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REVIEW ARTICLE

Molecular Genetics of Autosomal-Dominant Axonal Charcot-Marie-Tooth Disease

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Abstract

The autosomal-dominant axonal peripheral neuropathies comprise a genetically heterogeneous group of disorders that are clinically subsumed under Charcot-Marie-Tooth disease type 2 (CMT2). A significant increase in the number of genes underlying major forms of CMT2 has improved the classification of specific CMT phenotypes. The molecular dissection of cellular functions of the related gene products has only begun and detailed pathophysiological models are still missing, but already the biological scope of genes linked to CMT2 is more diversified than CMT1. The known CMT2 genes present key players in these pathways and will likely prove as powerful tools in identifying eventual future targets for therapeutic intervention.

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Index Entries: HMSN II; Charcot-Marie-Tooth disease; axonal neuropathy; motor neuropathy, CMT2.

Traditionally, the Charcot-Marie-Tooth (CMT) neuropathies, also known as hereditary motor and sensory neuropathies (HMSN), are one of the most common inherited disorders, with its prevalence estimated at about 1 in 2500 individuals (Skre, 1974). CMT neuropathies initially were separated into two major groups: CMT1 with reduced nerve conduction velocities (NCV) and CMT2 with normal NCV but decreased conduction amplitudes. This classification has been used to label families and types of neuropathy as demyelinating (CMT1) or axonal (CMT2). The peripheral nerves rely on the presence of intact myelin to maintain their normal conduction velocity of about 40–50 m/s. Damage to myelin reduces this ability and lowers the NCV, leading to the association of CMT1 families with demyelination. In contrast, if the axon is initially damaged, then the number of axons contributing to the nerve impulse will drop, but those remaining should have "normal" myelination. In these cases, the NCV representing the remaining intact axons should be normal, but the amount of the

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impulse (the peak height), representing the total number of functional axons, should drop and these individuals should appear as CMT2. With increasing knowledge of gene defects in both CMT types, this relatively simple concept has recently broken down, as genetic overlap has become evident between the CMT1 and CMT2 phenotypes. Families previously classified as CMT1 and CMT2 by NCV, and even pathology, have now been found to have mutations in the same gene. In addition, overlap has been seen between CMT2 and related diseases, such as distal hereditary motor neuropathy (dHMN)/distal spinal muscular atrophy (dSMA) and hereditary spastic paraplegia (SPG).

Currently, the most accepted classification for CMT uses a sequential lettering scheme and is used for this review (Table 1). Clearly, new approaches are needed to allow those less familiar with the disease to navigate through the different groups. Interestingly, a number of the defective gene products share domain similarities, cellular localization, or biological function (Table 1), and may contribute to the phenotype overlap. The emerging pathways for axonal neuropathies will facilitate focused functional and genetic research and should ultimately enable the identification of targets for therapeutic intervention.

Harding and Thomas (1980b), in their *Brain* paper, used HMSN II/CMT2 as a general term describing all of the inherited axonal neuropathies they examined. They found, however, that only 6 of their 56 CMT families appeared to be autosomal-recessive in inheritance, whereas the rest had an autosomal-dominant pattern of inheritance. Those families with autosomal-recessive inheritance are currently classified as CMT4. This review focuses on families lying within the current CMT2 classification (i.e., autosomal-dominant inheritance).

Clinical Hallmarks of CMT2

The "classic" clinical hallmarks of CMT are an inherited motor/sensory neuropathy, where the motor signs and symptoms are much greater than its sensory manifestations. In any one individual, CMT2 cannot be distinguished from CMT1 with any practical reliability. Variations of this motor–sensory relationship exist, however, and define certain types of CMT2 (Table 1). The usual presentation begins with distal muscle weakness and atrophy of the lower extremities, followed by involvement of the upper limbs, sensory loss, decreased or absent deep tendon reflexes, and foot deformities such as *pes cavus* and hammer toes. Less frequent symptoms, such as cranial nerve involvement, scoliosis, vocal cord paresis, and glaucoma, have also been described. CMT2 does have a wider range of age of onset than CMT1, with the majority of cases presenting between 10 and 30 yr. Initial estimates by Harding and Thomas suggested using 38 m/s in NCV as the velocity separating CMT1 and CMT2. Using this cutoff revealed that CMT2 represented only one-third of all CMT cases (Harding and Thomas, 1980b). It seems likely that given the genetic overlap so far observed, this number will increase significantly in the future.

Genetic Heterogeneity of CMT2

Advances in genetic research have led to further diversification of CMT. All known CMT forms are characterized as Mendelian traits and with few rare exceptions show complete penetrance, although the severity and extent of disease can vary considerably, even within affected members of the same family. Autosomal-dominant, -recessive, and X-linked forms have been described. During the last 15 yr, more than 30 genes have been identified for CMT; however, only relatively recently 10 genes have been described underlying axonal neuropathies (http://www.molgen.ua.ac.be/CMTMutations). Six genes are now known to primarily cause autosomal-dominant axonal CMT: mitofusin 2 (*MFN2*; CMT2A), RAS-associated protein 7 (*RAB7*; CMT2B), glycyl-tRNA synthetase (*GARS*; CMT2D), neurofilament light (*NEFL*; CMT2E), heat-shock protein 27 (*HSP27*; CMT2F), and heat-shock protein 22 (*HSP22*; CMT2L). A number of genes, whose primary phenotype falls within other CMT types, have also been shown to cause axonal CMT in some cases. Patients with mutations in myelin protein zero (*MPZ*) (CMT1B), gap-junction protein β-1 (*GJB1*; CMTX), ganglioside-induced differentiation-associated protein 1 (*GDAP1*; CMT2H, K and CMT4A) and dynamin 2 (*DNM2*; DI-CMTB) have all fit within this relatively small, but important group.

Despite these recent gene identifications, a large number of CMT genes still await discovery. At least

Table 1
Known Autosomal-Dominant Axonal CMT Genes and Their Protein Products According to the Traditional Classification

Disease	Clinical phenotype	Gene	Gene product	Proposed function	Putative protein domains (NCBI Conserved Domain Database)	Chromosomal location	OMIM
CMT2A	Classic CMT2	*MFN2*	Mitofusin 2	Mitochondrial membrane fusion	Dynamin-like GTPase, Fzo_mitofusin	1p36.2	609260
CMT2A[a]	Classic CMT2	*KIF1B*	Kinesin family member 1B	Axonal transport	Kinesin motor domain, Pleckstrin homology, ATPase	1p36.2	609260
CMT2B	CMT2 with severe sensory involvement	*RAB7*	Ras-associated protein 7	Endosomal trafficking	Rab subfamily of small GTPases	3q21	600882
CMT2C	CMT2 with vocal cord and respiratory involvement	Unknown	Unknown			12q23	606071
CMT2D	CMT2 with predominant hand involvement	*GARS*	Glycyl-tRNA synthetase	tRNA processing	GARS	7p15	601472
CMT2E	CMT2 or intermediate to slow NCV	*NEFL*	Neurofilament light	Neuronal cytoskeleton	Intermediate filament protein	8p21	607684
CMT2F	Classic CMT2 or dHMN	*HSP27*	Small HSP27	Mitochondrial molecular chaperone	α-Crystalline	7q11–21	606595
CMT2G	Classic CMT2 with slow progression	Unknown	Unknown				
CMT2H	CMT2 with vocal cord paralysis or intermediate to slow NCV	*GDAP1*	Ganglioside-induced differentiation-associated protein 1	Mitochondrial glutathione transferase	Glutathione-*S*-transferase	8q13-q21.1	607731
CMT2I	Predominantly CMT1 but also late onset CMT2	*MPZ*	MPZ	Myelin structural protein	Immunoglobulin domain	1q22	607677
CMT2J	Predominantly CMT1 but also CMT2 with Adie's pupil	*MPZ*	MPZ	Myelin structural protein	Immunoglobulin domain	1q22	607736
CMT2K	CMT2 with vocal cord paralysis or intermediate to slow NCV	*GDAP1*	Ganglioside-induced differentiation associated protein 1	Mitochondrial glutathione transferase	Glutathione-*S*-transferase	8q13-q21.1	607831
CMT2L	Classic CMT2 or dHMN-II	*HSP22*	Small heat shock protein 22	Mitochondrial molecular chaperone	α-Crystalline	12q24.3	608673

[a]A KIF1B mutation has been reported in only one small Japanese pedigree.

two CMT2 genomic loci are unresolved; CMT2C on 12q23 and CMT2G on 12q13. Additional genetic heterogeneity is very likely (Klein et al., 2003; Nelis et al., 2004). Although this situation provides challenges for the efficient clinical application of molecular diagnosis in CMT patients, the wealth of information has presented opportunities to begin to decipher the major underlying pathways in CMT2.

CMT2A: Mitofusin 2 and Kinesin Motor Protein B

The CMT2A locus was the first axonal neuropathy locus mapped to a chromosome, located in the chromosome 1p36 region (Ben Othmane et al., 1993). Unfortunately, this genomic region is very unstable and the actual size of the region could not be determined until the very end of the human genome sequencing effort. While waiting for this feat to be accomplished, a mutation in the protein, kinesin motor protein B (KIF1B) was reported in a single Japanese CMT2A family, suggesting that this was the defect for CMT2A. In addition, heterozygous *Kif1b* knockout mice were found to have developed a neuropathy-like phenotype (Zhao et al., 2001), supporting this finding. However, subsequent studies by CMT investigators throughout the world could not find any KIF1B cases among the known CMT2A families.

Thus, once the genomic map in the candidate region for CMT2A became reliable, we sought to find the primary gene for this locus. After screening more than 15 different genes, the gene mitofusin 2 (*MFN2*) was identified as the underlying cause in all linked families reported thus far (Zuchner et al., 2004a). Just 1 yr after we initially reported mutations in *MFN2*, the gene was established as the most prevalent axonal CMT form, comprising up to 20% of CMT2 (Zuchner et al., 2004a; Kijima et al., 2005; Lawson et al., 2005). This makes it second in frequency only to the *PMP22* duplication (CMT1A) on chromosome 17 as a cause of the CMT phenotype. Clinically, it may be the only true "axonal" NCV phenotype in CMT, as almost all known *MFN2* cases have NCV greater than 38 m/s, the traditional cutoff originally proposed by Harding and Thomas (1980a).

Phenotypically, *MFN2* continues to encompass an increasing group of disorders. Recently, an Australian family with a new *MFN2* mutation presented clinical features that suggested involvement of the first motor neuron (thus within the central, not peripheral nervous system), a disorder described previously as HMSN V (Zhu et al., 2005). We have also reported a case with a *de novo* truncation mutation in *MFN2* resulting in CMT2 and optic atrophy, also known as HMSN VI (Zuchner et al., 2004a). Lawson et al. (2005) reported that 59% of affected individuals in their largest *MFN2* family had sensorineural hearing loss. Thus, as the spectrum of patients with *MFN2* mutations increases, it would be expected that variable expression of *MFN2* phenotypes will continue to expand.

MFN2 is a large dynamin-like GTPase that spans the outer mitochondrial membrane (Rojo et al., 2002). Several studies revealed that MFN2 is a major factor for the fusion of mitochondria, specifically the outer mitochondrial membrane (Chen and Chan, 2004). Mitochondria constitute a highly dynamic network that constantly undergoes fusion and fission events (Nunnari et al., 1997). Koshiba et al. (2004) recently showed that the oligomerization of mitofusins allow for tethering of mitochondrial membranes. MFN2 mutants and MFN deficiency have been shown to disperse mitochondria and also reduce mitochondrial mobility (Santel and Fuller, 2001). This reduced mobility could lead to insufficient axonal transport of mitochondria presumably in the extended axons of peripheral nerves. Recently, Pich et al. (2005) also linked MFN2 function with the regulation of differential expression of oxidative phosphorylation (OXPHOS) complexes. Thus, it is possible that different mutations in MFN2 could affect different functions of the protein.

Mitochondrial dysfunction is known to be involved in a number of neurodegenerative diseases including Parkinson's, Alzheimer's, and neuromuscular diseases. The importance of mitochondria ranges from energy supply to the initiation of apoptosis. Several genes have recently been identified for axonal CMT that are encoded by the nuclear genome but fulfill their main known biological function in mitochondria, such as *MFN2*, the genes for heat-shock 22-kDa protein (*HSP22*), heat-shock 27-kDa protein (*HSP27*), and ganglioside-induced differentiation-associated protein 1 (*GDAP1*). Thus, a number of axonal CMT forms could be viewed as mitochondrial diseases. Why these mutations manifest phenotypes primarily in the peripheral nerve

is currently unknown, but seems likely owing to the extraordinary length, energy, and transport requirements of the nerve.

Finally, given that only one case has been reported as a result of *KIF1B* variation, and all other known CMT2A families are clearly as a result of *MFN2* mutations, an international CMT consortium (Second Symposium of the North American CMT Consortium, May 19–21, 2005, University of Western Ontario, Canada) has recently suggested that *KIF1B* should not be considered as a significant nor viable candidate for the etiology of CMT2 in future studies, or in unknown CMT2 patients, until confirmation of the *KIFIB* mutation is found.

CMT2B: RAB7

The gene underlying CMT2B, *RAB7*, is a member of the RAB family of RAS-associated GTP-binding proteins (Verhoeven et al., 2003). CMT2B is clinically characterized by marked distal muscle weakness and wasting but also prominent sensory loss leading to severe foot ulcerations of the lower legs. In fact, as a result of recurrent infections, amputations are often required. Auer-Grumbach et al. (2000) reported that the onset of symptomatic disease ranged from 13 to 30 yr (mean 18.1 yr). Thus, CMT2B does not follow the traditional pattern of motor symptoms being greater than sensory complaints. Several authors have argued that CMT2B actually falls within the classification of hereditary sensory and autonomic neuropathies (Vance et al., 1996; Auer-Grumbach, 2004; Houlden et al., 2004) rather than CMT. However, the CMT designation has remained. Only a few of these families have been reported, and thus CMT2B appears to be rare. The main differential diagnosis would be hereditary sensory neuropathy type 1 (SPTLC1). RAB proteins are important regulators of vesicular transport and are located in specific endosomal compartments (Bottger et al., 1996). RAB7 has been localized to late endosomes and shown to be important in the late endocytic pathway (Feng et al., 1995; Meresse et al., 1995).

CMT2C: 12q23-24

CMT2C is a rare autosomal-dominant motor and sensory neuropathy involving limb, diaphragm, vocal cord, and intercostal muscles (Dyck et al., 1994; Nagamatsu et al., 2000). Sensory loss is also a dominant symptom in CMT2C. Among affected individuals the age of onset and clinical severity has been reported to be variable. Individuals presenting with symptoms in childhood tend to have a severe disease and shortened life span as a result of respiratory failure and secondary complications. Using a large family of British origin, Klein et al. (2003) performed linkage analysis and determined a peak two-point LOD score of 4.73 at chromosome 12q23-24. The putative chromosomal locus contains a number of genes for neuromuscular diseases, such as spinocerebellar ataxia type 2, scapuloperoneal spinal muscular atrophy, and congenital dSMA, but the CMT2C gene remains to be identified.

CMT2D: GARS

One of the more surprising genes where mutations lead to axonal CMT is *GARS*, which causes CMT2D and dSMA V/dHMN V (Antonellis et al., 2003). For proper protein translation in eukaryotic cells, tRNA molecules must be charged in both the cytoplasm and mitochondria. *GARS* is thought to charge $tRNA^{Gly}$ molecules in both cytoplasm and mitochondrial locations (Shiba et al., 1994). As it is ubiquitously expressed, it was unexpected that such a fundamental RNA process would play an important role in CMT. The reason for this is currently unclear, but should provide a better understanding of the underlying mechanisms.

A recent study suggested that in many patients the presenting symptoms in CMT2D were muscle weakness in the hands, often preceded by transient cramping and pain on exposure to cold or exertion (Sivakumar et al., 2005). Progressive weakness and atrophy of the thenar and first dorsal interossei muscles were the major complaints in each patient described. In some patients, hand weakness started unilaterally, but soon became bilaterally involved. This presentation is distinctly different than most cases of CMT, where weakness and symptoms usually present in the distal muscles of the lower leg. It should be noted, however, that in one of the Duke families used for the initial identification of *GARS*, the patients presented with the typical CMT lower extremity to upper extremity progression of weakness. Sensory loss has been found to be mild, or none at all in the dSMA/V patients. The onset of symptoms

occurred between 8 and 36 yr of age, with most patients developing symptoms during the second decade of life (Sivakumar et al., 2005).

Thus, CMT2D and dSMA/V are very similar in clinical appearance except for the presence of sensory symptoms and signs in patients with CMT2D. Interestingly, the isolated presence of both phenotypes was present in different individuals of the same family (Antonellis et al., 2003). The reasons for this are not yet known.

CMT2E: Neurofilament Light

Intracellular transport in extended axons is dependent on an intact cytoskeleton. Therefore, it was perhaps of little surprise that neurofilament light (*NEFL*) was the first gene identified for the hereditary axonal neuropathies of the peripheral nervous system (Mersiyanova et al., 2000). It has remained an uncommon cause of the phenotype. Jordanova et al. (2003) determined the prevalence of CMT2E within 323 patients with CMT or related peripheral neuropathies and identified six mutations (1.9%). The disease has been reported to begin with gait problems usually before the age of 13 yr. Some patients have had a very early onset and presented with delayed motor milestones. Paresis in the distal parts of the lower limbs varied from mild weakness to a complete paralysis. Sensory loss was a common symptom in these patients and included all sensory modalities. All patients described to date have had a *pes cavus* deformity. Surprisingly, the motor NCVs were moderately to severely reduced, ranging from 13 to 38 m/s for the median nerve (normal >42 m/s). The amplitudes of the compound motor action potentials were usually severely reduced (Jordanova et al., 2003). Slow to normal NCVs in CMT2E patients have been reported by others as well (Georgiou et al., 2002; Zuchner et al., 2004b). Fabrizi et al. (2004) reported an Italian family with a Pro22Ser mutation in NEFL manifesting electrophysiologically as CMT1 and pathologically as an axonopathy with giant axons and accumulation of disorganized neurofilament.

Neurofilaments are synthesized in the perikaryon and then transported to the axons where they assemble into filamentous networks. Neurofilaments comprise the most abundant intermediate filament in neurons. Pérez-Ollé et al. (2005) demonstrated in cell culture that mutant NEFL had a variety of negative effects on axonal transport in both directions and specifically on the transport of mitochondria. It is obvious that the trafficking of vesicles, mitochondria, and other membrane encased organelles along microtubules in the axons of peripheral nerves is a crucial factor for the function of neurons. Impairment of trafficking would affect the most distant segments of axons first. This is indeed what occurs in most axonal neuropathies and could explain why ubiquitously expressed proteins lead to exclusive neuronal damage.

CMT2F: HSP27

Two small heat-shock proteins HSP22 and HSP27, have been identified to underlie different forms of axonal CMT and distal HMN (Evgrafov et al., 2004; Irobi et al., 2004; Tang et al., 2004), although these have been reported in only a few families. Mutations in HSP27 cause CMT2F (Evgrafov et al., 2004). Two CMT2F families have been reported thus far, both showing the classic axonal CMT phenotypes. Patients had symmetrical weakness of the lower limb muscles, foot drop, foot deformity, and mild-to-moderate sensory impairments. Wasting of upper limbs muscles caused clawing of the hands several years later. Cranial nerves were not involved. The disease onset of patients to date has been between the age of 15 and 25 yr with a slowly progressive course (Ismailov et al., 2001).

Heat-shock proteins operate as molecular chaperones, preventing misfolding of proteins, especially in oxidative environments such as mitochondria (Ganea, 2001). A wide range of endo- or exogenous factors is able to induce expression of these genes, which are characterized by a conserved α-crystalline domain (Kappe et al., 2003). Small HSPs like HSP27 have been shown to protect against H_2O_2-mediated cell death (Mehlen et al., 1995). Neuronal cell lines transfected with mutant HSP22 (CMT2L) or HSP27 showed a reduced viability (Irobi et al., 2004; Carra et al., 2005). Also, Hsp27 has been shown to be directly responsible for a stabile mitochondrial membrane potential through an increase and maintenance of the reduced form of the redox modulator glutathione (*see* GDAP1 in CMT2H,K) (Preville et al., 1999).

CMT2G: 12q13

A genome-wide linkage analysis in a large Spanish CMT2 family revealed a new chromosomal locus

(CMT2G) at 12q12-13.3 (Nelis et al., 2004). The locus encompassed 12.8 CM of genomic distance. The age at onset in this family was between 9 and 76 yr, with the majority of patients developing initial symptoms in their 20s. The disease presented with foot deformity, difficulty walking, and very slow progression. Patients were mildly disabled. Mild stocking hypoesthesia was seen. The upper limbs were involved in only two patients. Scoliosis, pupillary abnormalities, foot ulcers, deafness, diaphragm and vocal cord paralysis, nerve enlargement, optic atrophy, tremor, and ataxia were absent (Berciano et al., 1986). A postmortem study of the proband showed a loss of neurons in the anterior and dorsal horn and degeneration of the *fasciculus gracilis*. Morphometric evaluation of L5 ventral and dorsal roots revealed significant loss of large myelinated fibers and regeneration (Berciano et al., 1986).

CMT2H and K: GDAP1, CMT2I, and CMT2J: MPZ

As discussed, the original CMT classification defined dominant, primarily demyelinating neuropathies as CMT1 and axonal phenotypes as CMT2. Recessive CMT forms are usually subsumed under CMT4. However, some clinical phenotypes were reported as new CMT forms (and labeled as such), but later on, the underlying genes causing these rarer phenotypes turned out to be major genes that primarily cause CMT1 or recessive CMT phenotypes.

The CMT2H and CMT2K subtypes are caused by mutations in the gene *GDAP1*. In 2001, Barhoumi et al. (2001) reported a large Tunisian family with autosomal-recessive axonal CMT and pyramidal features that they linked to the chromosomal area on chromosome 8q where the demyelinating CMT4A had been described previously by Ben Othmane et al. (1994). This axonal CMT form was consequently classified as CMT2H instead of CMT4A. In 2002 both our laboratory and Cuesta et al. (2002) demonstrated that mutations in *GDAP1* cause autosomal-recessive CMT type 4A (CMT4A) (Baxter et al., 2001). The finding was fascinating as the two reports differed in the phenotypes of their families. Cuesta et al. (2002) described families who had an axonal phenotype (OMIM 607706) whereas our families from Tunisia showed a demyelinating phenotype (Baxter et al., 2002) (OMIM 214400). Subsequent reports have confirmed the duality of *GDAP1* phenotypes, even for the same mutation site (Nelis et al., 2002). Senderek et al. (2003) also reported two *GDAP1* families with intermediate NCV and signs of both axonal and demyelinating pathology in sural nerve biopsies.

Relevant to this review, a recent study by Claramunt et al. (2005) identified six new *GDAP1* mutations in a sample of 106 Spanish CMT families, with two (Arg120Trp, Thr157Pro) suggesting an autosomal-dominant transmission pattern. The patients heterozygous for the Arg120Trp mutation had a disease onset at the end of the second decade, very slow progression, and an overall much milder phenotype than patients with a recessive GDAP1 form. The patient with the heterozygous *de novo* mutation, Thr157Pro, developed symptoms within the first year of life with moderately reduced distal strength in the lower limbs, absent tendon reflexes, and optic atrophy (Claramunt et al., 2005). Current evidence suggests that GDAP1 represents a unique class of glutathione transferases (Marco et al., 2003), and Pedrola et al. (2005) have demonstrated that it localizes within mitochondria.

Several families have been classified as CMT2I and CMT2J (by Online Mendelian Inheritance in Man [OMIM]), but were found to carry mutations in *MPZ*, which primarily leads to a demyelinating NCV phenotype (CMT1B). However, it is now established that *MPZ* mutations in certain families cause primarily axonal phenotypes. For CMT2I, Senderek et al. (1999) reported two autosomal-dominant CMT2 families with *MPZ* mutations (Asp61Gly, Tyr119Cys) characterized by late onset (range 47–60 yr), distal muscle weakness and atrophy, foot deformity, distal sensory loss, and hyporeflexia. An Austrian family and a single unrelated patient with very late-onset axonal CMT (range 65–70 yr) also were found to have mutations in the *MPZ* gene (Asp60His, Ile62Met) (Auer-Grumbach et al., 2003). The phenotype included prominent sensory involvement and led to marked disability within a few years. A number of additional axonal and intermediate *MPZ* families were reported (Marrosu et al., 1998; Boerkoel et al., 2002; Sowden et al., 2005).

Interestingly, an axonal phenotype associated with pupillary abnormalities has been associated with *MPZ*. This association was first noticed with the Thr124Met mutation in MPZ and has been designated as CMT2J by OMIM. As noted by De Jonghe et al. (1999), these patients have a distinct

phenotype characterized by late onset (fourth to fifth decade), marked sensory abnormalities, deafness, and pupillary abnormalities (Adie's pupil). NCVs varied from less than 38 m/s to normal values. Sural nerve biopsies revealed clusters of remyelinating axons consistent with chronic axonal regeneration. The Thr124Met mutation was subsequently found in different populations, including Japanese CMT patients (Misu et al., 2000). By analyzing haplotypes from Thr124Met individuals of European descent, Senderek et al. (1999) suggested that the Thr124Met change is a mutational hotspot rather than an ancient haplotype shared by all affected individuals. Seeman et al. (2004) reported a family with a Glu97Val change in MPZ where hearing impairment and pupillary abnormalities preceded the CMT phenotype. A Japanese study identified the Asp75Val mutation also associated with this distinct CMT phenotype (Misu et al., 2000). The molecular reasons for these varying phenotypes associated with certain *MPZ* mutations are not fully understood. A very informative recent review on *MPZ* genotype–phenotype correlations has been published by Shy et al. (2004).

CMT2L: Small HSP22

In 2004, Tang et al. reported a large Chinese family with autosomal-dominant axonal neuropathy. Eighteen individuals were diagnosed with CMT2. Onset of disease was between 15 and 33 yr of age. Symptoms included weakness of the lower limbs and mild sensory loss. Notably, three affected members of the family had scoliosis. The NCV of the median nerve was normal, ranging from 56.7 to 69.2 m/s (mean: 64.8 m/s). A whole genome analysis detected a maximal two point LOD score of 8.08 near the *HSP22* locus. After this finding, the gene for distal hereditary motor neuronopathy type II, *HSP22*, was identified at the same locus (Irobi et al., 2004). Eventually, Tang et al. (2005) detected a new *HSP22* mutation in their family and proved that CMT2L is caused by unique mutation in the same gene. A mutation screen of 114 Chinese CMT patients did not reveal a second *HSP22* mutation in this population (Zhang et al., 2005). This situation is very similar to CMT2F and CMT2D, where certain mutations in *HSP27* and GARS cause different dHMN/dSMA subtypes. HSP22 and HSP27 are members of the same protein family and presumably share disease pathways. Neuronal cell lines transfected with mutant HSP22 or HSP27 showed a reduced viability and mutant HSP22 promoted the formation of intra-cellular aggregates (Benndorf et al., 2001; Irobi et al., 2004; Carra et al., 2005).

Unclassified Overlap With Axonal Neuropathies

The more mutational data that is available for CMT genes, the more it becomes apparent that axonal phenotypes might be associated with a number of other CMT genes. The classic example is *GJB1* or *connexin 32*, which constitutes an X-linked CMT form. It has been known for a long time that *GJB1* mutations cause demyelinating, intermediate, and also axonal NCV phenotypes of CMT. Interestingly, *GJB1* is expressed in Schwann cells but not in the axon (Zhou and Griffin, 2003). The first identified gene for the so-called intermediate CMT forms, dynamin 2 (*DNM2*), also caused pure axonal phenotypes in some patients (Zuchner et al., 2005). Finally, a recent study found a new mutation (Thr49Met) in LITAF/SIMPLE (CMT1C) associated with an axonal neuropathy (Saifi et al., 2005).

Discussion

The number of genes identified that cause autosomal-dominant CMT2 has increased impressively over the last 4 yr. Key to this success has been the careful description and collection of affected families, as well as productive collaborations between different research sites. Also, with the completion of the Human Genome Project the inaccuracy of positional cloning is no longer a major barrier in CMT2. Whereas the axonal CMT phenotype still appears to be less common than CMT1, it seems highly likely that we will find CMT2 to be more common than the one-third of all CMT cases believed today. Indeed, the majority of "idiopathic" neuropathies presenting to the average neurology clinic are primarily axonal in nature, and these have yet to be screened in any number to see if sporadic CMT2 patients are more prevalent than currently known.

Major underlying pathways involved in CMT2 relate to mitochondrial function, endosomal

trafficking, and RNA processing (Table 1). Some of the causative proteins have been the subject of many molecular biology studies; however, not necessarily in relation to the nervous system. The results of future research promise to be of major relevance for the whole field of neurodegenerative diseases and also for the common nonhereditary axonal neuropathies.

As the identification of genes continues, it seems likely that it will continue to substantiate the notion that significant genetic–phenotypic overlap exists between traditionally separate disease entities and classifications. The current classification schemes are not designed to handle this overlap, though one of us previously proposed a new classification that would allow the needed flexibility (Vance, 2000). Table 1 reflects this confusion, as a number of subtypes currently designated by OMIM are only phenotypic variants or single reports of known gene defects. Clearly, a new classification scheme that is useful to both the clinician and geneticist needs to be designed.

CMT patients and neurologists still wait for real therapeutic options. Currently, only supportive therapies are available for CMT2. Before targeted therapy develops for axonal neuropathies, it seems likely that we will need more insight into key molecular questions. The identification of the underlying genes is therefore an important but only intermediate step toward resolving CMT at the bedside.

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