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REVIEW ARTICLE

Intermediate Forms of Charcot-Marie-Tooth Neuropathy

A Review

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Abstract

The Charcot-Marie-Tooth (CMT) neuropathies divide into two main electrophysiological groups with slow and near normal conduction velocities corresponding to Schwann cell and axonal pathology. An intermediate group also exists with nerve conduction velocities, which overlaps the two main groups. Families with intermediate CMT can be recognized in which different affected individuals in the same family have motor conduction velocities in both the CMT type 1 and 2 ranges (i.e., above and below 38 m/s). The intermediate group is caused by a limited number of distinct gene mutations in dynamin2 (*DNM2*), gap-junction protein 1 (*GJB1*), neurofilament light polypeptide (*NF-L*) genes, and a rare mutation and as yet unknown genes on chromosome 1 and 10 loci. Intermediate forms of CMT may be associated with unique disease mechanisms affecting both Schwann cells and axons. It is useful to recognize this unique group of neuropathies for diagnostic and management purposes.

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Introduction

The Charcot-Marie-Tooth (CMT) syndrome is a complex group of more than 50 disease entities split into two main groups (CMT types 1 and 2) that are separated by an intermediate group. The dividing line for the separation of the two groups and the presence of the intermediate group have been a controversial topic.

In his original description, Tooth described what we now call CMT as "peroneal muscular atrophy." Charcot and Marie called it a "special form of progressive muscular atrophy." To quote the first paragraph of Charcot and Marie's (1886) article, "Progressive muscular atrophy seems to subdivide into secondary groups which increase in number as clinical observation becomes more attentive and more precise." Charcot and Marie were referring

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to the general group of muscular atrophies including amyotrophic lateral sclerosis (ALS), but this quote is now a very apt description for the many different genetic entities comprising CMT.

Historical Classification of CMT Syndromes

Slowing of nerve conduction velocity (NCV) in CMT was first shown by Gilliat, who published a single case report (Gilliatt and Thomas, 1957). Other families have normal NCV (Dyck and Lambert, 1968; Thomas and Calne, 1974), which allowed the separation of CMT into two main groups called types 1 and 2 by Dyck (1975), corresponding to the presence of axonal and demyelinating pathology (Thomas and Calne, 1974).

By plotting motor NCV of probands in CMT families, a dividing line of 38 m/s between the two groups was initially suggested by Harding and Thomas, 1980). However, a continuum was reported in a Spanish population (Salisachs, 1974). This conclusion was supported by the finding of an "intermediate group of kinships" reported by Madrid et al. (1977), Davis et al. (1978), and Bouche et al. (1983). In the latter study, an intermediate group with distinct pathology was recognized with NCV between 30 and 40 m/s. The intermediate group had segmental demyelination and onion bulb formation, but nerve hypertrophy was not seen, and axonal degeneration and regeneration were prominent (Madrid et al., 1977; Gherardi et al., 1983).

The intermediate group defined by Madrid and Davis was distinguished from the hypertrophic neuropathy group by the absence of clinically observed nerve hypertrophy and by the presence of a number of clinical features, including a more rapidly progressive disease. It was concluded to be genetically separate and distinct from the neuronal group. Nerve biopsy studies supported this view (Madrid et al., 1977). There was a relationship between severity of the disease and the NCV, which was most evident in the intermediate group. The velocity fell in older patients in the intermediate group. (Davis et al., 1978)

Harding's figure of 38 m/s, the lower limit of type 2, has recently been supported by analysis of NCV from the most common form of CMT2 owing to mitofusin (MFN) 2 mutations (Timmerman, North American CMT Consortium meeting).

The figure of 38 m/s does not separate CMT 1A, which can be associated with motor NCVs as fast as 42 m/s. Median motor NCV in CMTX can be as fast as 55 m/s and as slow as 20 m/s, but CMTX has features of both demyelination and axonal degeneration. CMTX can therefore be regarded as an intermediate form of CMT with both primary demyelinating and probably secondary axonal pathology (Scherer et al., 2005). Asymptomatic or mildly affected females can have entirely normal motor conduction velocities, so that families with CMTX have a range of NCVs (if both males and females are included) extending into both the demyelinating and axonal ranges.

Selection of Nerve Conduction Values in Different Affected Individuals in a Family to Define Intermediate CMT

Because slow NCV can be found in axonal neuropathies as a result of the loss of large rapidly conducting fibers (Kimura, 2005), it is therefore important to exclude conduction results in which the motor action potential is reduced. Peroneal conduction velocities are slower than median conduction velocities, so for consistency and for historical reasons median motor NCV results only have been compared in this review.

The Term "Intermediate" Should Not Be Used to Describe a Single NCV

The use of the term "intermediate NCV" was first introduced by Davis, Madrid, and Bradley in 1977 (Madrid et al., 1977). They also used the term intermediate CMT for particular kindreds with axonal and demyelinating features and NCVs between 30 and 40 m/s. Application of the term "intermediate" to describe NCVs in the 30–40 m/s range has caused much confusion because NCVs in affected individuals with either CMT types 1 or 2 can also lie in that range and because of the overlap of values in axonal CMT2A and the demyelinating form of CMT and CMT1A (*see* Fig. 1). A single conduction velocity value is therefore not definitive of intermediate CMT. The term "intermediate" should only be applied to the form of CMT and not the NCV value. In addition, intermediate forms of CMT should also have evidence of dual demyelinating and axonal pathology.

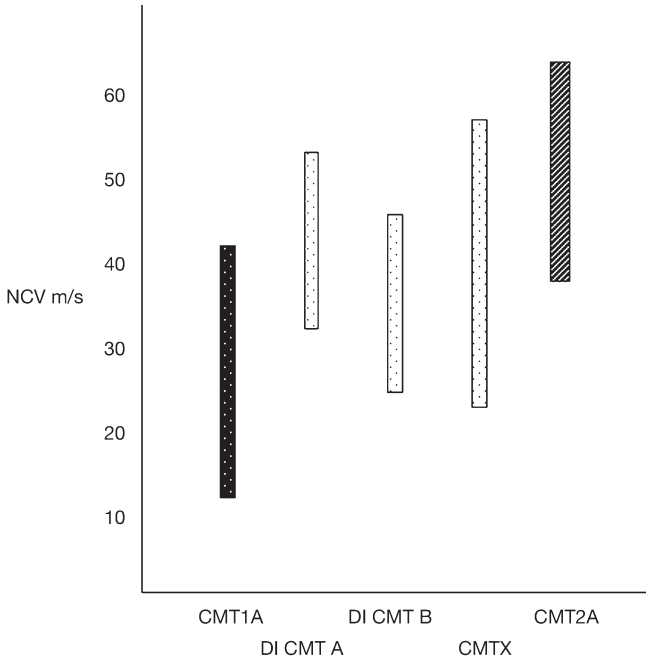

Fig. 1. Median motor nerve conduction ranges (NCV) reported for CMT1, (CMT1A a demyelinating CMT), and CMT2, (CMT2A an axonal form of CMT) and three forms of intermediate CMT, CMTX and DICMT A, and DICMT B.

However, families with intermediate forms of CMT can be recognized by the presence of individuals with median motor NCV in the unequivocal demyelinating range and other affected individuals with velocities in the axonal range, for example, less than 38 m/s in one affected individual and greater than 42 m/s in another affected individual in the same family. This may signal the presence of both axonal and demyelinating pathology in the CMT syndrome affecting the family.

When we selected intermediate CMT using these criteria (a range of NCVs in both the type 1 and 2 ranges), a number of families had no male-to-male inheritance. In approx 50% of these families, X-linked CMT (CMTX1) was confirmed by the presence of *GJB1* mutations. The other 50% of families with no male-to-male inheritance remained unclassified. These families may have other X-linked forms of CMT or may represent dominant intermediate CMT families that, by chance, had no male-to-male transmission. A similar proportion was reported by Rouger et al., who showed that 60% of intermediate families with no CMT1A duplication did not have *GJB1* mutations.

We next selected families with intermediate conduction velocities with autosomal-dominant inheritance (defined by male-to-male inheritance). The largest dominantly inherited CMT family with intermediate conduction velocities had sufficient affected individuals to map the chromosomal location of a gene by linkage analysis to chromosome 19 (Kennerson et al., 2001; Zhu et al., 2003). We then screened genes in the region and found a mutation in *DNM2* (Zuchner et al., 2005; Rouger et al., 1997.) There are a number of other forms of intermediate CMT with dominant inheritance.

Forms of CMT With Intermediate Features

Dominant intermediate CMT type A (DI-CMT A), an intermediate form of CMT, was reported in an Italian family (Rossi et al., 1985; Villanova et al., 1998) Linkage to chromosome 10q24.1-q25.1 was demonstrated by Verhoeven et al. (2001). Sural nerve biopsy in affected members of this family showed axonal degeneration, loss of large diameter fibers, rare segmental demyelination, and remyelination with onion bulb formation (i.e., a combination of Schwann cell and axonal changes).

The ultrastructural findings in peripheral nerve biopsies from two affected members of one family (DI-CMT A single Italian family) have been reported (Malandrini et al., 2001). Demyelinating features such as onion bulbs and myelin splits with uncompacted and irregularly enlarged lamellae, mostly at the Schmidt–Lantermann incisures and in paranodal region were shown. Signs of a chronic axonopathy such as regeneration clusters, large fiber loss, Bungner's bands, and unmyelinated fiber involvement were also seen. These authors concluded that the presence of both demyelinating and axonal findings, not found in other genetically determined types of CMT disease, confirmed a new entity of intermediate CMT (DI-CMT A).

Dominant Intermediate CMT Type B (DI-CMT B)

The gene mutation for our large dominantly inherited intermediate CMT family described earlier was initially mapped to chromosome 19 (Kennerson et al., 2001; Zhu et al., 2003) and this location was confirmed in a north American axonal CMT family. Zuchner, found a mutation in another GTPase, dynamin2 (*DNM2*) at the chromosome 19 locus (Zuchner et al., 2005) in these two families

and in a Belgian family. The American family had features of CMT 2 and no individuals with slow NCVs were found. This shows that the phenotype can vary between CMT2 and intermediate CMT. A fourth *DNM2* mutation was found in a Spanish family (Berciano, personal communication).

Dominant Intermediate CMT Type C (DI-CMT C)

Two unrelated families with intermediate CMT linked to a novel locus on chromosome 1p34-p35 (DI-CMTC) (Jordanova et al., 2003). The combined haplotype analysis in both families localized the DI-CMTC gene within a 6.3-cM linkage interval flanked by markers D1S2787 and D1S2830. The functional and positional candidate genes, syndecan 3 (SDC3), and lysosomal-associated multispanning membrane protein 5 (LAPTM5) were excluded for pathogenic mutations (Jordanova et al., 2003). A mutation has just been reported by Jordanova et al. (2006) in the *tyrosyl-tRNA* synthetase gene.

Other CMTs With Intermediate Features

The Asp6Tyr Mutation in the Myelin Protein Zero Gene

Mastaglia et al. (1999) reported a family with an *MPZ* mutation and with NCVs ranging from 24 to 41 m/s. The motor action potentials were not reported and the slowest value was from a severely affected individual aged 53. The nerve biopsy showed axonal degeneration and loss of myelinated fibers. The *MPZ* mutation produces an inferred amino acid change of aspartate to tyrosine at codon 6 (Asp6Tyr) of the processed protein in the extracellular domain and was present in all affected family members but not in 100 unrelated controls.

CMTX (see above)

X-linked CMT was originally suggested as a possible explanation for the existence of families with intermediate range NCV (Phillips et al., 1985). CMTX has dual pathology and both slow and normal value nerve conduction velocities in affected males even from the same family (Nicholson and Nash, 1993). These features can be used to efficiently select individuals with CMTX for mutation sequencing (Nicholson et al., 1998).

Females can vary from fully affected with slow nerve conduction velocities, to unaffected, according to the particular pattern of mosaicism of X chromosome inactivation. As a result of X-chromosome inactivation only one X-chromosome is expressed in a given cell, which can be either the mutant or normal allele. When patients with intermediate conduction velocities defined by median nerve conduction between 30 and 40 m/s but without 17p11.2 duplications were screened for *GJB1* coding region mutations, 40% had mutations (Rouger et al., 1997), suggesting that the remainder had another form of CMT, possibly some of the new intermediate forms described earlier.

Recessive CMT With GDAP1 Mutations

Recessive CMT with GDAP1 mutations was described by Cuesta et al. (2002) in three Spanish families and in Tunisian families by Baxter et al. (2002). The pathology in the Spanish families was predominantly axonal with some demyelinating features (Sevilla et al., 2003). Other families have an axonal phenotype (Azzedine et al., 2003). Axonal and demyelinating phenotypes in families with different mutations were described by Nelis et al. (Nelis et al., 2002; Birouk et al., 2003), described a family showing a marked reduction in myelinated fibres and signs of axonal regeneration, including frequent pseudo-onion bulb formations.

Neurophysiology and nerve pathology were heterogeneous in these cases: a subset of GDAP1 mutations was associated with peripheral nerve demyelination, whereas others resulted in axonal degeneration (Senderek et al., 2003). Patients with GDAP1 mutations displayed severe, early childhood- onset CMT neuropathy with prominent pes cavus equinovarus deformity and impairment of hand muscles. Nerve conduction velocities were between 25 and 35 m/s but the motor action potentials were not reported. Peripheral nerve pathology showed axonal as well as demyelinating changes (Senderek et al., 2003). These findings fit the definition of intermediate type CMT and further support the view that GDAP1 is vital for both, axonal integrity and Schwann cell properties.

CMT With NF-L Mutations

The first *NF-L* family was reported by Mersiyanova et al. (2000). Other patients were reported with *NF-L* mutations by Jordanova et al. (2003) and had an

Table 1
Intermediate Forms of CMT

Disease (OMIM nomenclature)	Gene mutation
Dominant intermediate	
CMT (DI CMT)	
DI CMT A chromosome 10q24	
DI CMT B chromosome 19p13-12	*DNM2* mutations
DI CMT C chromosome 1p34-35	
Other CMTs with intermediate features	
Dominantly inherited:	
CMT1F &2E	*NF-L* mutations
DI CMT D	*MPZ* Asp6Tyr
CMT2K	*GDAP1* mutations
X-linked:	
CMTX1	*GJB1* mutations
Recessive:	
RI CMT A (CMT4A)	*GDAP1* mutations

early onset and often a severe axonal clinical phenotype and electrophysiological examination showed moderately to severely slowed nerve conduction velocities. The nerve biopsy of a CMT patient with a *de novo* missense mutation in *NF-L* had mixed pathology with axonal regeneration clusters and onion bulb formations.

This disorder can have both slow or normal NCV and can be classified as an intermediate form of CMT (De Jonghe et al., 2001). Zuchner reported a family with a Glu397Lys mutation and median motor nerve conduction velocities in the axonal range, 59–63 m/s but pathological changes consisted of a reduction of predominantly large myelinated nerve fibers and various stages of onion bulb formation as typically seen in CMT1 (Zuchner et al., 2004).

Cell Biology of Intermediate CMTs

Although much is known about the cell biology of connexins, dynamin, and NF-L, little is known on about how mutations of these genes produce disturbances of Schwann cells and distal axons. Whether the phenotype is caused by loss of normal function or gain of a new deleterious effect is unknown. However in most dominant hereditary neuropathies in which the cell biology has been investigated, the mechanism has been found to be a gain of toxic function.

The dual pathology seen in intermediate CMTs could reflect expression of the mutation in both Schwann cells and neurones or changes in, for example, in Schwann cells with secondary effects in axons. There are five genes with mutations that are known to cause intermediate CMT: *DNM2*, *GJB*, *MPZ*, *GDAP1*, and *NF-L*.

The Connexins

Connexins are a family of transmembrane protein subunits, which form a connexin complex (two units) and enable gap junctions between cells to "dock." Connexins are encoded by a large gene family predicted to comprise of at least 20 isoforms in humans (Segretain and Falk, 2003, 2004). Gap junction biosynthesis and degradation is a complex and highly regulated process (Segretain and Falk, 2003). Mutations in Cx26 cause sensorineural deafness, in Cx43 cause cardiac abnormalities and mutations in *connexin32* cause CMTX1. *Connexin32* is expressed in both axons and Schwann cells which may explain why there is dual axonal and demyelinating pathology. *Connexin32* connects the various wrapped layers of the Schwann cell lamellae and is thought to provide a rapid radial communication but whether it connects Schwann cells to axons is unknown. *Connexin32* mutations produce pathology both by loss of channel function and by gain of toxic function predominantly by trafficking defects and by altering cell to cell communication. (Deschenes et al., 1997)

Secondary axonal degeneration in demyelinating diseases has long been known (Dyck 1975). Loss of myelin proteins can produce axonal degeneration in mouse models, for example, MAG deficient mice

(Fruttiger et al., 1995) and mutation of the myelin protein *PMP22* alters the localization of juxtaparanodal proteins and ion channels (Devaux and Scherer, 2005). It is therefore likely that the axonal degeneration in CMTX is a secondary effect to Schwann cell damage as it is in CMT1A.

The Dynamins

Dynamin 2 (*DNM2*) is a member of the dynamin superfamily. Dynamins are large mechanochemical GTPases that are central players in the clathrin-mediated endocytosis that interact with several functionally diverse SH3 domain-containing proteins. *DNM2* is ubiquitously expressed in all cell types. It has several cellular functions including receptor mediated endocytosis, a vesicle budding role in the endocytosis of caveolae, phagocytosis, trans-Golgi network budding, cytoskeleton regulation, apoptosis, cell proliferation, and cell division (Thompson et al., 2002, 2004). Receptor mediated endocytosis requires DNM2 GTPase activity to release energy for the process (Hinshaw 2000). The PH domain targets dynamin to the sites of vesicle budding, the GTPase effector domain is required for the ring assembly process around the neck of invaginating vesicles, whilst the proline rich domain regulates activity by phosphorylation (Hinshaw 2000; Tan et al., 2003; Larsen et al., 2004). How mutations in proteins such as *DNM2* expressed in axons, produce a demyelinating pathology is yet to be explored.

The Neurofilaments

Neurofilaments are the most abundant structural components of large myelinated axons. They are obligate heteropolymers in vivo made up of neurofilament light chain (NF-L), neurofilament medium chain (NF-M), and neurofilament heavy chain (NF-H) (Yuan et al., 2003). Neurofilament assembly begins with the association of two subunits into a coiled-coil dimmers which subsequently form tetramers and eventually higher-order forms of 10 nm filaments (Fuchs and Weber, 1994). The minimal requirement for this assembly is the polymerization of NF-L with either NF-M or NF-H subunits, but requirements for their axonal transport have long been debated (Yuan et al., 2003). Do they transport as an assembled unit or does each subunit (monomer or oligomer) transport separately to form a stable network in the axon (Baas and Brown, 1997; Hirokawa et al., 1997). It has recently been shown that neurofilament proteins require at least a hetero-oligomer formation for efficient axonal transport (Yuan et al., 2003). It is quite plausible to speculate that as *NF-L* is a backbone for neurofilament assembly and that mutations in this protein will have dramatic effects on axonal transport and myelin protein interaction.

The neurofilament light chain protein, *NF-L* is expressed in both Schwann cells and axons giving a possible explanation for combined myelin and axonal changes and an intermediate phenotype as observed in some *NF-L* mutations. Axonal contact is necessary for transient expression of *NF-L* in Schwann cells (Fabrizi et al., 1997). *NF-L*, interacts with MTMR2 (the gene mutated in the recessive CMT 4B1, a disease with focally folded myelin) in both Schwann cells and neurons (Previtali et al., 2003).

Conclusion

Although intermediate CMT was initially thought to be a nonexistent entity, a number of forms of CMT have been found with both axonal degeneration and myelin changes, signaled by different individuals in the same family having both slow and near normal range motor conduction velocities. It is still a difficult entity to diagnose as conduction slowing owing to large fibre fallout in axonal neuropathies must be excluded. The molecular mechanisms underlying these changes is not yet clear, although it is likely that, at least in the dominantly inherited intermediate CMTs, that the mechanism will be a gain of deleterious function mediated by the mutant protein with secondary effects on axons or Schwann cells.

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