassium channels at their extracellular surface. Sodium channel blocking in order to protect the neuron has also been proposed as a treatment in chronic demyelinating neuropathies (Waxman and Bangalore, 2004). This approach would not prevent conduction block or promote salutatory conduction. However, it would theoretically protect the neuron from "overwork" because it would inhibit the ability of the sodium channels to depolarize the axon in the generation of the action potential.

The third strategy to improve Schawnn cell–axonal interactions is to identify and manipulate specific signaling pathways between the Schwann cell and the axon. Nave and his colleagues have recently demonstrated that PNS myelin thickness is regulated by Nrg1 signaling from axons (Bao et al., 2003). Nrg1 is the founding member of the Nrg family that belongs to the epidermal growth factor (EGF) superfamily (Lemke, 1996). Nrg1, like other members of the EGF superfamily, binds to members of the ErbB receptor tyrosine kinase family. ErbB2 and ErbB3 are neuregulin receptors expressed in Schwann cells. Ligand binding to the receptors results in their dimerization and activation of signal transduction pathways including PI3-K and *rasl*MAP kinase (reviewed in Massicotte and Scherer, 2004). Although *Nrg1* mutations have not been shown to cause CMT, manipulations of this pathway could theoretically be used to manipulate myelin thickness in the future as a treatment modality, particularly because, as the authors point out, the Nrg1 C-terminal domain can be cleaved to become a signaling molecule itself (Bao et al., 2003).

Possible sites for other specific signaling interactions between Schwann cells and axons include the adaxonal internode, and the paranodal region of the myelinating Schwann cell. The axolemma is divided into a series of polarized domains in which particular molecules are expressed in specific areas such as the node of Ranvier, paranode, juxtaparanode, and internode (Salzer, 2003). A similar organization occurs in regions of adaxonal myelin that appose these domains. Further defining molecular

Table 3
Gene Therapy Delivery Systems

Group	Examples	Cellular target
RNA viral vectors	Retroviral vectors	Dividing cells
	Lentiviral vectors	Nondividing cells
DNA viral vectors	Herpes simplex	Neurons (sensory)
	Adenoviral	Nondividing cells
	Adeno-associated viral	Nondividing cells
Nonviral	Naked DNA	Nondividing cells
	Stem cells	Cellular replacement or trophic support

pathways through which the adaxonal myelin and underlying axolemma interact may provide therapeutic targets to prevent or minimize axonal degeneration in demyelinating neuropathies. However, no other specific signaling pathways between Schwann cells and axons that might prevent axonal degeneration have yet been identified.

Gene Therapy

Gene therapy can be defined as a strategy to transfer biologically relevant genetic material into somatic cells to treat disease (Hendriks et al., 2004). Gene therapy studies have been extensively carried out for more than a decade to develop treatments for neurodegenerative diseases. In general, approaches in gene therapy have followed two paths. The first is the development of delivery vectors or systems to target therapeutic genes to diseased Schwann cells or neurons. The second has been the development of genetically engineered cargoes to be carried by the constructs to treat the neuropathy or neurodegenerative disorder.

Gene Therapy Delivery Systems

Most gene therapy delivery systems utilize replication defective viruses to act as "Trojan horses" and carry therapeutic constructs to Schwann cells or neurons. Some of these systems are discussed below and are summarized in Table 3.

Retroviral Vectors

Retroviral vectors are derived from naturally occurring single-stranded RNA viruses and are used to insert genes into the genome of dividing cells. Most of the early generation retroviral vectors were derived from the Maloney murine leukemia virus that requires the host cell to enter the S phase of the cell cycle for the integration of viral genes to enter the host genome. Retroviral vectors are made replication deficient by removing viral structural genes such as *pol*, *gag*, and *env* from the viral genome that are then replaced with mRNA encoding the minigene to be transduced by the vector. Dividing cells to be infected are cultured with the vector along with "packaging cells" that express the necessary *pol*, *gag*, and *env* genes *in trans* to allow the vector to infect the target cell. Following reverse transcription in the host cell, the resultant DNA is incorporated into the host genome in which it is transcribed from the chosen promoter. Because retroviral vectors only infect dividing cells, they will not ordinarily infect neurons or Schwann cells in vivo. They have been used to introduce a *lacZ* gene in cultured Schwann cells that were then successfully introduced into regenerating nerves in vivo (Feltri et al., 1992).

Herpes Simplex Vectors

Herpes simplex vectors (HSV) were among the first vectors used in gene therapy studies of the nervous system because of their tropism for sensory neurons and their life-long latency in neurons following deletion of immediate early (IE) genes. During the latency phase of HSV infection, the viral genome remains in an episomal state in which almost all of the viral genome is silenced. However, a portion of the "long repeat" region of the viral genome is transcribed during latency, generating a population of RNA transcripts termed "latency-associated transcripts" (LAT). Researchers have used the LAT

promoter to drive inserted transgenes in gene therapy approaches. The large cloning capacity of the region (152 Kb) is a particularly attractive feature of the HSV as is the fact that the vectors can infect postmitotic cells (reviewed in Hendriks et al., 2004). HSV expressing Bcl2 and GDNF have prevented motor neuron death following ventral root avulsion in rodents (Yamada et al., 2001). NGF-expressing HSV have also demonstrated therapeutic effects on rat superior cervical ganglia following axotomy (Federoff et al., 1992). Concerns with HSV have involved their limited tropism for neurons other than sensory neurons, the transient nature of transgene expression, and their toxicity to target cells.

Adenoviral Vectors

Recombinant adenoviral vectors (AdV), based on naturally occurring DNA adenoviruses, have been widely utilized for gene therapy approaches for more than a decade. AdV can transiently express transgene proteins (reviewed in Berkner 1992) but, unlike retroviral vectors, they can also infect nondividing cells. This is important in the PNS because the Schwann cells that ensheath axons are no longer dividing and neurons are postmitotic. Following infection with AdV, the majority of the viral DNA remains episomal in the nucleus, which avoids possible disruption of the host genome, although it limits the use of AdV in proliferating cells. Early "first-generation" and "second-generation" AdV were generated by partially replacing portions of the viral coding region with the appropriate transgene. These early generation vectors proved capable of introducing transgenes directly into in vivo Schwann cells following intraneural injection (Shy et al., 1995; Sorensen et al., 1998; Guenard et al., 1999; Jani et al., 1999; Colby et al., 2000). In addition, AdV vectors proved capable of introducing genes into both muscle and neurons after intramuscular injection (Acsadi et al., 1995; Gravel et al., 1997; Boulis et al., 1999), suggesting that adenovirus or its gene products can be delivered to the motor neuron cell body by retrograde axonal transport. However, the potential use of early generation AdV has been clearly limited by a marked cellular immune response against viral proteins, leading to diminished levels of transgene expression, reduced duration of viral-mediated gene expression, and probably also fatal complications in a patient.

As a result of these concerns, attempts have been made to generate "gutted" AdV, in which adenoviral genes have been entirely eliminated (reviewed in Cao et al., 2004). These "gutted" AdVs, with all viral sequences deleted, are not only able to accommodate larger size inserts, but the lack of viral coding sequences leads to decreased host immune response to viral proteins (neoantigens), lower cellular toxicity, and a prolonged transgene expression. A great deal of effort has recently been directed toward determining whether the "gutted" AdV can provide long-term gene expression in muscle and other tissues without the use of immunosuppression (Kochanek et al., 1996; Kumar-Singh and Chamberlain, 1996).

Adeno-Associated Viral Vectors

Adeno-associated virus serotype (AAV)-2 vectors were developed to overcome some of the immunological and transient expression difficulties associated with AdV. AAV-2 is a nonpathogenic, noncytotoxic, replication-defective virus with a broad host range (for review *see* Xiao et al. 1998; Zolotukhin et al., 2002; Grimm et al., 2003). It is capable of infecting both dividing and nondividing cells of multiple tissue origin (muscle, liver, brain, intestine, eye, and lung) (Chao et al., 2000; Kay et al., 2000; Leone et al., 2000; Sun, Li, and Xiao, 2000; Duan et al., 2001; Grimm, 2002; Xiao, 2002; Kaspar et al., 2003). It is easy to manipulate and grows to high titers as a vector (Xiao et al., 1998; Grimm, 2002; Zolotukhin et al., 2002; Grimm et al., 2003) and can lead to persistent gene transfer in the infected cell as an integrated provirus or as an episomal form (Rabinowitz et al., 2002; Grimm et al., 2003; Hoke et al., 2003). These promising features made it possible to begin human clinical gene therapy trials for hemophilia and Canavan disease with AAV-2 (Kay et al., 2000; Leone et al., 2000). There were, however, some limitations found with the use of AAV-2 vectors for human application. First, transgene transduction has been inefficient in certain clinically relevant cell types such hepatocytes and some hematopoietic cells (reviewed in Xiao, 2002). Second, as a result of prior exposure to wild-type virus, neutralizing antibodies are prevalent in the majority of the human population that will likely interfere with efficient AAV-2 vector-mediated gene transfer (Duan et al., 2001). Third, it has been shown in

animal experiments that there are also antibodies generated against the AAV-2 capsid protein that prevent the repetitive administration of the virus that may be required to either increase the level of transgene expression or to deliver two or more genes to the same recipient (Chao et al., 2000). Fourth, there are size limitations on the packaging capacity of the AAV (<4.7 kb) that has just been extended to genes larger than 5 kb (Sun et al., 2000). Finally, retrograde transport has generally been poor with AAV-2 (Kaspar et al., 2003), which is a potential significant problem for projects requiring retrograde transport.

For these reasons, newer AAV vectors have been developed, based on alternative AAV serotypes. There are now at least eight well-characterized members of the AAV family (Rutledge et al., 1998). AAV type 5 (AAV-5) is grossly similar in genetic structure and biological properties to AAV2 (Chiorini et al., 1999). However, its transduction efficiency in muscle is greater and it does not appear to induce neutralizing antibodies. It has also been shown to be highly efficient for directly transducing neuronal cells and expression was also observed at a distance from the site of delivery. AAV-6 vectors, using vectors pseudotyped with serotype 6 capsid proteins have transduced skeletal muscle of mice at levels greater than 500-fold higher than that achievable with AAV-2 vectors following direct injection (Blankinship et al., 2004).

Lentiviral Vectors

Lentiviral vectors are derived from retroviruses but are able to infect and introduce genes into nondividing cells. Original lentiviral vectors were derived from HIV (Naldini et al., 1996). Subsequent vectors have been generated from simian and feline immunodeficiency viruses (Mitrophanous et al., 1999; Trono 2000; Mazarakis et al., 2001). Core viral particles are usually pseudotyped with vesicular stomatitis viral glycoprotein G (VSVG) to permit infection of a larger number of cell types (Naldini et al., 1996; Watson et al., 2002). Particular attractive features of lentiviral vectors are the long-term expression of their transgenes and the minimal immune response they induce in the host. GDNF expression has been detected 8 mo following injection of a lentiviral vector into the basal ganglia of monkeys (Kordower et al., 2000) and β-Gal expression was detected 6 mo following injection of a lentivirus into rat brain (Blomer et al., 1997). Among other studies, the trophic factor VEGF delivered by a lentiviral vector prolonged the life of the SOD1 mouse model of FALS (Azzouz et al., 2004).

Naked DNA

A nonviral approach to gene therapy involves introducing plasmids directly into tissue under the control of a relevant promoter. Typically, this involves physical receptor-mediated or cationic liposome-mediated introduction of the plasmid DNA into the target cells (Yang et al., 2001; Wang et al., 2005). Advantages to the use of naked DNA are the minimal host immune responses associated with the treatment because there are no viral proteins to be concerned with. Limiting factors to the use of naked DNA have been poor delivery efficiency and the transient nature of transgene expression (Hendriks et al., 2004).

Stem Cells

Embryonic stem cells that could potentially develop into Schwann cells or neurons have generated a great deal of excitement among families with CMT, as well as among investigators in CMT research. However, there are formidable challenges to the use of stem cells in the inherited neuropathies. It would be a difficult challenge for stem cells to differentiate into neurons and then generate axons that would travel down limbs more than a meter before reaching their appropriate neuromuscular junction or sensory endings. Similarly, it will be difficult for stem cells to differentiate into Schwann cells and then contact axons because many mutated Schwann cells will still be ensheathing axons in demyelinating forms of CMT, even if they are not generating a normal myelin sheath. However, another potential use of stem cells might be as a source of trophic support for inherited neuropathies. Stem cells could be engineered to differentiate relevant trophic factors or other molecules and then transplanted into the PNS (Liu et al., 1999; Himes et al., 2001). For example, a clone of neural stem cells genetically modified with a retroviral vector continued to produce NT3 for at least 2 mo after being transplanted into rat spinal cord (Liu et al., 1999). Subsequent injections of the NT3 expressing stem cells partially prevented the loss of neurons in

Clarke's nucleus following transection of their axons (Himes et al., 2001).

Gene Therapy Cargo Strategies

Gene Replacement

Mutant genes that cause neuropathy by a simple loss of function of the normal gene are among the most promising targets for gene therapy. This is because the treatment should require simple gene replacement rather than repair of an abnormal toxic gain of function mechanism caused by the mutant gene. Candidate disorders for "simple" gene replacement include the autosomal-recessive forms of CMT. As with other recessive diseases, most CMT4 cases are thought to result from loss-of-function of the normal gene. In addition, a gene replacement would appear an appropriate strategy for dominant forms of CMT caused by clear loss-of-function mutations. One obvious example is HNPP, caused by the deletion of one of the two PMP22 alleles. Additional targets for gene replacement are nonsense mutations that cause premature termination of the mutant protein, and "nonsense-mediated decay" in which truncated mRNAs are degraded (Inoue et al., 2004). Finally, CMTX1 may prove susceptible to simple gene replacement. Despite the fact that there are more than 300 distinct *GJB1* mutations that cause CMTX1, many of the phenotypes appear similar to those expressed in families who have the entire *GJB1* gene deleted, suggesting that their neuropathies are caused by simple loss of function (Hahn et al., 2005).

Gene Dosage Reduction

CMT1A, the most common form of CMT, is caused by an increase rather than a decrease of PMP22. As a result, a gene therapy approach to CMT1A needs to reduce the amount of PMP22 mRNA and protein. An emerging strategy for this is the field of posttranscriptional gene silencing. Small double-stranded RNAs (dsRNA), of about 21 nucleotides, are used in plants and animals to degrade mRNA in a sequence-specific manner. Similar small inhibitory RNAs (siRNAs) can be genetically engineered to reduce the expression of target mRNAs such as PMP22 in patients with CMT1A. Catalytic RNA molecules known as ribozymes and antisense oligonucleotides, also have the potential to downregulate levels of mRNAs in a sequence-specific manner (Scherer and Rossi, 2004). In fact, an antisense oligonucleotide to *Pmp22* mRNA has been combined with an inducible promoter to generate transgenic mice in which *Pmp22* mRNA levels, and peripheral neuropathy, can be modulated by feeding the animals tetracycline (Huxley et al., 1998). In theory, because all of these approaches involve sequence specificity they could be utilized to reduce expression of genes containing missense mutations causing gain-of-function abnormalities.

Perspective

At the time of this review, no genetically affected Schwann cell or neuron has been cured of disease by gene therapy, although there have been some successes with gene therapy in animal models and in tissue culture studies. Nevertheless, it seems premature to conclude that the concept of gene therapy is a failure. Gene therapy is still a relatively new field. Newer vector systems continue to be developed. New methods of introducing gene therapy vectors are continuously emerging. For example, intravascular administration of viral vectors in the presence of vasodilators appears capable of transducing many more cells than was previously possible (Arruda et al., 2005). Moreover, it is difficult to imagine future cures for genetic neurodegenerative disorders such as CMT that will not require some form of gene therapy.

Outcome Measures

Therapeutic trials in CMT require the use of outcome measures as end points to evaluate the effectiveness of the agent in question. This is particularly challenging in CMT because disease progression is often insidious and because little natural history data on the various forms of CMT is available. Presently, there are at least three composite scoring systems that have been proposed to measure disease progression in CMT. In addition, there are a number of other measurements of disease severity, such as nerve conduction velocities, that have been used in other disorders that are potential outcome measures for CMT. Some of these outcome measures are discussed below.

CMT Neuropathy Score

The CMT Neuropathy Score (CMTNS) is based on the Total Neuropathy Score (Cornblath et al., 1999) and consists of a combined scoring system of neuropathy symptoms, signs, and neurophysiological measurements. The CMTNS has a total of 36 points and patients can be separated into mild, moderate, or severe disability depending on whether the CMTNS is less than 10, between 10 and 20, or more than 20. The CMTNS has been validated by investigators who care for large numbers of CMT patients, and has been demonstrated to have excellent inter- and intraexaminer reliability. There are no published longitudinal studies demonstrating the ability of the CMTNS to measure disease progression. However, preliminary studies have suggested that the most common form of CMT, CMT1A, progresses by about 1 point on the CMTNS over a 2-yr period (Lewis et al., 2005).

Neuropathy Impairment Score

The NIS is a quantifiable, standardized neurological examination originally called the Neurology Disability Score (Dyck et al., 1980). The NIS has a more than 250 points and has been used in multiple studies as a measure of weakness and sensory loss in peripheral neuropathies (Dyck et al., 1995). In a series of CMT1 patients published before the identification of known genetic causes for CMT, the NIS was shown to progress over a 15-yr period by what, on the average, was about 1 point per year. The NIS has also been shown to correlate with the CMTNS (Shy et al., 2005). Preliminary studies have also suggested that the NIS can identify statistically significant disease progression in a 2-yr period (Lewis et al., 2005).

Study Short Form-36 Quality-of-Life Scale

The Medical Outcome Study short form (SF)-36 is a quality-of-life (QoL) scale consisting of eight subscales that are separated into physical (PCS-36) and mental (MHC-36) scores. Because the SF-36 is a generic, as opposed to a disease-specific QoL scale, it allows comparison of specific disease groups with the population at large. Vinci and colleagues (2005) recently demonstrated that CMT patients as a group have significantly lower PCS-36 and MHC-36 scores than the general Italian population. They also found differences between older and younger patients, men and women, and patients in and out of work, but not between patients with demyelinating and axonal forms of CMT. Unlike the CMTNS or NIS, which focus entirely on physical disability, the SF-36, and potentially other QoL scales, provides insight into patients views of their disability. The addition of QoL measures in clinical trials will be essential to obtain trial participants' views regarding the effectiveness of an intervention, especially in trials in which the primary outcome measure may be one that lacks obvious relevance for patients. Examples of such outcomes include percentage reduction in muscle strength scoring or changes in sensory scores. QoL measures will also allow assessment of cost–benefit ratios of an intervention. This assessment will be important for interventions that are invasive or have major side effects. Indeed, in this light, QoL scores might prove to be the most important outcome measure for many trials (Shy and Rose, 2005).

Other Potential Outcome Measures

There are a number of other measurements of disease severity that have been used or have been proposed to be used in CMT clinical trials. Some of these are listed here, however, this list is not meant to be inclusive.

- Median and ulnar compound muscle action potential (CMAP) amplitudes have been used as measures of axonal loss. They have been used to demonstrate that disability in CMT1A correlates more with secondary axonal loss than with the primary demyelination of the disorder (Krajewski et al., 2000). In theory, therefore, CMAP amplitudes could be used as a measure of disease progression in both demyelinating and axonal forms of CMT.
- The 9-Hole Peg test is a timed test to extract and replace pegs in a 9-hole platform. It has been used extensively in literature on multiple sclerosis (Cohen et al., 2001) and has been shown to correlate with disability in CMT1A (Krajewski et al., 2000).
- The Ambulation Index or timed walk is based on the patient's timed ability to walk a distance of 25 ft and whether the patient requires orthotics or ambulation aids to walk this distance. The Ambulation Index has been shown to correlate with disability in CMT1A (Krajewski et al., 2000).

- The pinch test is a small handheld device that measures the strength generated by squeezing the device between the thumb and index finger or between the thumb and the tips of both the index and middle fingers. Two trials are performed for each hand. It is being utilized because it measures strength in intrinsic hand muscle groups particularly affected in CMT.
- Grip testing is chosen because it is an easily performed test that can be performed in either hand. It has been particularly sensitive in evaluating peripheral neuropathy weakness (Hahn, personal communication).

Prenatal Genetic Diagnosis

Couples at risk for transmitting genetic disorders are increasingly interested in research advances that can ensure that their children will not be born with or develop significant phenotypic abnormalities. Past options for these couples have included deciding to remain childless, considering gamate donation or adoption, or having a prenatal diagnosis followed by termination in case of an affected fetus (reviewed in Ogilvie et al., 2005). There are obviously personal ethical considerations involved in these and related issues designed to ensure that a developing fetus is not born with a particular disease. However, recent advances in preimplantation genetic diagnosis (PGD) have given parents an additional option to reduce the likelihood of transmitting inherited diseases such as CMT.

PGD utilizes standard techniques of in vitro fertilization (IGF) and in vitro embryo culture. The fertilized egg typically undergoes reductive cell division and reaches the eight-cell stage around 3 d postfertilization. By the time the embryo reaches the blastocyst stage, the discrete clump of cells destined to become the fetus is identifiable. PGD requires the biopsy of either the oocyte or this developing embryo. The biopsied material is tested for the genetic disorder, usually by a form of polymerase chain reaction (PCR) or fluorescence *in situ* hybridization (FISH), and unaffected embryos are transferred to the uterus. As an alternative, first and second polar bodies are biopsied and results used to infer the genetic diagnosis although these results may be less certain. The most widely used approach is to biopsy single blastomeres from embryos on day 3 (reviewed in Ogilvie et al., 2005).

PGD has been used in CMT, although its use is not widespread and there is only a little data available to judge its effectiveness. One recent report documents the effective use of PGD in five couples with CMT1A, caused by the duplication on chromosome 17. Fluorescence-based PCR-based tests were utilized to ensure that healthy, unaffected embryos were transferred to the uterus (De Vos et al., 2003). In theory, a multiplex PCR-based approach should prove capable for detecting missense mutations causing other forms of CMT, although data supporting the effectiveness of such studies in CMT is not yet available.

Traditional Therapies

Patients, physicians, and scientists are understandably concerned with new and future treatments directed at curing CMT and other genetic neurodegenerative diseases. However, patients need treatment that is currently available. Unfortunately, an all too common experience is that patients with CMT are told that there is nothing that can be done for them. Therapy that is currently available can help patients ambulate independently for many years, and plan for their families by knowing who is at risk to develop CMT.

Genetic Counseling

Competent genetic counseling is an extremely important element in the management of patients with inherited neuropathy and their families. Such counseling requires knowledge of the genetic and neurological features of the underlying disorder. It is based on the concept that a nondirective counseling approach is best for handling the complex issues that can arise when the diagnosis of an inherited condition is made. Patients with inherited conditions often seek further information regarding various decisions, including those concerning family planning. All options available to the patient, including prenatal testing, PGD, sperm and egg donation, adoption, having children without any testing, and having no children, can be explored. Patients are aided in making decisions that best fit their beliefs, values, culture, and lifestyle. This approach can be difficult, especially when the clinician faces the inevitable question, "What would you do?" Although it is tempting to answer this

question with an opinion, it is important to keep in mind that the answer given may not be the best solution for a given patient. Nondirective counseling is based on the principle of autonomy and the belief that an individual is the person who knows what decisions are best for his or her life.

One mechanism for neurologists to deal with these time-consuming issues is to extend the multidisciplinary approach for neuromuscular disease to include a genetics professional. The need for a multidisciplinary approach in the care of patients with neuromuscular disease has been recognized for some time. For example, psychiatry, physical and occupational therapy, orthopedics, and pulmonary support have greatly contributed to patient care. Similarly, the presence of a geneticist or genetic counselor can greatly enhance the ability of a neuromuscular clinic to provide quality care to patients with inherited disease. Genetic counselors are specially trained to help individuals deal with the complex issues that arise when the diagnosis of a genetic disease is made or is being considered.

Physical Therapy and Exercise

Rehabilitation plays an essential role in preserving the quality of life of CMT patients, but data on which approaches should be standard is limited, owing to the insufficient amount of scientific studies in this field. Weakness and wasting, involving the foot intrinsic muscles and later the leg muscles, are responsible for susceptibility to ankle sprains, poor balance, pain, clumsy gait, stepping gait, and foot deformities including high arch foot (*pes cavus*), *pes equinovarus*, and hammer toes. Weaknesses of proximal muscles of the lower limbs are less commonly observed. In the upper limbs, the impairment of the intrinsic muscles of the hands starts later and is almost never extreme, nonetheless many patients complain of reduced dexterity. Therefore, a good rehabilitation program should increase muscle strength of those muscles that still function, improve mobility, prevent joint deformities and falls, and ameliorate hand function.

The role of exercise has been addressed in a few studies in which CMT patients were included. In one of these, a 12-wk moderate resistance (30% of maximum isometric force) exercise program led to an improvement of muscular strength ranging from 4 to 20% without any notable deleterious effects. The same group of patients underwent a 12-wk high-resistance (training at the maximum weight a subject could lift 12 times) exercise program, without further benefit compared with the moderate resistance program, and with evidence of overwork weakness in some of the participants (Chetlin et al., 2004a, 2004b). Overwork weakness is a potential concern in CMT, as in other neuromuscular diseases. In this respect, any exercise program that causes muscles to feel weaker within 30 min after exercise, or that causes excessive muscle soreness or severe muscle cramping is discouraged. Certain low-impact aerobic exercise such as walking and swimming can improve cardiovascular performance, increase muscle efficiency, and help control body weight. Thus, these are useful with many patients with CMT. Gentle stretching may help to fight muscle contractures.

Assistive Devices

Depending on the level of muscle weakness and wasting, different devices can be recommended. An initial footdrop with varus deformity and foot inversion can benefit from a lateral wedge to induce eversion and redistribute loading to a larger area of the foot. A more pronounced footdrop usually requires ankle–foot orthoses (AFOs) that should be custom-made and possibly of a lightweight material like polypropylene, carbon-fiber resin, or sylicone, and fit intimately to provide good stability and prevent pressure sores. The older traditional double metal upright AFOs built into the shoe may be too heavy. Most patients require a short course of physical therapy after the braces are made to help them use the braces effectively.

Medications and CMT

Medications to Avoid

A common concern of CMT patients is whether particular medications might exacerbate their neuropathy. In general, medications that have clear neurotoxic affects such as vincristine or cisplatin should be avoided, if medically possible, in CMT patients because they are likely to exacerbate the already existing neuropathy. There have been reports of severe, Guillain Barré-type weakness in patients with CMT who were given vincristine. For other medications the situation is less clear. The

CMT Association publishes a list of medications on its website and in its newsletter that may exacerbate CMT. The degree of risk varies with the individual medication and in some cases, the risk may be small compared with the medical need. Good judgment by the physician on the risk–benefit ratio of a given medication can probably suffice as a useful guide for the use of these medicines. The literature of medications exacerbating CMT has been recently reviewed (Weimer, in press).

Pain Management

Most patients with CMT do not develop neuropathic pain, characterized by burning or painful dysesthesias in their feet or hands. Nevertheless, a minority of patients do experience these symptoms and the subject is briefly addressed. Neuropathic pain can be quite difficult to treat and usually requires combination therapy in the opinion of this author. Some of the more effective agents are listed here.

Topical Agents

Lidocaine and prilocaine emulsion are used topically for the treatment of painful neuropathies and are primarily effective on areas they are in direct contact with, such as the surface of the feet.

Tricyclic Antidepressant

Amitriptyline, nortriptyline, and desipramine are low-cost, efficacious medications. Amitriptyline is the most frequently administered drug belonging to this category. The dosage required for pain control may be significantly lower compared with the doses normally used for antidepressive purposes. Patients typically begin with lower doses, at bedtime, which are decreased until they prove effective or induce toxicity. The main side effects include orthostatic hypotension, dry mouth, urinary retention, confusion, and somnolence. They may also increase the risk of cardiac arrhythmias.

Antiepileptic Drugs

Gabapentin is an anticonvulsivant that has been recently approved by the Food and Drug Administration for the treatment of neuropathic pain. The mechanism of action of this molecule is not completely clear, but it was designed as a precursor of γ-aminobutyric acid and was shown to increase the γ-aminobutyric acid content in brain synapses. It is also supposed to decrease the influx of calcium ions into neurons. The main side effects of dizziness, gait problems, and somnolence usually disappear after 10 d of treatment and may be minimized by slow increases in dosage. The only contraindication is renal failure.

Lamotrigine

Lamotrigine acts through the inhibition of voltage-gated sodium channels and has been demonstrated to be efficacious in the painful neuropathy associated with HIV. The usual dose is 200–500 mg/d divided in two doses. The starting dose is 50 mg, which should be incremented of 50 mg every 2 wk. The reason for slow increments is to prevent hypersensitivity. Side effects include dizziness, ataxia, and nausea. If a skin rash appears, the drug should be discontinued because more serious allergic reactions may develop. Multiorgan failure or blood dyscrasias are rare but serious concerns.

Carbamazepine

Carbamazepine is mainly used for trigeminal neuralgia, but has been proposed also for other painful neuropathies. The dosage is 200–400 mg, divided into two to three doses daily. The most common side effects of carbamazepine are dizziness, ataxia, and dyspepsia and may be prevented by slow increments. A complete blood count should be carefully monitored to detect blood dyscrasias, including agranulocytosis and aplastic anemia, which may occur as a result of idiosyncrasy (reviewed in Grandis and Shy, in press). Although beyond the scope of this review, many CMT patients develop chronic, aching pain in joints as a result of arthritic damage exacerbated by their CMT. These pains are typically treated with medications such as nonsteroidal anti-inflammatory drugs that are used to treat chronic osteoarthritis pain in patients who do not have CMT.

Conclusions

All too often, patients with heritable neuropathies have been told by their physicians that nothing can be done for their neuropathies and that they simply have to learn to live with their disabilities. There may have been some truth to this before 1991, when the first genetic cause of CMT was identified. However, since then, advances in molecular genetics and molecular biology have

made this nihilistic advice clearly outdated. It is now possible to offer precise genetic diagnosis to many patients and to predict who in their families are at risk for developing CMT. It is also now realistic to discuss with patients the possibilities of biologically based strategies to treat and eventually cure these neuropathies, if not for them than for their children. If scientific advances over the next 15 yr, match those of the past 15 yr physicians will hopefully begin to be able to offer effective, rational treatments for not only CMT but for related neurodegenerative diseases as well.

Acknowledgment

This work was supported in part by grants from the NIH/NINDS, Muscular Dystrophy Association and the Charcot-Marie-Tooth Association.

References

Acsadi G., Anguelov R. A., Yang H., et al. (2002) Increased survival and function of SOD-1after GDNF gene transfer. *Hum. Gene. Ther.* **13,** 1047–1059.

Acsadi G., Massie B., and Jani A. (1995) Adenovirus-mediated gene transfer into striated muscles. *J. Mol. Med.* **73(4),** 165–180.

Antonellis A., Ellsworth R. E., Sambuughin N., et al. (2003) Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. *Am. J. Hum. Genet.* **72(5),** 1293–1239.

Apfel S. C. (2002) Is the therapeutic application of neurotrophic factors dead? *Ann. Neurol.* **51(1),** 8–11.

Apfel S. C. (2002) Nerve growth factor for the treatment of diabetic neuropathy: what went wrong, what went right, and what does the future hold? *Int. Rev. Neurobiol.* **50,** 393–413.

Arroyo E. J. and Scherer S. S. (2000) On the molecular architecture of myelinated fibers. *Histochem. Cell Biol.* **113(1),** 1–18.

Arruda V. R., Stedman H. H., Nichols T. C., et al. (2005) Regional intravascular delivery of AAV-2-F.IX to skeletal muscle achieves long-term correction of hemophilia B in a large animal model. *Blood* **105(9),** 3458–3464. Epub 2004 Oct 12.

Azzouz M., Ralph G. S., Storkebaum E., et al. (2004) VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature* **429(6990),** 413–417.

Bao J., Wolpowitz D., Role L. W., and Talmage D. A. (2003) Back signaling by the Nrg-1 intracellular domain. *J. Cell Biol.* **161(6),** 1133–1141.

Bennett C. L., Shirk A. J., Huynh H. M., et al. (2004) SIMPLE mutation in demyelinating neuropathy and distribution in sciatic nerve. *Ann. Neurol.* **55(5),** 713–720.

Bergoffen J., Scherer S. S., Wang S., et al. (1993) Connexin mutations in X-linked Charcot-Marie-Tooth disease. *Science* **262(5142),** 2039–2042.

Berkner K. L. (1992) Expression of heterologous sequences in adenoviral vectors. *Curr. Top Microbiol. Immunol.* **158**, 39–66.

Bhattacharya R., Kang-Decker N., Hughes D. A., et al. (2005) Regulatory role of dynamin-2 in VEGFR-2/KDR-mediated endothelial signaling. *FASEB J.* **19**, 1692–1694.

Bird T. D., Ott J., and Giblett E. R. (1982) Evidence for linkage of Charcot-Marie-Tooth neuropathy to the Duffy locus on chromosome 1. *Am. J. Hum. Genet.* **34(3),** 388–394.

Blankinship M. J., Gregorevic P., Allen J. M., et al. (2004) Efficient transduction of skeletal muscle using vectors based on adeno-associated virus serotype 6. *Mol. Ther.* **10(4),** 671–678.

Blomer U., Naldini L., Kafri T., Trono D., Verma I. M., and Gage F. H. (1997) Highly efficient and sustained gene transfer in adult neurons with a lentivirus vector. *J. Virol.* **71(9),** 6641–6649.

Bomont P., Cavalier L., Blondeau F., et al. (2000) The gene encoding gigaxonin, a new member of the cytoskeletal BTB/kelch repeat family, is mutated in giant axonal neuropathy. *Nat. Genet.* **26(3),** 370–374.

Boulis N. M., Turner D. E., Dice J. A., Bhatia V., and Feldman E. L. (1999) Characterization of adenoviral gene expression in spinal cord after remote vector delivery. *Neurosurgery* **45(1),** 131–137; discussion 137, 138.

Bunge M. B., Williams A. K., Wood P. M., Uitto J., and Jeffrey J. J. (1980) Comparison of nerve cell and nerve cell plus Schwann cell cultures, with particular emphasis on basal lamina and collagen formation. *J. Cell Biol.* **84(1),** 184–202.

Bunge R. P. (1987) Tissue culture observations relevant to the study of axon-Schwann cell interactions during peripheral nerve development and repair. *J. Exp. Biol.* **132,** 21–34.

Cao H., Koehler D. R., and Hu J. (2004) Adenoviral vectors for gene replacement therapy. *Viral Immunol.* **17(3),** 327–333.

Chance P. F., Alderson M. K., Leppig K. A., et al. (1993) DNA deletion associated with hereditary neuropathy with liability to pressure palsies. *Cell* **72(1),** 143–151.

Chao H., Liu Y., Rabinowitz Li C., Samulski R. J., and Walsh C. E. (2000) Several log increase in therapeutic transgene delivery by distinct adeno-associated viral serotype vectors. *Mol. Ther.* **2(6),** 619–623.

Charcot J. and Marie P. (1886) Sur une forme particulaire d'atrophie musculaire progressive souvent familial debutant par les pieds et les jamber et atteingnant plus tard les mains. *Rev. Med.* **6**, 97–138.

Chen Y. Z., Bennett C. L., Huynh H. M., et al. (2004) DNA/RNA Helicase Gene Mutations in a Form of Juvenile Amyotrophic Lateral Sclrosis (ALS4*) Am. J. Hum. Genet.* **74(6),** 1128–1135. Epub 2004 Apr 21.

Chetlin R. D., Gutmann L., Tarnopolsky M. A., Ullrich I. H., and Yeater R. A. (2004a) Resistance training effectiveness in patients with Charcot-Marie-Tooth disease, recommendations for exercise prescription. *Arch. Phys. Med. Rehabil.* **85(8),** 1217–1223.

Chetlin R. D., Gutmann L., Tarnopolsky M. A., Ullrich I. H., and Yeater R. A. (2004b) Resistance training exercise and creatine in patients with Charcot-Marie-Tooth disease. *Muscle Nerve* **30(1),** 69–76.

Chiorini J. A., Afione S., and Kotin R. M. (1999) Adeno-associated virus (AAV) type 5 Rep protein cleaves a unique terminal resolution site compared with other AAV serotypes. *J. Virol.* **73(5),** 4293–4298.

Cohen J. A., Cutter G. R., Fischer J. S., et al. (2001) Use of the multiple sclerosis functional composite as an outcome measure in a phase 3 clinical trial. *Arch. Neurol.* **58(6),** 961–967.

Colby J., Nicholson R., Dickson K. M., et al. (2000) PMP22 Carrying the Trembler or Trembler-J Mutation Is Intracellularly Retained in Myelinating Schwann Cells. *Neurobiol. Dis.* **7(6 Pt B),** 561–573.

Cornblath D. R., Chaudhry V., Carter K., et al. (1999) Total neuropathy score, validation and reliability study. *Neurology* **53(8),** 1660–1664.

De Vos A., Sermon K., De Rijcke M., et al. (2003) Preimplantation genetic diagnosis for Charcot-Marie-Tooth disease type 1A. *Mol. Hum. Reprod.* **9(7),** 429–435.

de Waegh S. and Brady S. T. (1990) Altered slow axonal transport and regeneration in a myelin-deficient mutant mouse: the trembler as an in vivo model for Schwann cell–axon interactions. *J. Neurosci.* **10(6),** 1855–1865.

Dejerine H. and Sottas J. (1893) Sur la nevritte interstitielle, hypertrophique et progressive de l'enfance. *CR Soc. Biol. Paris* **45**, 63–96.

Ding J., Liu J. J., Kowal A. S., et al. (2002) Microtubule-associated protein 1B: a neuronal binding partner for gigaxonin. *J. Cell Biol.* **158(3),** 427–433. Epub 2002 Jul 29.

Duan D., Yan Z., Yue Y., Ding W., and Engelhardt J. F. (2001) Enhancement of muscle gene delivery with pseudotyped adeno-associated virus type 5 correlates with myoblast differentiation. *J. Virol.* **75**(16), 7662–7671.

Dyck P. J. and Lambert E. H. (1968) Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic, and electrophysiologic findings in hereditary polyneuropathies. *Arch. Neurol.* **18(6),** 603–618.

Dyck P. J. and Lambert E. H. (1968) Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. II. Neurologic, genetic, and electrophysiologic findings in various neuronal degenerations. *Arch. Neurol.* **18(6),** 619–625.

Dyck P. J., Litchy W. J., Lehman K. A., Hokanson J. L., Low P. A., and O'Brien P .C. (1995) Variables influencing neuropathic endpoints: the Rochester Diabetic Neuropathy Study of Healthy Subjects. *Neurology* **45(6),** 1115–1121.

Dyck P. J., Sherman W. R., Hallcher L. M., et al. (1980) Human Diabetic Endoneurial Sorbitol, Fructose, and myo-Inositol Related to Sural Nerve Morphometry. *Ann. Neurol.* **8,** 590–596.

Federoff H. J., Geschwind M. D., Geller A. I., and Kessler J. A. (1992) Expression of nerve growth factor in vivo from a defective herpes simplex virus 1 vector prevents effects of axotomy on sympathetic ganglia. *Proc. Natl. Acad. Sci. USA* **89(5),** 1636–1640.

Feltri M. L., Scherer S. S., Wrabetz L., Kamholz J., and Shy M. E. (1992) Mitogen-expanded Schwann cells retain the capacity to myelinate regenerating axons after transplantation into rat sciatic nerve. *Proc. Natl. Acad. Sci. USA* **89(18),** 8827–8831.

Fortun J., Dunn W. A., Jr., Joy S., Li, J., and Notterpek L. (2003) Emerging role for autophagy in the removal of aggresomes in Schwann cells. *J. Neurosci.* **23(33),** 10,672–10,680.

Fortun J., Li J., Go J., Fenstermaker A., Fletcher B. S., and Notterpek L. (2005) Impaired proteasome activity and accumulation of ubiquitinated substrates in a hereditary neuropathy model. *J. Neurochem*. **92(6),** 1531–1541.

Grandis M. and Shy M. E. Peripheral Neuropathies. In*:* Rackel R. and Bope E. T. (eds.), *Conn's Current Therapy 2006*. Elsevier, Philadelphia, in press.

Gravel C., Gotz R., Lorrain A., and Sendtner M., (1997) Adenoviral gene transfer of ciliary neurotrophic factor and brain-derived neurotrophic factor leads to long-term survival of axotomized motor neurons. *Nat. Med.* **3(7),** 765–770.

Griffin J. W. and Watson D. F. (1988) Axonal transport in neurological disease. *Ann. Neurol.* **23(1),** 3–13.

Grimm D. (2002) Production methods for gene transfer vectors based on adeno-associated virus serotypes. *Methods* **28(2),** 146–157.

Grimm D., Kay M. A., and Kleinschmidt J. A. (2003) Helper virus-free, optically controllable, and two-plasmid-based production of adeno-associated virus vectors of serotypes 1 to 6. *Mol. Ther.* **7(6),** 839–850.

Grimm D., Zhou S., Nakai H., et al. (2003) Preclinical in vivo evaluation of pseudotyped adeno-associated virus vectors for liver gene therapy. *Blood* **102(7),** 2412–2419. Epub 2003 Jun 05.

Guenard V., Schweitzer B., Flechsig E., et al. (1999) Effective gene transfer of lacZ and P0 into Schwann cells of P0-deficient mice. *Glia* **25(2),** 165–178.

Gurunathan S., David D., and Gerst J. E. (2002) Dynamin and clathrin are required for the biogenesis of a distinct class of secretory vesicles in yeast. *EMBO J.* **21(4),** 602–614.

Hahn A., Ciskind C., Krajewski K., Lewis R., and Shy M. E. (2005) Genotype–Phenotype Correlations in CMTX1 (Abstract) *J. Peripheral Nervous System* **10(Suppl. 1),** 31, 32.

Harding A. E. and Thomas P. K. (1980) The clinical features of hereditary motor and sensory neuropathy types I and II. *Brain* **103(2),** 259–280.

Harding A. E. and Thomas P. K. (1980) Genetic aspects of hereditary motor and sensory neuropathy (types I and II) *J. Med. Genet.* **17(5),** 329–336.

Harrison R. G. (1924) Neuroblast versus sheath cell in the development of peripheral nerves. *J. Comp. Neurol.* **37,** 123–194.

Hayasaka K., Himoro M., Sato W., et al. (1993) Charcot-Marie-Tooth neuropathy type 1B is associated with mutations of the myelin P0 gene. *Nat. Genet.* **5(1),** 31–34.

Hendriks W. T., Ruitenberg M. J., Blitis B., Boer G. J., and Verhaagen J., et al. (2004) Viral vector-mediated gene transfer of neurotrophins to promote regeneration of the injured spinal cord. *Prog. Brain Res.* **146**, 451–476.

Himes B. T., Liu Y., Solowska J. M., Snyder E. Y., Fischer I., and Tessler A. (2001) Transplants of cells genetically modified to express neurotrophin-3 rescue axotomized Clarke's nucleus neurons after spinal cord hemisection in adult rats. *J. Neurosci. Res.* **65(6),** 549–564.

Hoke A., Ho T., Crawford T. O., LeBel C., Hilt D., and Griffin J. W. (2003) Glial cell line-derived neurotrophic factor alters axon schwann cell units and promotes myelination in unmyelinated nerve fibers. *J. Neurosci.* **23(2),** 561–567.

Huxley C., Passage E., Manson A., et al. (1996) Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum. Mol. Genet.* **5(5),** 563–569.

Huxley C., Passage E., Robertson A. M., et al. (1998) Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. *Hum. Mol. Genet.* **7(3),** 449–458.

Inoue K., Khajavi M., Ohyama T., et al. (2004) Molecular mechanism for distinct neurological phenotypes conveyed by allelic truncating mutations. *Nat. Genet.* **36(4),** 361–369. Epub 2004 Mar 7.

Jani A., Menichella D., Jiang H., et al. (1999) Modulation of cell-mediated immunity prolongs adenovirus-mediated transgene expression in sciatic nerve. *Hum. Gene Ther.* **10(5),** 787–800.

Kaspar B. K., Llado J., Sherkat N., Rothstein J. D., and Gage F. H. (2003) Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* **301(5634),** 839–842.

Kay M. A., Manno C. S., Ragni M. V., et al. (2000) Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector. *Nat. Genet.* **24(3),** 257–261.

Khajavi M., et al. (2005) Curcumin treatment abrogates endoplasmic reticulum retention and aggregation-induced apoptosis associated with neuropathy-causing myelin protein zero-truncating mutants. *Am. J. Hum. Genet.* **77(5),** 841–850. Epub 2005 Sep 30.

Kochanek S., Clemens P. R., Mitani K., Chen H. H., Chan S., and Caskey C. T. (1996). A new adenoviral vector: replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. *Proc NAtl Acad Sci USA* **93(12),** 5731–5736.

Kordower J. H., Emborg M. E., Bloch J., et al. (2000) Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson disease. *Science* **290(5492),** 767–773.

Krajewski K. M., Lewis R. A., Fuerst D. R., et al. (2000) Neurological dysfunction and axonal degeneration in Charcot-Marie-Tooth disease type 1A. *Brain* **123(Pt 7),** 1516–1527.

Kretz A., Happold C. J., Marticke J. K., and Isenmann S. (2005) Erythropoietin promotes regeneration of adult CNS neurons via Jak2/Stat3 and PI3K/AKT pathway activation. *Mol. Cell Neurosci.* **29(4),** 569–579.

Kumar-Singh R. and Chamberlain J. S. (1996). Encapsidated adenovirus minichromosomes allow delivery and expression of a 14 kb dystrophin cDNA to muscle cells. *Hum Mol Genet* **5**(7):913-922

Le Douarin N. M. and Dupin E. (1993) Cell lineage analysis in neural crest ontogeny. *J. Neurobiol.* **24,** 146–161.
Lemke G. (1996) Neuregulins in development. *Mol. Cell Neurosci.* **7(4),** 247–262.
Leone P., Janson C. G., Bilaniuk L., et al. (2000) Aspartoacylase gene transfer to the mammalian central nervous system with therapeutic implications for Canavan disease. *Ann. Neurol.* **48(1),** 27–38.
Lewis R. A., McDermott M., Krajewski K., Herrmann D. N., and Shy M. E. (2005) CMT Neuropathy Score identifies two year progression in CMY1A patients. *J. Peripheral Nervous Syst. (Abstr.)* **10(Suppl. 1),** 52.
Li J., Krajewski, K., Lewis R. A., and Shy M. E. (2004) Loss-of-function phenotype of hereditary neuropathy with liability to pressure palsies. *Muscle Nerve* **29(2),** 205–210.
Li X., Gonias S. L., and Campana W. M. (2005) Schwann cells express erythropoietin receptor and represent a major target for Epo in peripheral nerve injury. *Glia* **51(4),** 254–265.
Liu Y., Himes B. T., Solowska J., et al. (1999) Intraspinal delivery of neurotrophin-3 using neural stem cells genetically modified by recombinant retrovirus. *Exp. Neurol.* **158(1),** 9–26.
Lupski J. R., de Oca-Luna R. M., Slaugenhaupt S., et al. (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. *Cell* **66(2),** 219–232.
Magyar J. P., Martini R., Ruelicke, et al. (1996) Impaired differentiation of Schwann cells in transgenic mice with increased PMP22 gene dosage. *J. Neurosci.* **16(17),** 5351–5360.
Manabe Y., Nagano I., Gazi M. S., et al. (2002) Adenovirus-mediated gene transfer of glial cell line-derived neurotrophic factor prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. *Apoptosis* **7(4),** 329–334.
Massicotte C. and Scherer S. S. (2004) Neuropathies—Translating Causes into Treatments. In: Waxman S. G. (ed.), *Neuroscience, Molecular Medicine, and the Therapeutic Transformation of Neurology.* Elsevier Science, London, pp. 401–414.
Mazarakis N. D., Azzouz M., Rohll J. B., et al. (2001) Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum. Mol. Genet.* **10(19),** 2109–2121.
McArthur J. C., Yiannoutsos C., Simpson D. M., et al. (2000) A phase II trial of nerve growth factor for sensory neuropathy associated with HIV infection. AIDS Clinical Trials Group Team 291. *Neurology* **54(5),** 1080–1088.
Mirsky R. and Jessen K. R. (1996) Schwann cell development, differentiation and myelination. *Curr. Opin. Neurobiol.* **6(1),** 89–96.
Mitrophanous K., Yoon S., Rohll, et al. (1999) Stable gene transfer to the nervous system using a non-primate lentiviral vector. *Gene Ther.* **6(11),** 1808–1818.
Moreira M. C., Klur S., Watanabe M., et al. (2004) Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat. Genet.* **36(3),** 225–227. Epub 2004 Feb 8.
Naldini L., Blomer U., Gage F. H., Trono D., and Verma I. M. (1996) Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc. Natl. Acad. Sci. USA* **93(21),** 11,382–11,388.
Naldini L., Blomer U., Gallay P., et al. (1996) In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science* **272(5259),** 263–267.
Navon R., Seifried B., Gal-On N. S., and Sadeh M. (1996) A new point mutation affecting the fourth transmembrane domain of PMP22 results in severe de novo Charcot-Marie-Tooth disease. *Hum. Genet.* **97(5),** 685–687.
Nishimura A. L., Mitne-Neto M., Silva H. C., et al. (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* **75(5),** 822–831. Epub 2004 Sep 15.
Ogilvie C. M., Braude P. R., and Scriven P. N. (2005) Preimplantation genetic diagnosis—an overview. *J. Histochem. Cytochem.* **53(3),** 255–260.
Okado-Matsumoto A. and Fridovich I. (2001) Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu, Zn-SOD in mitochondria. *J. Biol. Chem.* **276(42),** 38,388–38,393. Epub 2001 Aug 15.
Pareek S., Notterpek L., Snipes G. J., et al. (1997) Neurons promote the translocation of peripheral myelin protein 22 into myelin. *J. Neurosci.* **17(20),** 7754–7762.
Pasinelli P., Belford M. E., Lennon N., et al. (2004) Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron* **43(1),** 19–30.
Passage E., Norreel J. C., Noack-Fraissignes P., et al. (2004) Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. *Nat. Med.* **10(4),** 396–401. Epub 2004 Mar 21.
Pedrola L., Espert A., Wu X., Claramunt R., Shy M. E., and Palau F. (2005) GDAP1, the protein causing Charcot-Marie-Tooth disease type 4A, is expressed

in neurons and is associated with mitochondria. *Hum. Mol. Genet.* **14(8),** 1087–1094.

Pitt M., Houlden H., Jacobs J., et al. (2003) Severe infantile neuropathy with diaphragmatic weakness and its relationship to SMARD1. *Brain* **126(Pt 12),** 2682–2692. Epub 2003 Sep 23.

Pradat P. F. (2003) Treatment of peripheral neuropathies with neutrotrophic factors: animal models and clinical trials. *Rev. Neurol. (Paris)* **159(2),** 147–161.

Puls I., Jonnakuty C., LaMonte B. H., et al. (2003) Mutant dynactin in motor neuron disease. *Nat. Genet.* **33(4),** 455, 456.

Rabinowitz J. E., Rolling F., Li C., et al. (2002) Cross-packaging of a single adeno-associated virus (AAV) type 2 vector genome into multiple AAV serotypes enables transduction with broad specificity. *J. Virol.* **76(2),** 791–801.

Raeymaekers P., Timmerman, V., Nelis E., et al. (1991) Duplication in chromosome 17p11.2 in Charcot-Marie-Tooth neuropathy type 1a (CMT 1a) The HMSN Collaborative Research Group. *Neuromuscul. Disord.* **1(2),** 93–97.

Reid E., Kloos M., Ashley-Koch A., et al. (2002) A kinesin heavy chain (KIF5A) mutation in hereditary spastic paraplegia (SPG10) *Am. J. Hum. Genet.* **71(5),** 1189–1194. Epub 2002 Sep 24.

Roa B. B., Garcia C. A., Suter U., et al. (1993) Charcot-Marie-Tooth disease type 1A. Association with a spontaneous point mutation in the PMP22 gene. *N. Engl. J. Med.* **329(2),** 96–101.

Roussy G. and Levy G. (1926) A sept cas d'une maladie familiale particulaire. *Rev. Neurol.* **33,** 427–450.

Russell J. W., Windebank A. J., and Harper C. M. (1995) Treatment of stable chronic demyelinating polyneuropathy with 3,4-diaminopyridine. *Mayo Clin. Proc.* **70(6),** 532–539.

Rutledge E. A., Halbert C. L., and Russell D. W. (1998) Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. *J. Virol.* **72(1),** 309–319.

Sahenk Z., Nagaraja H. N., McCracken B. S., et al. (2005) Neurotrophin-3 Promotes Nerve Regeneration and Improvements in sensory function in CMT1A Results from animal models and a pilot clinical study. *Neurology* **65,** 681–685**.**

Salzer J. L. (2003) Polarized domains of myelinated axons. *Neuron* **40(2),** 297–318.

Scherer L. and Rossi J. J. (2004) Recent applications of RNAi in mammalian systems. *Curr. Pharm. Biotechnol.* **5(4),** 355–360.

Scherer S. S. (1997) The biology and pathobiology of Schwann cells. *Curr. Opin. Neurol.* **10(5),** 386–397.

Sereda M., Griffiths I., Puhlhofer, et al. (1996) A transgenic rat model of Charcot-Marie-Tooth disease. *Neuron* **16(5),** 1049–1060.

Sereda M. W., Meyer Zu Horste G., Suter U., Uzma N., and Nave K. A. (2003) Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A) *Nat. Med.* **9(12),** 1533–1537.

Shames I., Fraser A., Colby J., Orfali W., and Snipes G. J. (2003) Phenotypic differences between peripheral myelin protein-22 (PMP22) and myelin protein zero (P0) mutations associated with Charcot-Marie-Tooth-related diseases. *J. Neuropathol. Exp. Neurol.* **62(7),** 751–764.

Shy M. E., Blake J., Krajewski K., et al. (2005) Reliability and validity of the CMT neuropathy score as a measure of disability. *Neurology* **64(7),** 1209–1214.

Shy M. E., and Rose M. R., (2005) Charcot Marie Tooth disease impairs quality of life but why? (and how do we improve it?), Editorial. *Neurology* **65,** 790, 791.

Shy M. E., Tani M., Shi Y .J., et al. (1995) An adenoviral vector can transfer lacZ expression into Schwann cells in culture and in sciatic nerve. *Ann. Neurol.* **38(3),** 429–436.

Sorensen J., Haase G., Krarup C., Gilgenkrantz H., Kahn A., and Schmalbruch H. (1998) Gene transfer to Schwann cells after peripheral nerve injury: a delivery system for therapeutic agents. *Ann. Neurol.* **43(2),** 205–211.

Southwood C. M., Garbern J., Jiang W., and Gow A. (2002) The unfolded protein response modulates disease severity in Pelizaeus-Merzbacher disease. *Neuron* **36(4),** 585–596.

Street V. A., Bennett C. L., Goldy J. D., et al. (2003) Mutation of a putative protein degradation gene LITAF/SIMPLE in Charcot-Marie-Tooth disease 1C. *Neurology* **60(1),** 22–26.

Sun L., Li J., and Xiao X. (2000) Overcoming adeno-associated virus vector size limitation through viral DNA heterodimerization. *Nat. Med.* **6(5),** 599–602.

Suter U., Moskow J. J., Welcher A. A., et al. (1992) A leucine-to-proline mutation in the putative first transmembrane domain of the 22-kDa peripheral myelin protein in the trembler-J mouse. *Proc. Natl. Acad. Sci. USA* **89(10),** 4382–4386.

Suter U. and Scherer S. S. (2003) Disease mechanisms in inherited neuropathies. *Nat. Rev. Neurosci.* **4(9),** 714–726.

Suter U., Welcher A. A., Ozcelik T., et al. (1992) Trembler mouse carries a point mutation in a myelin gene. *Nature* **356(6366),** 241–244.

Thomas P. K., Marques W., Davis M. B., et al. (1997) The phenotypic manifestations of chromosome 17p11.2 duplication. *Brain* **120(Pt 3),** 465–478.

Tobler A. R., Notterpek L., Naef R., Taylor V., Suter U., and Shooter E. M. (1999) Transport of Trembler-J mutant peripheral myelin protein 22 is blocked in the intermediate compartment and affects the transport of the wild-type protein by direct interaction. *J. Neurosci.* **19(6),** 2027–2036.

Tooth H. (1886) *The Peroneal Type of Progressive Muscular Atrophy*. Lewis, London.

Trono D. (2000) HIV-based vectors: getting the best out of the worst. *J. Gene Med.* **2(1),** 61–63.

Valentijn L. J., Bass F., Wolterman R. A., et al. (1992) Identical point mutations of PMP-22 in Trembler-J mouse and Charcot- Marie-Tooth disease type 1A. *Nat. Genet.* **2(4),** 288–291.

Vallat J. M., Sindou P., Preux P. M., et al. (1996) Ultrastructural PMP22 expression in inherited demyelinating neuropathies. *Ann. Neurol.* **39(6),** 813–817.

Vinci P. A., Serrao M., Millul A., et al. (2005) Quality of life in patients with Charcot Marie Tooth disease. *Neurology* **65,** 922–924**.**

Wang X. D., Tang J. G., Xie X. L., et al. (2005) A comprehensive study of optimal conditions for naked plasmid DNA transfer into skeletal muscle by electroporation. *J. Gene Med.* **3,** 1235–1245.

Watson D. J., Kobinger G. P., Passini M. A., Wilson J. M., and Wolfe J. H. (2002) Targeted transduction patterns in the mouse brain by lentivirus vectors pseudotyped with VSV, Ebola, Mokola, LCMV, or MuLV envelope proteins. *Mol. Ther.* **5(5 Pt 1),** 528–537.

Waxman S. G. and Bangalore L. (2004) Electrophysiologic consequences of myelination. In: Lazzarini R. A. (ed.), *Myelin biology and disorders*. Elsevier, London, vol. 1, pp. 117–143.

Webster H. (1993) Development of peripheral nerve fibers. In: Dyck P. J., P. K. T., Low P. A. and Poduslo J. F. P*eripheral Neuropathy*. W.B. Saunders, Philadelphia, PA, 243–266.

Weimer L. H. Medication-induced exacerbation of neuropathy in Charcot Marie Tooth Disease. *J. Neurol. Sci.*, in press.

Xia C. H., Roberts E. A., Her L. S., et al. (2003) Abnormal neurofilament transport caused by targeted disruption of neuronal kinesin heavy chain KIF5A. *J. Cell Biol.* **161(1),** 55–66. Epub 2003 Apr 7.

Xiao X. (2002) Adeno-associated Viruses. *Encyclopedia of Life Sciences*. Macmillan Publishers Ltd, Nature Publishing Group/www.els.net**:** 1–9.

Xiao X., Li J., and Samulski R. J. (1998) Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. *J. Virol.* **72(3),** 2224–2232.

Yamada M., Natsume A., Mata M., et al. (2001) Herpes simplex virus vector-mediated expression of Bcl-2 protects spinal motor neurons from degeneration following root avulsion. *Exp. Neurol.* **168(2),** 225–230.

Yang J., Dai C., and Liu Y. (2001) Systemic administration of naked plasmid encoding hepatocyte growth factor ameliorates chronic renal fibrosis in mice. *Gene Ther.* **8(19),** 1470–1479.

Yuen E. C. (2001) The role of neurotrophic factors in disorders of peripheral nerves and motor neurons. *Phys. Med. Rehabil. Clin. N. Am.* **12(2),** 293–306, viii.

Zolotukhin S., Potter M., Zolotukhin., et al. (2002) Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods **28(2),** 158–167.

Zuchner S., Mersiyanova I. V., Muglia M., et al. (2004) Mutations in the mitochondrial GTPase mitofusin 2 cause Charcot-Marie-Tooth neuropathy type 2A. *Nat. Genet.* **36(5),** 449–451. Epub 2004 Apr 4.

Zuchner S. and Vance J. M. (2005) Emerging pathways for hereditary axonopathies. *J. Mol. Med.* **83**, 935–943.