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Diagnosis and Therapy in Neuromuscular Disorders: Diagnosis and new treatments in Genetic Neuropathy

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Diagnosis and New Treatments in Genetic Neuropathies

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INTRODUCTION

The genetic neuropathies are a clinically and genetically heterogeneous group in which an accurate genetic diagnosis is increasingly possible. Research into their pathogenesis has revolutionized our understanding of the peripheral nervous system and allowed the development of rational approaches to therapy.

The identification of more than 30 causative genes for inherited neuropathies has raised important questions as to how to approach their diagnosis; the move towards developing gene specific therapies will make accurate genetic diagnoses even more important (1).

The genetic neuropathies can broadly be classified into two groups; those in which the neuropathy is the sole or primary part of the disorder (e.g. Charcot Marie Tooth disease, CMT) and those in which the neuropathy is part of a more generalized neurological or multisystem disorder (Table 1). Although there have been major advances in both the diagnosis and the treatment of the latter group (e.g. liver transplantation for Familial Amyloid Polyneuropathy (FAP), this review will concentrate on CMT.

DIAGNOSIS OF GENETIC NEUROPATHIES

Although more than 30 causative genes have been identified, only about 10 are routinely available for diagnostic purposes. In many countries genetic testing is expensive and the specific genetic tests requested must be selected carefully. Accurate diagnosis and appropriate genetic testing are based on the careful evaluation of the clinical phenotype (mainly encompassing history, examination, neurophysiology and in selected cases a nerve biopsy), and a detailed family tree (2). Many algorithms have been developed to help the busy clinician choose the appropriate genetic test based on the phenotype and family history (2).

Classification

CMT is separable into autosomal dominantly (AD) inherited demyelinating (CMT1) or axonal (CMT2) neuropathies, which are also historically classified as hereditary motor and sensory neuropathies (HMSN) I and II (3, 4). Severely affected infants were classified as having congenital hypomyelinating neuropathies (CHN) or

Dejerine Sottas Neuropathy (DSN) which were thought to be autosomal recessive (AR) disorders and labeled CMT3 or HMSNIII. Since many of these patients actually have de novo AD neuropathies we now use CHN or DSN to indicate severely affected infants, ignore CMT3, and use CMT4 to characterize AR neuropathies. Table 2 is summary of currently known main CMT genes (space constraints do not allow all loci to be included). Hereditary Sensory and Autonomic Neuropathy (HSAN) describes forms of inherited neuropathies characterised by primary sensory or autonomic abnormalities (Table 3) and distal Hereditary Motor Neuropathies (dHMN) describes purely motor forms (Table 4). Subtypes (CMT1A, CMT2A etc) are used to characterize specific genetic causes of each of the larger categories. This system is not perfect. For example HSAN1 and CMT2B are clinically identical despite being caused by mutations in different genes, and certain genes for dHMN also cause axonal CMT (GARS, HSP27, HSP22).

Is the neuropathy genetic (CMT / HNPP / HSAN / dHMN)?

The first step is to determine whether the patient has a genetic neuropathy. Sometimes the answer is clear as when there is also an affected parent making either autosomal dominant (AD) or X-linked (if there is no definite male-to-male transmission) inheritance probable, or when there are affected siblings from a consanguineous marriage making autosomal recessive (AR) inheritance likely. In other patients recognizing CMT can be more difficult. There may be no family history or families may be small and extensive family histories not available. Factors that help the clinician decide whether the neuropathy is genetic include presentations in infancy, long, slow progression, the presence of foot deformities, and in an adults, the lack of positive sensory symptoms (dysesthesias, paresthesias) in the presence of clear sensory signs.

CMT

CMT is common with a prevalence of 1:2500 (5) and is usually classified as either demyelinating if the median (or ulnar) nerve motor conduction velocity (MCV) is less than 38 m/s, or axonal if the median MCV is above 38 m/s (Table 2). Motor conduction velocities in upper extremities are usually uniformly slow in demyelinating CMT whereas there is often patchy, asymmetric slowing in acquired demyelinating

neuropathies (AIDP, CIDP) (6, 7). Intermediate forms of CMT with median or ulnar MCVs between 25-45 m/s are also present and may help direct genetic diagnosis (see below). Sporadic CMT patients occur often and are usually found to have mutations in the common AD genes (*de novo* mutations) or in the AR genes. In most UK / north European and US populations about 90% of cases of CMT are either AD or X-linked, whereas in countries with a higher rate of consanguineous marriages, AR CMT accounts for about 40% (8). The diagnostic approach will therefore vary in specific countries and specific ethnic groups. CMT1 is consistently reported to be more common than CMT2 but as 75% of the genes have yet to be identified for CMT2, the true prevalence of CMT2 is unknown.

CMT1; Autosomal dominant demyelinating neuropathies

This is the most common form of CMT in most populations. Patients usually present with a "classical CMT phenotype" that includes lower limb motor symptoms (difficulty walking / foot deformity) beginning in the first two decades accompanied by distal weakness, atrophy, sensory loss, hyporeflexia and foot deformity. Patients have normal life spans, frequently need ankle-foot orthotics (AFOs) and rarely require wheel chairs for routine ambulation. Median and ulnar MCVs are below 38 m/s and the sensory action potentials (SAPs) are either reduced or absent. Nerve biopsy demonstrates demyelination and onion bulb formation. However biopsies are not necessary to make the diagnosis.

Rational approaches to genetic diagnosis require careful clinical examination, neurophysiology and an appreciation of the frequency with which a particular gene causes CMT1 (Table 2, Figure 1). Classic phenotypes and MCV around 20 m/s are strongly suggestive of CMT1A, caused by a 1.4Mb duplication on 17p11.2 (9, 10). Sporadic cases occur in about 10% of CMT1A cases. Thus the lack of family history does not exclude CMT1A. In European populations CMT1A accounts for 70% of all CMT1 cases (11). Mutations in the *PMP22* gene can also cause CMT1, but with a wide spectrum of phenotypes including DSN and HNPP (see below).

CMT1B, caused by mutation in *myelin protein zero (MPZ)* comprise about 10% of CMT1. Patients can present with the classical CMT1 phenotype but are more likely to

have either a more severe early onset form of CMT with MCV <10 m/sec or a late onset form of CMT with median MCVs in the axonal range (12).

EGR2 and *SIMPLE* mutations are rare (<1%) causes of CMT1 (13, 14). EGR2 patients usually present with DSN and SIMPLE patients frequently resemble those with CMT1A. Mutations in *NEFL* were originally described as a cause of CMT2 (15), although some patients have MCVs in the demyelinating range (16), though the gene is expressed in neurons but not Schwann cells.

HNPP

HNPP is an AD neuropathy usually caused by a deletion of the same 1.4 Mb portion of chromosome 17 that is duplicated in CMT1A (17). Nonsense or frameshift mutations that truncate *PMP22* are rare causes of HNPP. Patients typically present with transient, recurrent episodes of focal weakness or sensory loss in the distribution of individual nerves or plexus (18). Nerve conduction studies often show focal areas of slowing around sites subject to compression (19). Screening patients with isolated pressure palsies such as carpal tunnel syndromes for HNPP is not warranted.

X-linked CMT1

This is the second commonest form of CMT and is caused by mutations in the *gap junction protein beta 1 (GJB1) gene* encoding connexin 32 (Cx32) (20). Males are usually more severely affected than females. Cx32 is expressed in myelinating Schwann cells, not neurons (see (21)). However, nerve conductions are only mildly slowed in both men and women with CMT1X with values often in the intermediate range (25-40 m/sec)(22). Occasional CMT1X patients have asymmetric MCV reminiscent of CIDP (23, 24). Although oligodendrocytes also express Cx32, the CNS is most often only occasionally involved in CMT1X (extensor plantars, mild deafness, abnormal brainstem evoked potentials) (25). However, occasionally transient severe CNS involvement characterized by ataxia and dysarthria has been described (26, 27). There are over 300 causative GJB1 mutations (http://www.molgen.ua.ac.be/CMTMutations/default.cfm). Unlike distinct *PMP22* and *MPZ* mutations, virtually all *GJB1* mutations have similar age related phenotypes that resemble those in which the gene is entirely deleted (28).

CMT2; autosomal dominant axonal neuropathies

CMT2 can be difficult to distinguish from an idiopathic axonal neuropathy when there is no family history. Eight causative genes have been identified (Table 2) accounting for about 25% of all the CMT2 cases. CMT2 can be subdivided into three distinct phenotypes (Figure 2). In the first, and most common phenotype, patients present with the "classical CMT" phenotype although much later ages of onset may sometimes be seen than with CMT1. Such patients are indistinguishable from CMT1 without neurophysiology. Nerve biopsies are rarely helpful diagnostically but if done show an axonal neuropathy without any specific diagnostic features.

Mutations in *mitofusin 2 (MFN2)* cause CMT2A (29), which represents about 20% of all CMT2 cases. CMT2A patients generally have a severe phenotype that may severely impair them in childhood. Twenty percent of *MFN2* mutations are *de novo*. Occasional CMT2A patients also have optic atrophy (HMSN VI in previous classifications (30)), brisk reflexes and minor white matter changes on brain MRI (30, 31). Small heat shock protein genes HSP27 (HSPB1) and HSP22 (HSPB8) are rare causes of CMT2 but usually have minimal sensory involvement (these two genes also cause a purely motor phenotype, dHMN type II (reviewed in (32)). A homozygous mutation in *HSP27* has also recently been described to cause AR CMT (33). Mutations in *MPZ* and *NEFL* can also cause CMT2 phenotypes.

Profound sensory impairment, often including "ulceromutilations" characterizes the second CMT2 phenotype (34). The causative gene is *RAB7* and patients are classified as having CMT2B (35). CMT2B patients are difficult to distinguish from those with HSAN1, caused by mutations in the *SPTLC1* gene (36, 37). If patients present with prominent sensory features, both the *RAB7* and *SPTLC1* genes should be initially considered.

The third phenotype for CMT2 is exemplified in patients with CMT2D. Such patients present with atrophy and weakness of the small muscles of the hand (this can be unilateral and misdiagnosed as thoracic outlet syndrome) and much later involvement of the distal lower limb muscles. CMT2D is caused by mutations in *GARS* (38). Some patients have no sensory involvement and have been classified as dHMN type V, an allelic condition. The dHMN V / CMT2D phenotype has subsequently been shown to be

more commonly due to mutations in the *BSCL2* gene (39) which usually causes Silver syndrome (spastic legs and distal amyotrophy of the upper limbs) but can present (33% of cases) with just amyotrophy of the upper limbs.

CMT4; Autosomal Recessive CMT

Thirteen genes have been identified that cause autosomal recessive CMT4 (including 3 genes - *PMP22, MPZ* and *EGR2* - that more commonly cause CMT1) (Table 2). Demyelinating forms of CMT4 are more frequent. No one algorithm is suitable for the evaluation of CMT4, but there are simple clinical rules that can be used to aid diagnosis. Usually CMT4 cases have early, infantile onset (DSN or CHN) and are severe. Weakness often progresses to involve proximal muscles and result in early loss of ambulation. Recent comprehensive reviews of demyelinating (8) and axonal CMT4 are available (40). Nerve biopsies can be useful in certain cases because specific features make a particular genetic diagnosis more likely (Figure 3). Particular points to consider are:

AR neuropathies can be difficult to identify since few cases have been identified, polymorphisms are frequent, and compound heterozygous mutations can be disease causing. Often multiple family members must be screened to ensure mutations are disease causing.

Identifying demyelination by MCV can be difficult in CMT4 because motor and sensory amplitudes are often unobtainable at routine recording sites in these severely affected patients. Conduction studies of nerves innervating proximal muscles may be necessary to identify slow MCV.

Nerve biopsies showing focally folded myelin are characteristic of CMT4B1 (*MTMR2* mutations) and CMT4B2 (*MTMR13* mutations) but can also be seen with *MPZ* mutations and in CMT4F secondary to periaxin mutations.

Severe and early scoliosis may be seen with CMT4C due to mutations in the *KIAA1985* gene. Several patients have had characteristic nerve biopsy features including basal membrane onion bulbs and multiple cytoplasmic processes of the Schwann cells ensheathing unmyelinated axons (Figure 3)(41).

Two forms of AR CMT are largely confined to patients of Balkan gypsy origin.

CMT4D secondary to *NDRG1* mutations is characterized by a demyelinating neuropathy with a high prevalence of deafness. Tongue atrophy has also been described. CCFDN (congenital cataract, facial dysmorphism, and neuropathy syndrome) secondary to *CTDP1* mutations is also found in gypsies.

Predominant sensory involvement and variable phenotypes are characteristic of CMT4F (periaxin mutations).

Only two known causative genes have been identified for autosomal recessive axonal CMT4, (*LMNA* and *GDAP1* (table 2)). Most patients with mutations in lamin A/C (*LMNA*) (42) present in the second decade with a severe CMT phenotype including proximal muscle involvement although some have a milder phenotype. Lamin A/C mutations have been associated with a wide spectrum of other phenotypes including Emery-Dreifuss muscular dystrophy, cardiomyopathy, and Dunniugan-type familial partial lipodystrophy.

Intermediate CMT

Certain forms of CMT characteristically present with MCVs in the intermediate range (25-45 m/s) These include dominant intermediate (DI)-CMTB caused by *DNM2* mutations (43), DI-CMTC caused by *YARS* mutations (44), and DI-CMTA in which only linkage has been identified, at 10q24.1-25.1. In addition, patients with CMT1X, CMT2E, late onset CMT1B and patients with CMT4A often present with intermediate MCV.

Hereditary Sensory and Autonomic Neuropathy (HSAN)

The hereditary sensory and autonomic neuropathies are rare but many of the genes have been identified (Table 3). Autonomic abnormalities are often minimal and motor involvement can be present. The sensory loss can lead to severe complications including recurrent injuries, ulcerations, osteomyelitis and amputations. The commonest AD form is HSAN1 (or HSN1) caused by *SPTLC1* mutations. Patients usually present in the second decade with distal lower limb sensory loss and many have neuropathic pain. Motor involvement can be significant especially later in the disease course (Figure 4). MCVs can be in the demyelinating range with males being more severely affected than females (45). This disease is very difficult to differentiate from CMT2B secondary to

RAB7 mutations although the lancinating pain in patients with *SPTLC1* mutations can be a useful guide to this diagnosis.

HSAN II is an early onset autosomal recessive severe sensory neuropathy with prominent sensory complications, due to mutations in the *HSN2* gene.

HSAN 111 (Riley-Day syndrome) is a autosomal recessive neuropathy seen in Askenazi Jews and characterized by mainly autonomic involvement, but it also involves the peripheral nervous system, particularly the sensory nerves. The causative gene is the *IKBKAP* gene.

HSAN IV and V are both AR neuropathies characterized by congenital insensitivity to pain. HSAN IV (also called congenital insensitivity to pain with anhidrosis) presents with a severe sensory neuropathy, anhidrosis and mental retardation and is due to mutations in the *NTRK1* gene. HSAN V is similar but without the mental retardation or significant anhidrosis, described with both *NTRK1* and also *NGFB* mutations.

Recently the identification of homozygous mutations in the SCN9A gene as a rare cause of congenital insensitivity to pain (46) has been of great interest as heterozygous mutations in the same gene cause hereditary erythermalgia (47) and paroxysmal extreme pain disorders (48).

Distal Hereditary Motor Neuropathyies (dHMNs)

The dHMNs are a complex group of disorders (Table 4) that typically present with length dependent weakness and no sensory loss. DHMN II is the classic form of AD dHMN and is due to mutations in the *HSP27* and *HSP22* genes, which also cause CMT2F and CMT2L. There are many other forms but the genes are only known for several:

Mutations in *GARS* and *BSCL2* cause dHMN V (also CMT2D).

dHMN VI, an unusual severe AR form of dHMN, presents in infancy with respiratory and distal limb involvement (called spinal muscle atrophy with respiratory distress type 1). This is due to mutations in *IGHMBP2*.

Mutations in dynactin (*DCTN1*) cause one form of dHMN type VII, which is characterized by vocal cord paralysis and progressive weakness and atrophy of the face, hands and legs.

Missense mutations in senataxin (*SETX*) can cause a form of dHMN with pyramidal features whereas nonsense mutations in the same gene cause autosomal recessive ataxia oculomotor apraxia type 2 (AOA2).

TREATMENT OF GENETIC NEUROPATHIES

There are no specific therapies available for any of the genetic neuropathies discussed in this review. Therapy to date has focused on physical therapies, use of orthotics, orthopaedic interventions (e.g. for scoliosis or foot deformity), pain management and providing genetic counseling for diagnostic, predictive, prenatal and more recently pre-implantation testing. Although many patients regularly receive physiotherapy, it is not known what therapies are best suited to this group of patients and studies are ongoing in this area. This review will concentrate on new disease specific therapies being developed by summarizing the major emerging pathogenic mechanisms and the therapies that are evolving from these.

Biological insights into demyelination and neuronal degeneration from CMT

The >30 CMT genes and their proteins constitute a human "microarray" of molecules that are necessary for the normal function of myelinated axons in the peripheral nervous system (PNS). In some of the demyelinating neuropathies, the causal proteins were predictable since PMP22, MPZ, and periaxin (CMT4F) were known components of the PNS myelin sheath, and EGR2 (CMT1D) was known to be an essential transcription factor for the development of myelinating Schwann cells (13, 49). Similarly, neurofilament light (CMT2E) was known to be an essential component of axonal neurofilaments. However in many other forms of CMT the identification of the causal protein has come as a surprise. Cx32 was not known to be a component of myelin sheaths until *GJB1* mutations were found to cause CMT1X (20). GDAP1 had no known function prior to being recognized as the cause of CMT4A (50, 51). Subsequently it has been found that GDAP1 is a nuclear encoded protein that participates in mitochondrial fragmentation, or fission (52-54), a process not previously recognized as necessary for maintaining axonal integrity. *MFN2* (CMT2A)(29), *LITAF/SIMPLE* (CMT1C)(14), *RAB7* (CMT2B)(35), *GARS* (CMT2D)(38), *DMN2* (DI-CMTB)(43), *YARS* (DI-

CMT1C)(44), *MTMR2* (CMT4B1)(55), *MTMR13* (CMT4B2)(56), *SETX* (ALS4)(57) and *RAB7* (CMT2B)(35) encode widely expressed proteins that were not known to have special roles in myelin or axons until mutations in each were found to cause inherited neuropathies. These mutations have illuminated important intracellular pathways leading to demyelination or axonal degeneration, including intracellular protein trafficking, axonal transport, regulation of transcription, and mitochondrial fusion/fission, to name a few. Some forms of inherited neuropathies affect primarily motor neurons and their axons. These include proteins involved in DNA/RNA processing (IGHMBP2, SETX), protein synthesis (GARS, YARS, BSCL2), apoptosis (HSP27), stress response (HSP22 and HSP27), or axonal transport (HSP27, DCTN1) (reviewed in (32)). Conversely, mutations in several other genes cause predominantly sensory peripheral neuropathies/neuronopathies. *SPTLC1*, involved in ceramide synthesis, causes HSAN1 (36, 37); *IKBKAP*, a component of the human elongator acetylase complex that may play a role in tubulin acetylation and microtubule-based protein trafficking (58), causes HSANIII (59); *NTRK1*, the receptor for NGF, causes HSANIV (60, 61); and NGF, the ligand for TrkA, causes HSANV. Taken together, investigations of disease mechanisms in various genetic forms of inherited neuropathies are providing insights into the pathogenesis not only of CMT but also into neurodegenerative diseases in general. An overview of many of the cellular processes disrupted in CMT is provided in the elegant figure from Nieman et al (Figure 5) (62). Manipulation of these processes offers a rational therapeutic approach to many forms of inherited neuropathy.

Gene dosage and regulating myelination: Therapy for CMT1A

The most common form of CMT, CMT1A, is caused by over-expression, rather than mutation of a gene, the result of a 1.4 Mb duplication on chromosome 17 in the region carrying the gene encoding *PMP22* (9, 10, 63). In contrast, deletion of the same 1.4 Mb region on chromosome 17 that is duplicated in CMT1A causes HNPP (17), a distinct disorder characterized by focal episodes of weakness and/or sensory loss (18). Decreased expression of PMP22 is the cause of HNPP (17). 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 degradation before reaching the myelin sheath (64). Nevertheless, treatment strategies are currently being devised to regulate *PMP22* mRNA levels as a method of treating CMT1A. Approaches such as the use of siRNA or antisense oligonucleotides may permit alteration of PMP22 as these techniques are perfected. However, investigators are also turning towards 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

Progesterone has been shown to increase expression of PMP22 and MPZ mRNA levels in cultured Schwann cells (65). Therefore, its ability to modulate *Pmp22* levels in a rat model of CMT1A was investigated. The CMT1A rat was generated by specific over-expression of a *Pmp22* cDNA. Heterozygous animals develop a progressive, demyelinating neuropathy with clinical, neurophysiological and pathological features that resemble CMT1A (66). Daily administration of progesterone to these CMT1A rats elevated the steady-state levels of *Pmp22* and *Mpz* mRNA in sciatic nerves, as would have been predicted from the tissue culture studies. The result was 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 in the animals and improved their CMT phenotype, without obvious side effects. Taken together, these data provided proof of principle that the progesterone receptor of myelin-forming Schwann cells is a promising pharmacological target for therapy of CMT1A. Unfortunately, onapristone is toxic in 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

Schwann cells co-cultured with neurons derived from dorsal root ganglia (DRG) have been used to investigate PNS myelination in a number of classic studies (67, 68). Schwann cells only 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 (67). Investigators therefore treated a 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 that necessary to induce the disease phenotype (69). As a result, clinical trials using various doses of ascorbic acid to treat CMT1A are underway at several centers throughout the world.

High Throughput Screens and CMT1A

Both ascorbic acid and progesterone antagonists are currently available compounds chosen by investigators because of their demonstrated ability to alter PMP22 expression. Additional compounds may currently exist that also alter PMP22 expression, perhaps more effectively than ascorbic acid or onapristone. It is currently feasible to rapidly test libraries containing hundreds of thousands of candidate medications for CMT1A using what are termed high throughput screens (HTS). Cell lines expressing PMP22-reporter constructs are created and treated with candidate compounds in robotic, computerized systems and compounds found to lower PMP22 expression can then be further tested in rodent CMT1A models. These HTS promise to rapidly generate additional potential therapies for CMT1A patients.

Membrane fusion, fission and protein transport

In addition to MFN2 and GDAP1, several forms of CMT and related disorders appear to be caused by abnormalities in the fusion and fission of cellular membranes. GTPase dynamin 2 (DNM2) mutations cause dominant intermediate CMT type B (DI-CMTB). The role of DNM2 appears to be in aiding the separation of newly formed endosomes from the cell membrane (70-72). Additionally, the vesicle-associated protein B participates (VAPB) in membrane fusion and has recently been shown to cause ALS in several Brazilian families (73). VAPB contains a v-SNARE domain. SNARE refers to soluble NSF attachment protein receptor. 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 (74).

Mutations in the putative protein degradation protein LITAF/SIMPLE cause CMT1C (75). Although the precise function of SIMPLE is unknown its murine

orthologue interacts with Nedd4, a E3 ubiquiton ligase. Mono-ubiquitination 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 (76). 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 (77) and Leu147Arg (78) cause demyelinating neuropathies in humans and the naturally occurring demyelinating trembler J (Tr^J) (79) and trembler (Tr) (80) mouse mutants. Since both of these mutations are more severe in humans than HNPP (also more severe than CMT1A caused by *PMP22* duplication) they cause an abnormal gain of function 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 Pmp22 were retained in a cytoplasmic compartment that co-localized with the endoplasmic reticulum (ER) (81). Other studies have also shown that mutant Tr and Tr^J proteins aggregate abnormally in transfected cells (82). 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 contribute to the pathogenesis of the neuropathy (83). More recent studies have shown that there are abnormalities of proteosome function resulting in the accumulation of ubiquitinated substrates in the Tr^J model (84). Transfection studies have also demonstrated that other PMP22, as well as some MPZ mutations result in mutant proteins being retained in intracellular compartments (85-87). 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) (88) are areas of active investigation that may lead to future treatments.

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 (89)). Mitochondrial morphology is determined by a dynamic equilibrium between organelle fusion and fission. MFN2, the cause of CMT2A (29), is a highly conserved, nuclear encoded mitochondrial GTPase that is a component of the outer mitochondrial membrane and an essential regulator of mitochondrial fusion (90, 91). MFN2 and the related MFN1 form both homotypic and heterotypic oligomers that promote mitochondrial fusion (92). Fusion requires either MFN1 or MFN2, as mitochondria lacking both MFN1 and MFN2 cannot fuse (93). In addition, it has been suggested that *MFN2* mutations result in impaired axonal transport of mitochondria, depriving the distal axon of a necessary source of energy (94). Alternatively, it has been proposed that *MFN2* mutations impair the ability of mitochondria to dock with kinesin KIFIB, the axonal motor that carry mitochondria along microtubules, or that the *MFN2* mutations impair oxidative phosphorylation by mitochondria (reviewed in (62)). Interestingly, mutations in *OPA1*, which encodes a mitochondrial GTPase localized to the inner mitochondrial membrane, cause dominantly inherited optic atrophy (95). OPA1 interacts directly with MFN1 to mediate the fusion of the inner mitochondrial membrane (92, 96). Whatever the mechanism(s) by which *MFN2* mutations cause neuropathy, they likely involve a toxic gain of function by the mutant protein because the neuropathies are often severe in the heterozygous state (see above), and heterozygous *Mfn2*-deficient mice have no obvious phenotype (93). Alternatively, mutations in *Ganglioside-Induced Differentiation Protein 1* (*GDAP1*) cause CMT4A, the most frequent autosomal recessive form of CMT (8, 97, 98). GDAP1 is expressed predominantly in neurons (53, 54), although there is evidence of Schwann cell expression, as well. Both demyelinating (50) and axonal (51) mutations have been described; whether particular mutations disrupt myelin more than axons is unknown. GDAP1 is localized to the outer mitochondrial membrane (52, 54). In contrast to *MFN2* mutations, *GDAP1* mutations cause mitochondrial fragmentation; this can be counteracted by MFN2. The GDAP1 mutants associated with CMT4A are no longer targeted to mitochondria and cannot induce fragmentation; these data further demonstrate the importance of mitochondrial

fusion/fission for the health of PNS axons.

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

Schwann cell-axonal interactions

Schwann cell-axonal interactions are necessary for normal axonal function and are disrupted in demyelinating inherited neuropathies. Consequences of these disruptions include changes in the phosphorylation status and packing density of neurofilaments and abnormal axonal transport (99). Ultimately, axonal degeneration occurs, which may contribute more to disability than the initial demyelination (100). Therefore strategies directed towards preventing axonal degeneration even in demyelinating neuropathies have been undertaken. Currently these strategies are focusing in three areas, one of which is to provide trophic factor support to degenerating axons. Several families of trophic factors have been extensively used in recent years to treat neurodegenerative diseases, including CMT. (reviewed in (101)). Despite successes in animal models results in human trials with trophic factors have been disappointing to date. Part of the reasons for disappointing results with these proteins may relate to methods of delivery. For example, half-lives of many trophic factors are only for several minutes and they have frequently been administered to patients by subcutaneous injection. Targeting the trophic factors specifically to neurons or Schwann cells may also be necessary to induce beneficial effects. Finally, it may be necessary to coordinately deliver multiple trophic factors to induce axonal regeneration in neurodegenerative disorders.

A second strategy is based on the hypothesis that demyelination places increased energy demands on neurons. Thinning or absence of myelin reduces its ability to maintain a charge separation resulting 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 (102). 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 down their concentration gradient, also making it more difficult for depolarization to occur at the node of Ranvier (102). 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 (103). However, more specific potassium channel blockers are becoming available including agents that are capable of blocking the channels from inside the axolemma.

A third strategy to improve Schawnn cell-axonal interactions is to identify and manipulate specific signaling pathways between the Schwann cell and the axon. PNS myelin thickness is regulated by neuregulin 1 type III (Nrg1*) signaling from axons (104). Nrg1, like other members of the EGF superfamily (105), 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 (101)). While *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 since, as the authors point out, the Nrg1 C-terminal domain can be cleaved to become a signaling molecule itself (104).

Potential additional sites for other specific signaling interactions between Schwann cells and axons include the adaxonal internode, the paranodal region of the myelinating Schwann cell, and their underlying underlying axolemma. The axolemma is divided into a series of polarized domains in which specific molecules are expressed in specific areas such as the node of Ranvier, paranode, juxtaparanode and internode (106). A similar organization occurs in regions of adaxonal myelin that appose these domains. Further defining molecular pathways through which the adaxonal myelin and underlying axolemma interact may provide therapeutic targets to prevent or minimize axonal degeneration in demyelinating neuropathies. Nectin-like cell adhesion molecules Necl4 expressed by Schwann cells and Necl1 expressed by neurons bind to each other along the myelin internode and appear necessary for myelination (107, 108). Whether these or

other molecules involved in neural-glial interactions can be manipulated to prevent axonal degeneration is being actively investigated.

Gene Therapy

Gene therapy can be defined as a strategy to transfer biologically relevant genetic material (usually genes or proteins) into affected cells in the body to treat disease. Approaches in gene therapy have focused in two areas. The first is to identify molecular abnormalities causing disease and to develop appropriate therapeutic molecules to repair the abnormalities. The second is to design delivery systems, or vectors, to target therapeutic molecules to the diseased neurons or Schwann cells (Table 5). This latter area of research has proven at least as challenging as determining the molecular basis for the various forms of CMT.

Gene Therapy Delivery Systems

Viral vectors

Most gene therapy delivery systems use parts of viruses that have been modified so they cannot cause disease but will still carry the therapeutic gene to the cells that the virus usually infects (109). Unfortunately, viral vectors have often caused immunological reactions in animal disease models and even in patients. Overcoming these immune reactions is a major challenge that currently limits their more widespread. For example one young man died several years ago because of an immunological reaction to an adenoviral vector used to treat his liver disease.

Plasmid DNA

A non-viral approach to gene therapy involves directly introducing DNA itself, without a vector, directly into tissue. The DNA is introduced in the form of plasmids, which is how DNA is stored in bacteria. Direct introduction of plasmid DNA into an animal causes minimal immunological reactions. Challenges for the use of plasmid DNA have been poor delivery efficiency and the fact that the proteins made from the plasmids have only been made in target organs for a short time.

Stem cells

The use of embryonic or other types of stem cells to treat neurodegenerative diseases is generating great excitement among families with CMT as well as among researchers. However, there are formidable challenges to the use of stem cells in the inherited neuropathies. It will be a difficult challenge for stem cells to differentiate into neurons and then generate axons that need to travel down limbs more than a meter in length to reach their appropriate neuromuscular junction or sensory target. Similarly, it will be a challenge for stem cells to differentiate into Schwann cells that contact and ensheath all demyelinated axons in patients with demyelinating CMT. However, another potential use of stem cells may be to provide trophic support for inherited neuropathies. Stem cells could be engineered to secrete trophic factors or molecules discussed above following the introduction of the stem cells into patients.

CONCLUSION

The diagnosis of the genetic neuropathies has been revolutionised by the major advances in identifying the causative genes. Despite a genetic diagnosis being possible in many patients the accurate phenotyping of patients remains crucial in the diagnosis and in the development of gene specific outcome measures for future therapies. The emerging knowledge of the pathogenetic mechanisms underlying these neuropathies has led not only to the first human trials being undertaken and to the investigation of many therapies at the pre-clinical stage but also has advanced our knowledge of the development and maintenance of the peripheral nervous system and given us insights to neurodegeneration more generally. The next decade should see the emergence of therapies for some of these neuropathies.

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FIGURE LEGENDS

Figure 1. Algorithm for molecular diagnosis of AD and X-linked CMT1.

Figure 2. Algorithm for molecular diagnosis of AD CMT2.

Figure 3. EM from a 24 year old woman with CMT4C due to a KIAA1985 mutation showing typical abnormal Remak fibers. The unmyelinated axons appear normal but the associated Schwann cells form unusually attenuated processes linking the axons. Bar = 1 micron.

Figure 4. Hands of a patient with HSN1 secondary to the C133W *SPTLC1* mutation showing severe wasting and weakness.

Figure 5. Schematic representation of CMT causing proteins and intracellular pathways involved in CMT. (reprinted with permission from Neuromolecular Medicine: 2006 (62)).

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TABLE 1: Classification of the Genetic Neuropathies

Neuropathies in which the Neuropathy is the sole or primary part of the disorder

Charcot-Marie-Tooth disease (CMT)
Hereditary neuropathy with liability to pressure palsies (HNPP)
Hereditary sensory and autonomic neuropathies / Hereditary sensory neuropathies (HSAN/HSN)
Distal hereditary motor neuropathies (dHMN)
Hereditary neuralgic amyotrophy (HNA)

Neuropathies in which the neuropathy is part of a more widespread neurological or multisystem disorder

Familial amyloid polyneuropathy (FAP)
Disturbances of lipid metabolism
Porphyrias
Disorders with defective DNA
Neuropathies associated with mitochondrial diseases
Neuropathies associated with hereditary ataxias
Miscellaneous

TABLE 2: Classification of Charcot-Marie-Tooth disease

Type	Gene/locus	Specific phenotype
Autosomal dominant CMT1 (AD CMT1)		
CMT 1A	Dup 17p (PMP22)	Classic CMT1
	PMP22 (point mutation)	Classic CMT1 / DSN / CHN / HNPP
CMT 1B	MPZ	CMT1/ DSN / CHN / intermediate / CMT2
CMT 1C	LITAF	Classic CMT1
CMT 1D	EGR2	Classic CMT1 / DSN / CHN
CMT 1	NEFL	CMT2 but can have slow MCVs in CMT1 range +/- early onset severe disease
Hereditary neuropathy with liability to pressure palsies (HNPP)		
HNPP	Del 17p (PMP-22)	Typical HNPP
	PMP-22 (point mutation)	Typical HNPP
X-linked CMT1 (CMT 1X)		
CMT 1X	GJB1	intermediate +/- patchy MCVs / male MCVs < female MCVs
Autosomal Recessive demyelinating (CMT4)		
CMT4A	GDAP1	CMT1 or CMT2 usually early onset and severe / vocal cord and diaphragm paralysis described / rare AD CMT2 families described
CMT4B1	MTMR2	Severe CMT1 / facial/bulbar/focally folded myelin
CMT4B2	MTMR13	Severe CMT1 / glaucoma/focally folded myelin
CMT4C	KIAA1985 (SH3TC2)	Severe CMT1 / scoliosis/cytoplasmic expansions
CMT4D (HMSNL)	NDRG1	Severe CMT1 / gypsy/deafness/tongue atrophy
CMT4E	EGR2	Classic CMT1 / DSN / CHN
CMT4F	PRX	CMT1 / more sensory/focally folded myelin
CMT4H	FGD4	CMT1
CMT4J	FIG4	CMT1
CCFDN	CTDP1	CMT1 / gypsy / cataracts / dysmorphic features
HMSN Russe	10q22-q23	CMT1
CMT1	PMP22 (point mutation)	Classic CMT1 / DSN / CHN / HNPP
CMT1	MPZ	CMT1 / DSN / CHN / intermediate / CMT2
Autosomal dominant CMT2 (AD CMT 2)		
CMT 2A	KIF1Bβ	Classic CMT2
CMT 2A	MFN 2	CMT2 / usually severe / optic atrophy
CMT 2B	RAB7	CMT2 with predominant sensory involvement and sensory complications
CMT 2C	12q23 – q24	CMT2 with vocal cord and respiratory involvement
CMT 2D	GARS	CMT2 with predominant hand wasting / weakness or dHMN-V
CMT 2E	NEFL	CMT2 but can have slow MCVs in CMT1 range +/- early onset severe disease
CMT 2F	HSP27 (HSPB1)	Classic CMT2 or dHMN-II
CMT 2G	12q12-q13.3	Classic CMT2
CMT 2L	HSP22 (HSPB8)	Classic CMT2 or dHMN-II
CMT 2	MPZ	CMT1/ DSN / CHN / intermediate / CMT2
CMT 2 (HMSNP)	3q13.1	CMT2 with proximal involvement

Autosomal recessive CMT 2 (also called CMT4)

AR CMT2A	LMNA	CMT2 proximal involvement and rapid progression described / also causes muscular dystrophy / cardiomyopathy / lipodystrophy
AR CMT2B	19q13.1-13.3	Typical CMT2
AR CMT2	GDAP1	CMT1 or CMT2 usually early onset and severe / vocal cord and diaphragm paralysis described / rare AD CMT2 families described

Dominant intermediate CMT (DI-CMT)

DI-CMTA	10q24.1 – 25.1	Typical CMT
DI-CMTB	DNM2	Typical CMT
DI-CMTC	YARS	Typical CMT

Hereditary Neuralgic Amytrophy (HNA)

HNA	SEPT9	Recurrent neuralgic amyotrophy

Abbreviations: AD= autosomal dominant; AR= autosomal recessive; Dup = duplication; Del = deletion; PMP-22= peripheral myelin protein 22; MPZ- myelin protein zero; LITAF= Lipopolysaccharide-induced tumor necrosis factor; EGR2= early growth response 2; GJB1= Gap junction protein beta1; GDAP1= ganglioside-induced differentiation-associated protein 1; MTMR2= myotubularin-related protein 2; MTMR13= myotubularin-related protein 13; SH3TC2 = SH3 domain and tetratricopeptide repeats 2, NDRG1= N-myc downstream-regulated gene 1; PRX= periaxin; CTDP1 = CTD phosphatise subunit 1; FGD4 = FYVE; RhoGEF and PH domain containing 4; FIG4 = FIG 4 homolog; KIF1Bß =Kinesin family member 1B-ß; MFN2= Mitofusin 2; RAB7= RAB7, member RAS oncogene family; GARS = glycyl-tRNA synthetase; NEFL= neurofilament, light polypeptide 68kDa ; HSP 27 = heat shock 27kDa protein 1; HSP 22 = heat shock 22kDa protein 8; LMNA = Lamin A/C; DMN2 = dynamin 2; YARS = tyrosyl-tRNA synthetase; SEPT9 = septin 9.

TABLE 3: Classification of the hereditary sensory and autonomic neuropathies

Type	Inheritance	Gene/locus	Specific phenotype
HSAN I	AD	SPTLC1	Mainly sensory, sensory complications, motor involvement variable, males may be more severe
CMT2B	AD	RAB7	Sensorimotor, sensory complications, no pain
HSAN 1B	AD	3p22-p24	Sensory, cough, gastroesophageal reflux
HSAN II	AR	HSN2	Severe sensory complications, mutilations, onset first 2 decades
HSAN III	AR	IKBKAP	Familial dysautonomia or Riley-Day syndrome, prominent autonomic, absence fungiform papillae of the tongue
HSAN IV	AR	NTRK1	Congenital insensitivity to pain with anhydrosis (CIPA), severe sensory, anhydrosis, mental retardation, unmyelintated fibers mainly affected
HSAN V	AR	NTRK1	Congenital insensitivity to pain with mild anhydrosis, no mental retardation, small myelinated fibers mainly affected
HSAN V	AR	NGFB	Congenital insensitivity to pain, minimal autonomic, no mental retardation, mainly unmyelinated fibers affected.
Channelopathy associated insensitivity to pain	AR	SCN9A	Congenital insensitivity to pain.

Abbreviations; SPLTC1 = Serine palmitoyltransferase, long chain base subunit-1; HSN2 = Hereditary sensory neuropathy type II gene; IKBKAP = Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein; NTRK1 = Neurotrophic tyrosine kinase receptor type 1; NGFB = nerve growth factor beta polypeptide; SCN9A = Sodium channel, voltage-gated, type IX, alpha subunit.

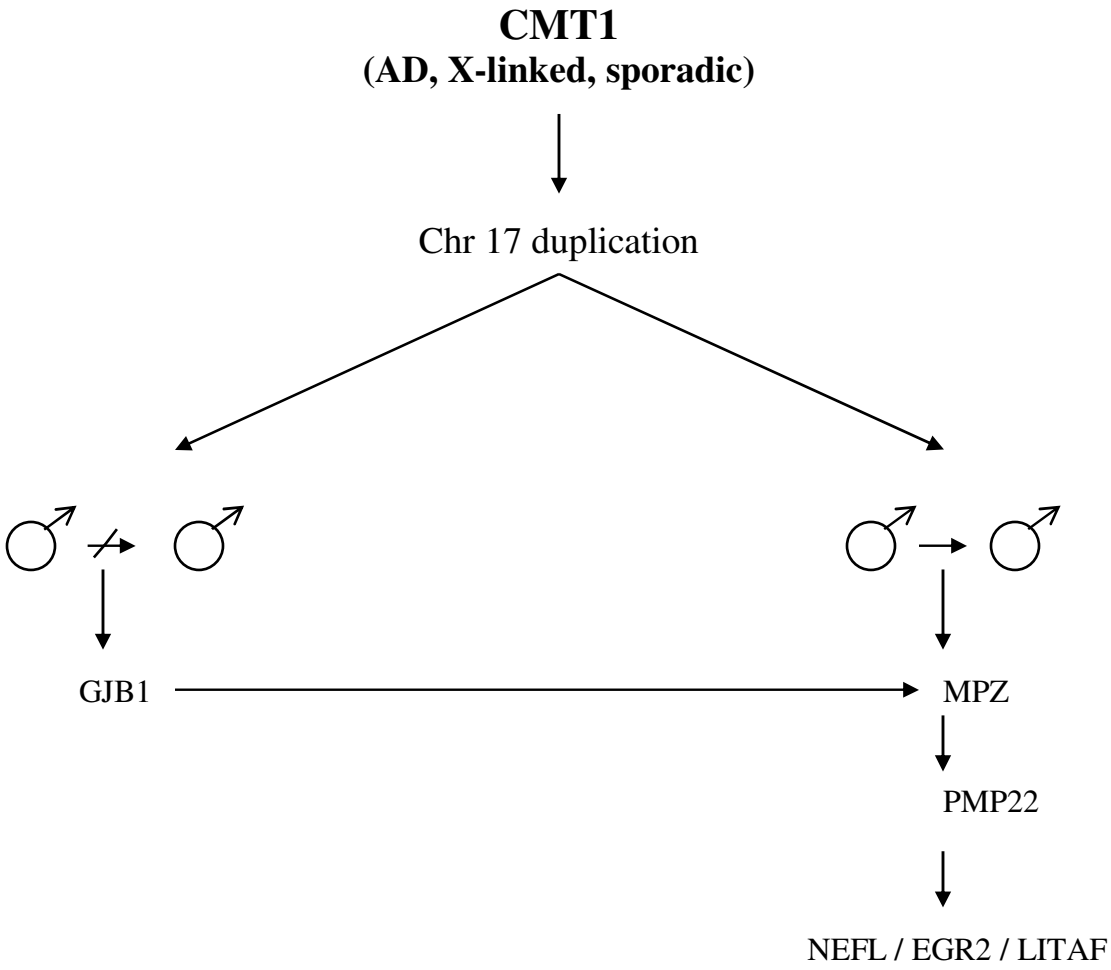
TABLE 4 : Classification of the distal hereditary motor neuropathies

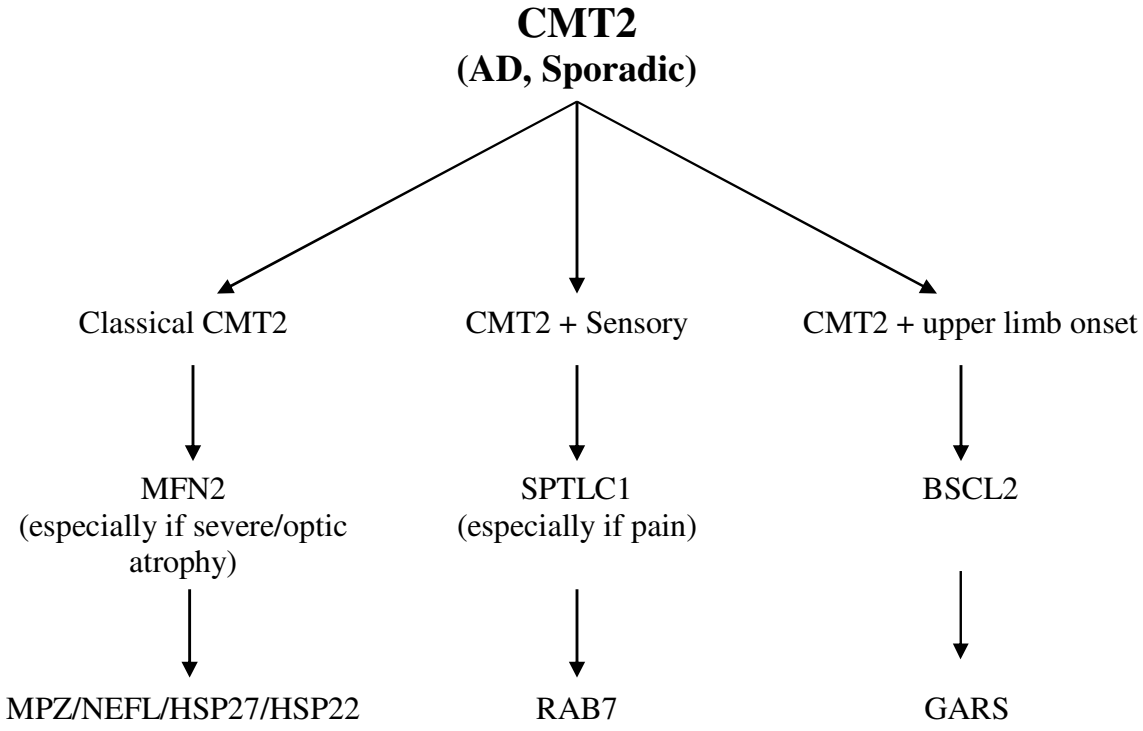
Type	Inheritance	Gene/locus	Specific phenotype
HMN I	AD	unknown	juvenile onset dHMN
HMN II	AD	HSP27(HSPB1)	Adult onset typical dHMN / CMT2F
HMN II	AD	HSP22(HSPB8)	Adult onset typical dHMN / CMT2L
HMN III	AR	11q13	Early onset, slowly progressive
HMN IV	AR	11q13	Juvenile onset, diaphragmatic involvement
HMN V	AD	GARS	Upper limb onset, slowly progressive / CMT2D
HMN V	AD	BSCL2	Upper limb onset, +/-spasticity lower limbs / Silver syndrome
HMN VI	AR	IGHMBP2	Spinal muscle atrophy with respiratory distress (SMARD1), infantile onset respiratory distress
HMN VIIA	AD	2q14	Adult onset, vocal cord paralysis
HMN VIIB	AD	DCTN1	Adult onset/vocal cord paralysis/facial weakness
HMN/ALS4	AD	SETX	Early onset, pyramidal signs
HMN-J	AR	9p21.1-p12	Juvenile onset, pyramidal features, Jerash
Congenital distal SMA	AD	12q23-12q24	Antenatal onset, arthrogryposis

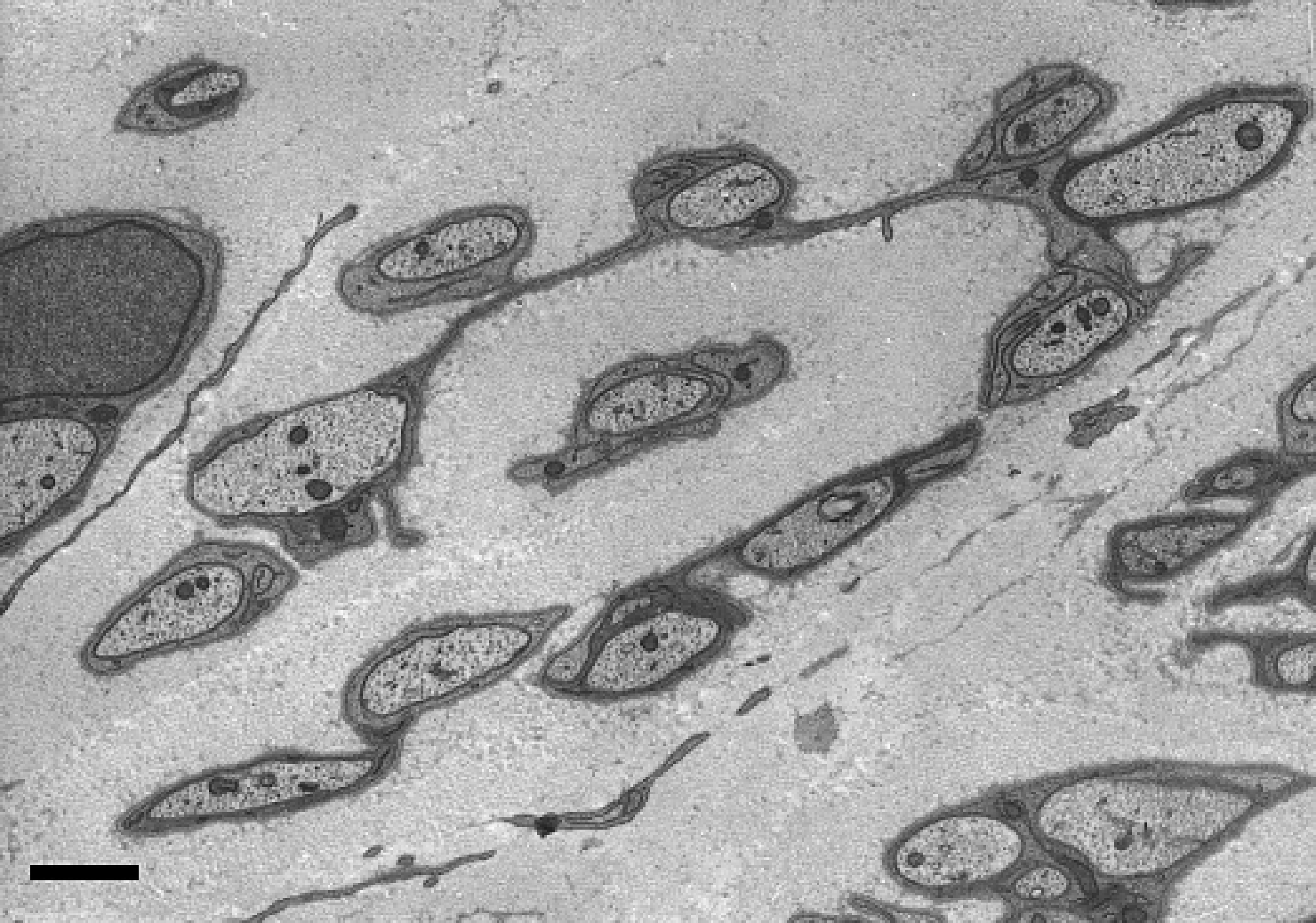
Abbreviations: HSP 27 = heat shock 27kDa protein 1; HSP 22 = heat shock 22kDa protein 8; GARS = glycyl-tRNA synthetase; BSCL2 = Berardinelli-Seip congenital lipodystrophy 2 (Seipin); IGHMBP2 = immunoglobulin mu binding protein 2; DCTN1 = dynactin1; SETX = sentaxin.

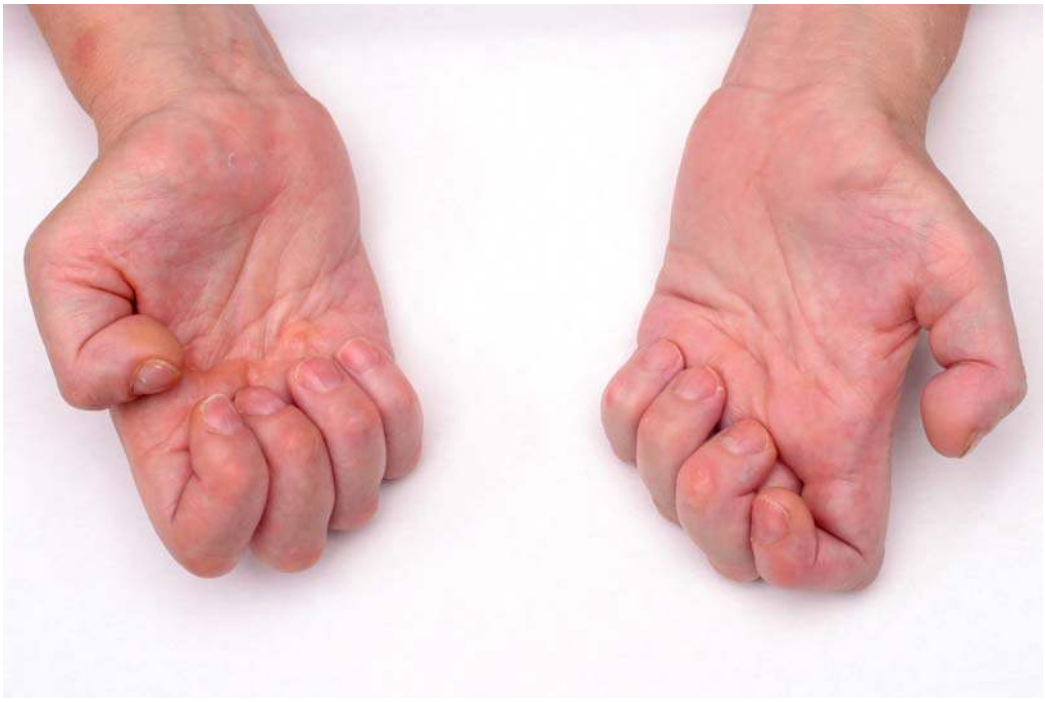
TABLE 5: Gene therapy delivery systems

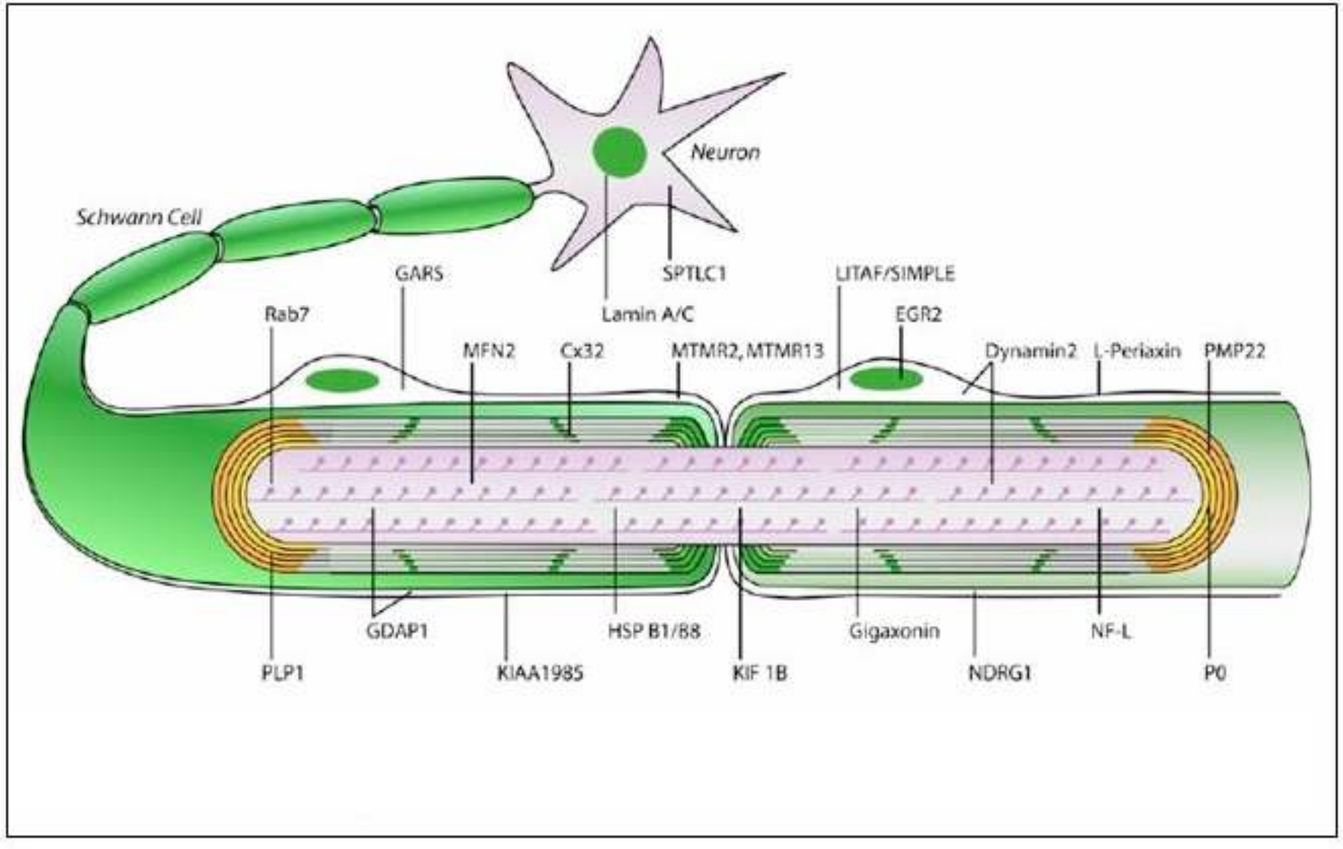
GROUP	EXAMPLES	CELLULAR TARGET
RNA viral vectors		
	Retroviral vectors	Dividing cells
	Lentiviral vectors	Non-dividing cells
DNA viral vectors		
	Herpes Simplex	Neurons (sensory)
	Adenoviral	Non-dividing cells
	Adeno-Associated viral	Non-dividing cells
Non-viral		
	Naked DNA	Non-dividing cells
	Stem Cells	Cellular replacement or trophic support

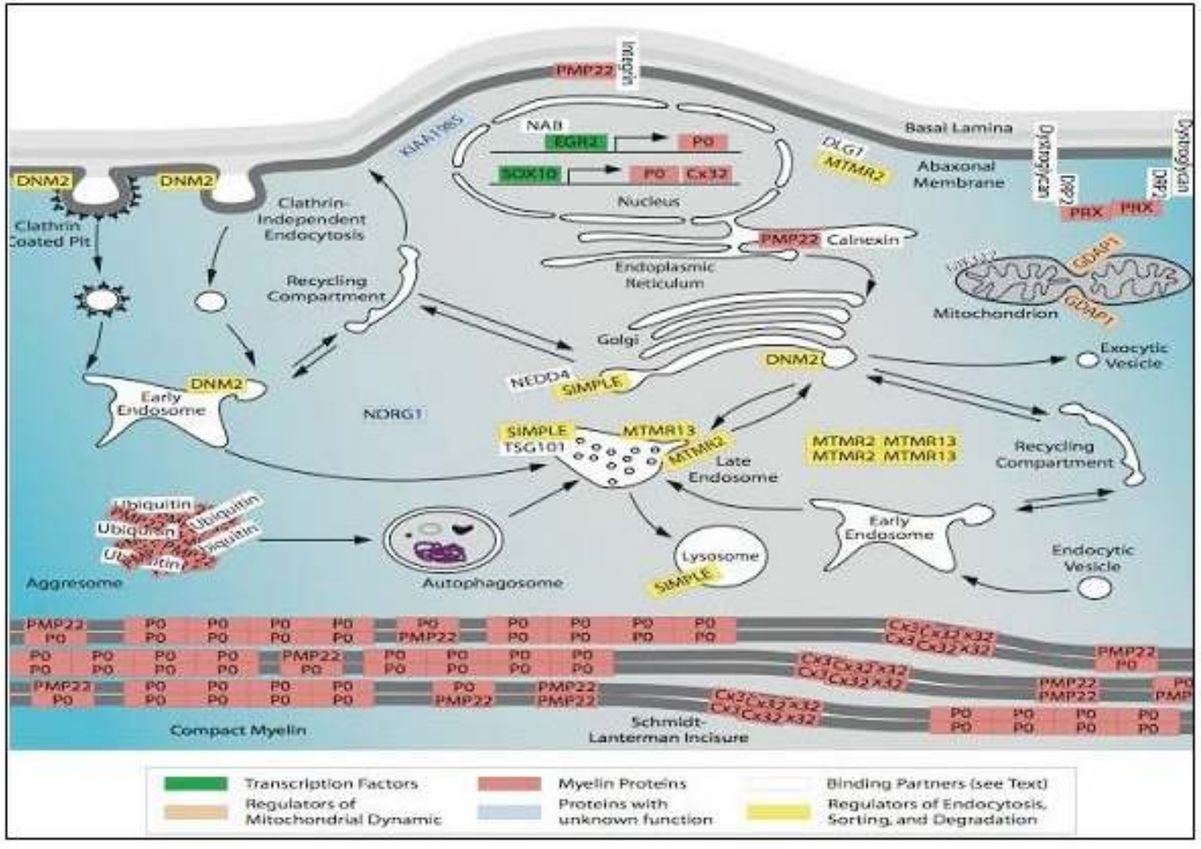

CMT1
(AD, X-linked, sporadic)
Chr 17 duplication
GJB1
MPZ
PMP22
NEFL / EGR2 / LITAF


CMT2
(AD, Sporadic)
Classical CMT2
CMT2 + Sensory
CMT2 + upper limb onset
MFN2
(especially if severe/optic atrophy)
SPTLC1
(especially if pain)
BSCL2
MPZ/NEFL/HSP27/HSP22
RAB7
GARS






Neuron
Schwann Cell
GARS
SPTLC1
Rab7
Lamin A/C
LITAF/SIMPLE
EGR2
MFN2
Cx32
MTMR2, MTMR13
Dynamin2
L-Periaxin
PMP22
GDAP1
HSP B1/88
Gigaxonin
NF-L
PLP1
KIAA1985
KIF 1B
NDRG1
P0


PMP22
Integrin
NAB
EGR2
P0
SOX10
P0
Cx32
Nucleus
Basal Lamina
Abaxonal Membrane
Dystroglycan
Dystrophin
DRP2
PRX
PRX
DLG1
MTMR2
KIAA1985
DNM2
DNM2
Clathrin Coated Pit
Clathrin-Independent Endocytosis
Recycling Compartment
PMP22
Calnexin
Endoplasmic Reticulum
Golgi
Mitochondrion
GDAP1
GDAP1
DNM2
Exocytic Vesicle
NEDD4
SIMPLE
Early Endosome
NDRG1
SIMPLE
MTMR13
TSG101
MTMR2
Late Endosome
MTMR2 MTMR13
MTMR2 MTMR13
Recycling Compartment
Ubiquitin
Ubiquitin
Ubiquitin
Ubiquitin
Aggresome
Autophagosome
Lysosome
SIMPLE
Early Endosome
Endocytic Vesicle
Compact Myelin
Schmidt-Lanterman Incisure
Transcription Factors
Regulators of Mitochondrial Dynamic
Myelin Proteins
Proteins with unknown function
Binding Partners (see Text)
Regulators of Endocytosis, Sorting, and Degradation