Neurosci Bull December 1, 2014, 30(6): 999–1009. http://www.neurosci.cn
DOI: 10.1007/s12264-014-1475-7

·Review·

Intermediate Charcot-Marie-Tooth disease

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Charcot-Marie-Tooth (CMT) disease is a common neurogenetic disorder and its heterogeneity is a challenge for genetic diagnostics. The genetic diagnostic procedures for a CMT patient can be explored according to the electrophysiological criteria: very slow motor nerve conduction velocity (MNCV) (<15 m/s), slow MNCV (15–25 m/s), intermediate MNCV (25–45 m/s), and normal MNCV (>45 m/s). Based on the inheritance pattern, intermediate CMT can be divided into dominant (DI-CMT) and recessive types (RI-CMT). *GJB1* is currently considered to be associated with X-linked DI-CMT, and *MPZ*, *INF2*, *DNM2*, *YARS*, *GNB4, NEFL,* and *MFN2* are associated with autosomal DI-CMT. Moreover, *GDAP1*, *KARS*, and *PLEKHG5* are associated with RI-CMT. Identification of these genes is not only important for patients and families but also provides new information about pathogenesis. It is hoped that this review will lead to a better understanding of intermediate CMT and provide a detailed diagnostic procedure for intermediate CMT.

Keywords: Charcot-Marie-Tooth disease; intermediate CMT; dominant type CMT; recessive type CMT; diagnostic procedure

Introduction

Charcot-Marie-Tooth disease (CMT), also known as hereditary motor and sensory neuropathy, is one of the most common inherited neurologic disorders, with an estimated prevalence of 17 to 40 per 10,000[1]. Currently, ~53 loci or 49 different genes are associated with CMT (http://neuromuscular.wustl.edu/time/hmsn.html). CMT can be transmitted through autosomal dominant, autosomal recessive, or X-linked inheritance[2]. Clinically, it is characterized by progressive muscle weakness and atrophy of the distal extremities, distal sensory loss, reduced or absent deep tendon reflexes, and foot deformities[3]. Uniformly slow motor nerve conduction velocity in the median nerve (mMNCV) (<38 m/s) is characteristic of the CMT1 phenotype (demyelinating form) and an MNCV above this cutoff is typical of the CMT2 phenotype (axonal form). Classification as CMT1 or CMT2 is a milestone and is still useful for the majority of patients. However, increasing numbers of CMT patients manifest features of both types. Intermediate MNCVs (25 to 45 m/s) are often found in males with X-linked CMT (CMT1X phenotype), and different intermediate CMT types have therefore been identified. Moreover, different mutations of some genes can result either in demyelinating CMT with slow MNCVs, or in axonal CMT with normal MNCVs[4]. Therefore, the concept of intermediate CMT with mMNCVs of 25 to 45 m/s appeared in the 1970s and has recently become more popular due to the importance of direct genetic diagnosis[5]. Previously, autosomal dominant intermediate (DI) CMT was only classified into three types: DI-CMTA, DI-CMTB, and DI-CMTC[6-8]. Currently, *GJB1* is considered to be associated with X-linked DI-CMT, and *MPZ*, *NEFL*, *GNB4*, *INF2*, and *MFN2* are associated with autosomal DI-CMT. In addition, *GDAP1*, *KARS*, and *PLEKHG5* are associated with autosomal recessive intermediate CMT (RI-CMT). The information on these genes is shown in Table 1 and statistics of their mutations and phenotypes are listed in Table 2.

Table 1. Genes associated with intermediate CMT

Phenotypes	Genes	Loci	OMIM number	Inheritance	Specific features	Other types
DI-CMTA	Not cloned	10q24	606483	AD	None	NE
DI-CMTB	*DNM2*	19p13.2	606482	AD	Cataracts neutropenia	CMT2M
DI-CMTC	*YARS*	1p35.1	608323	AD	None	NE
DI-CMTD	*MPZ*	1q23.3	607791	AD	Adie's pupil	CMT1B CMT2I\J
DI-CMTE	*INF2*	14q32.33	614455	AD	FSGS	NE
DI-CMTF	*GNB4*	3q26.33	615185	AD	None	NE
CMT-X	*GJB1*	Xq13.1	302800	X-linked	None	NE
DI-CMT?	*NEFL*	8p21	?	AD	None	CMT1F CMT2E
DI-CMT?	*MFN2*	1p36.22	?	AD	None	CMT2A2 CMT1?
RI-CMTA	*GDAP1*	8q21.11	608340	AR	Severity (CMTNS>20)	CMT4A CMT2H\K
RI-CMTB	*KARS*	16q23.1	613641	AR	Vestibular Schwannoma	NE
RI-CMTC	*PLEKHG5*	1p36.31	615376	AR	None	NE

AD, autosomal dominant; AR, autosomal recessive; NE, not existed; FSGS, focal segmental glomerulosclerosis; CMTNS, Charcot-Marie-Tooth neuropathy score; "?", not classified or defined.

Table 2. Statistics of gene mutations in different phenotypes

Genes	Total mutation number (*n*)	Mutations with EMG information (*n*)	Mutations in CMT1 (*n*)	Mutations in CMT2 (*n*)	Mutations in intermediate CMT (*n*)
GJB1	405	226	38	30	158
MPZ	166	144	70	17	39
INF2	28	19	0	0	19
DNM2	23	8	0	6	2
YARS	3	3	0	0	3
GNB4	2	2	0	0	2
NEFL	23	18	1	14	1
MFN2	83	83	2	80	1
GDAP1	42	24	19	1	1
KARS	2	2	0	0	2
PLEKHG5	3	3	0	0	3

CMT1, demyelinating form of CMT; CMT2, axonal form of CMT; EMG, electromyography.

Dominant Intermediate CMT and Associated Genes

GJB1 and the X-linked DI-CMT Phenotype

GJB1 (gap junction protein beta-1), also known as *Cx32* (connexin 32), is located on chromosome Xq13.1 and encodes a gap junction protein in myelinated Schwann cells. More than 400 *GJB1* mutations, predicted to affect all regions of the GJB1 protein, have been described (www.molgen.ua.ac.be/CMTMutations/Mutations/). About 70% of the mutations are associated with intermediate CMT. Six connexins arranged around a central pore form a hemichannel (or connexon)[9], and two apposed hemichannels form a functional channel which provides a contiguous pathway between adjacent cells or cellular compartments. The channel diameter is too small to allow the transfer of nucleic acids or proteins, but is large enough to allow the diffusion of ions and other small particles (<1000 Da)[10]. Many of the connexin channels are located in the cell membrane, where they are called gap junctions. In *GJB1* mutants, the formation of functional gap junctions is disrupted or abnormal gap junctions are formed, and this is considered to be the main pathological mechanism of *GJB1* mutation[11]. Affected males with the CMT1X phenotype have moderate to severe symptoms, whereas heterozygous females are usually less affected with mild symptoms. The mMNCV typically ranges from 30 to 40 m/s in affected males with *GJB1* mutation and 30–50 m/s in affected females with the CMT1X phenotype, which is faster than in patients with the demyelinating form and slower than in patients with the axonal form of CMT[11-13]. In 2014, we discussed the clinical and electrophysiological features in a cohort of 59 Chinese CMT1X patients. We found that the mean MNCV of male patients is 34.5 ± 6.3 m/s and that of female patients 43.5 ± 7.2 m/s[14]. As intermediate CMT with mMNCVs between 25 and 45 m/s is often found in CMT1X patients[15], *GJB1* mutation analysis is the first step to genetically diagnose a CMT patient with an intermediate MNCV if there is no sign of male-to-male transmission.

MPZ and DI-CMTD Phenotype

MPZ is located on chromosome 1q23.3 and is 7 kb in size[16,17]. It has six exons encoding myelin protein zero (P_0). The mature protein contains 219 amino-acids and has a cytoplasmic domain, a single transmembrane domain, and an extracellular domain[17,18]. The majority of *MPZ* mutations are located on exon 2 or 3 which corresponds to the immunoglobulin-like extracellular domain, whereas mutations in the intracellular and transmembrane domains are less frequent[19]. P_0 is the major protein constituent of peripheral myelin, comprising ~50% of all peripheral nervous myelin proteins[20]. P_0 is essential for the commencement of myelination and the maintenance of myelin compaction[21]. The compaction of myelin lamellae relies on homotypic interactions between the extracellular domains of P_0 [22]. Therefore, mutant P_0 can not only directly influence the formation and compaction of the myelin sheath, but also indirectly result in axonal degeneration. So far, >160 *MPZ* mutations have been reported, giving rise to demyelinating, axonal, and intermediate CMT forms with a wide range of severity[23]. Approximately 30% of *MPZ* mutations are associated with intermediate CMT and this form is defined as a DI-CMTD phenotype. In 2010, Banchs *et al.* described a four-generation CMT family with *MPZ* mutations, with mMNCVs between 35 and 43 m/s[24]. In the same year, Schneider-Gold *et al.* reported a German family whose members exhibited the intermediate CMT phenotype with a missense mutation c.700G>T p.Asp234Tyr (deviant nomenclature: c.670G>T, p.Asp224Tyr) in the intracellular domain of P_0[25]. In 2012, Ramirez *et al.* described an English CMT family of four affected generations with a novel heterozygous mutation (Trp101X) on exon 3, which codes for a portion of the extracellular domain of P_0, resulting in premature termination of protein translation[23]. Electrophysiological and pathological studies in this English CMT family revealed demyelination and axonal loss with mMNCVs in the intermediate range (38–43 m/s)[23]. In 2013, we presented four CMT families and one CMT patient with *MPZ* mutations. Three were classified as intermediate CMT with mMNCVs ranging from 32.8 to 45 m/s[26]. It has been suggested that *MPZ* is the second most frequent gene resulting in intermediate CMT (Table 2). Therefore, *MPZ* mutation should be tested in CMT families with intermediate mMNCVs (25–45 m/s), especially when *GJB1* mutations are negative or there is male-to-male transmission[24,27].

INF2 and DI-CMTE Phenotype

*INF*2 is located on chromosome 14q32.33, and encodes inverted formin-2 (INF-2), a member of the diaphanous-related formin family, which is involved in remodeling the microtubule cytoskeleton and actin[28]. INF2 interacts

with Rho-GTPase CDC42, lymphocyte protein, and myelin, which are implicated in essential steps of the commencement of myelination and maintenance[29-31]. Like other diaphanous-related formins, INF2 contains an N-terminal diaphanous-inhibitory domain, two formin-homology domains, and a C-terminal diaphanous-autoregulatory domain[32]. Immunohistochemical analysis has revealed that INF2 is expressed in the cytoplasm of Schwann cells and kidney podocytes, so it is involved in diseases affecting both the peripheral nervous system and the renal glomerulus[31]. *INF2* mutations appear to cause focal segmental glomerulosclerosis (FSGS)-associated intermediate CMT. It has been reported that *INF2* gene mutations account for 12–17% of isolated FSGS cases transmitted through autosomal dominant inheritance, and 75% of affected CMT-FSGS cases[31,33,34]. So far, ~18 intermediate CMT patients (French, French-Canadian, Spanish, Korean, and Italian) with *INF2* mutations have been reported[31,35,36]. This form is defined as a DI-CMTE phenotype and is supposed to be the third most frequent intermediate CMT. In 2014, Mathis *et al.* studied the pathologic features of 6 *INF2*-related DI-CMTE patients (4 females and 2 males), and found chronic demyelination and remyelination associated with progressive axonal loss[37]. In the same year, Park *et al.* described a novel *INF2* mutation (p.L132P) in a family with intermediate CMT and FSGS in two affected members; this was the first reported case from Korea[35]. The clinical features of DI-CMTE include age at onset 5–28 years, proteinuria, mild to severe weakness, pes cavus, reduced tendon reflexes, sensory loss in 50% of patients, sensorineural hearing loss in 30% of patients, cramps, and mMNCV 23–45 m/s.

DNM2 and DI-CMTB Phenotype

DNM2 is located on 19p13.2, and encodes dynamin-2 protein (DNM2)[7]. DNM2 possesses five characteristic domains: a highly conserved N-terminal GTPase domain, a middle domain that binds to γ-tubulin, a pleckstrin-homology domain that interacts with phosphoinositides, a GTPase effector domain, and a proline/arginine-rich domain at the C-terminus[38-40]. DNM2 belongs to a family of large GPases that regulate membrane trafficking, actin-based dynamics, and the membrane fusion and fission of vesicles, peroxisomes, and mitochondria[41]. *DNM2* mutations cause not only DI-CMTB, but also the CMT2M phenotype. The majority of DNM2 mutations are located in the pleckstrin-homology domain, and other mutations with similar phenotypes have been reported in the Pro/Arg-rich domain and the middle domain[42]. Patients with DI-CMTB usually have the classic phenotype of teenage onset (in the first or second decade) and an intermediate mMNCV from 26 m/s to normal values, and only ~3% of patients are wheel-chair bound[42]. Some patients also have combined nuclear and cortical cataracts that occur in early life. In some families with *DNM2* mutation, the patients have also been segregated with neutropenia, ptosis, and ophthalmoparesis[42,43].

YARS and DI-CMTC Phenotype

YARS encodes tyrosyl-tRNA synthetase (TyrRS) and is located on chromosome 1p35.1[44]. Aminoacyl-tRNA synthetases (ARSs) charge amino-acids onto their homologous tRNAs during protein translation. First, studies suggested that insufficient enzyme activity in protein synthesis is the mechanism of ARS-related CMT. Second, the mutant proteins were thought to lead to ubiquitin-positive misfolded protein aggregates and the formation of toxins[45]. Mutations in glycyl-, tyrosyl-, and alanyl-tRNA synthetases have been associated with CMT[45]. TyrRS is expressed ubiquitously and is an essential enzyme for protein biosynthesis. It catalyzes the aminoacylation of $tRNA^{Tyr}$ with tyrosine in two steps: tyrosine activation by ATP to form tyrosyl-adenylate, and transfer of the activated form to cognate $tRNA^{Tyr}$[46]. The intermediate CMT caused by *YARS* mutations is defined as the DI-CMTC phenotype. Some studies have shown that the pathogenesis of this phenotype is not due to haploinsufficiency of aminoacylation activity, but most probably to an increased functional alteration of the mutant TyrRS[47]. In 2006, Jordanova *et al.* reported two mutations in *YARS,* p.Gly41Arg and p.Glu196Lys, in two unrelated DI-CMTC families from North America and Bulgaria, and a 12-bp in-frame deletion (153–156delVKQV) in an affected individual from Belgium[6]. In the North American and the Bulgarian families with *YARS* mutations, the clinical features were age at onset between 10 and 60 years, intermediate MNCV ranging from 25 to 58 m/s, slow progression, and moderate severity[44].

GNB4 and DI-CMTF Phenotype

GNB4 is located on chromosome 3q26.33 and encodes

guanine-nucleotide-binding protein subunit beta-4 ($G\beta_4$). Guanine-nucleotide-binding proteins are heterotrimeric, composed of α, β, and γ subunits. They can act in concert with different G-protein-coupled receptors to transmit extracellular signals into cells. And these receptors can be activated by many different signaling factors, such as hormones, neurotransmitters, and cytokines[48]. $G\beta_4$ is co-localized with neurofilament heavy chain, indicating that it is abundant in Schwann cells and axons in peripheral nerves[49]. In 2013, Yi-Chung Lee *et al*. reported that *GNB4* mutation is a cause of DI-CMT using genome-wide linkage and exome sequencing in a Chinese family (six affected individuals, five unaffected individuals, and two married-in spouses). They revealed a heterozygous mutation, c.158G>A (p.Gly53Asp), in *GNB4* in two affected first cousins[49]. The disease status of this family included age of onset ranging from 10 to 45 years, clinical severity from asymptomatic to wheelchair-bound (only one patient needed a wheelchair), mMNCVs from 16.5 to 45.7 m/s, and men tending to be more severely affected[49]. Subsequently, another *GNB4 de novo* mutation, c.265A>G (p.Lys89Glu), was identified in a Chinese CMT patient[49]. The frequency of *GNB4* mutations in the CMT population in that study was estimated to be ~0.8%[49]. The intermediate CMT caused by *GNB4* mutation is defined as a DI-CMTF phenotype.

NEFL and the Associated Phenotype

NEFL is located on chromosome 8p21[50] and encodes neurofilament light-chain polypeptide (NEFL). So far, ~20 mutations have been identified, and span all functional domains of the protein. NEFL, one of the most abundant cytoskeletal components of the neuron, can assemble with neurofilaments of higher molecular mass, medium- and heavy-chain polypeptides, into intermediate filaments in the cytoplasm to form an extensive fibrous network. There is a consensus that the radial accumulation of neurofilaments leads to the lateral growth of axons during myelination, and therefore can determine the axon diameter and the conduction velocity of peripheral nerves to some extent[51,52]. It is supposed that NEFL mutants not only influence the normal assembly of neurofilaments, but also cause aggregate formation that prevents transport or other physiological functions of the axon[53]. Moreover, NEFL mutants impede neurofilament assembly and transport *in vivo* and affect mitochondrial morphology by interfering with mitochondrial dynamics in a cellular model of the CMT2E/1F phenotype[54,55]. *NEFL* mutations can result in CMT neuropathies with variable clinical and electrophysiological features. The p.Gln333Pro mutation caused a mild CMT2E phenotype in a Russian family; the p.Pro8Arg mutation led to a classical and more severe CMT phenotype in a Belgian kindred[56]. According to the electrophysiological criteria, most Belgian CMT patients in this study could be diagnosed as CMT1 phenotype, but as a whole the family could be classified into intermediate CMT[57]. Taken together, *NEFL* mutations can cause CMT1, CMT2, and intermediate CMT.

10q24.1-q25.1 Locus and DI-CMTA Phenotype

Kristien Verhoeven *et al.* performed a genome-wide search in an Italian family exhibiting autosomal DI-CMT and mapped the locus on chromosome 10q24.1-q25.1[8]. This family was clinically characterized by early-onset (in the first or second decade of life), slow progression, slight-to-moderate severity, no wheelchair-bound individuals, and intermediate MNCVs (25–45 m/s)[58]. Loss of large myelinated axons and occasional onion bulbs were found in nerve biopsies[59]. So far, the pathogenic gene for DI-CMTA has not been cloned and cases of the DI-CMTA phenotype have rarely been reported.

Genes Associated with Recessive Intermediate CMT

GDAP1 and RI-CMTA Phenotype

GDAP1 is located on chromosome 8q21.11 and spans ~17 kb of genomic DNA[60]. Its encoded protein, ganglioside-induced differentiation associated-protein 1 (GDAP1), has five domains: two glutathione-S-transferase (GST) domains, an α-helical domain (also called a 4-5 loop), a hydrophobic domain, and a transmembrane domain[61-63]. This protein is anchored to the outer mitochondrial membrane of neurons and Schwann cells through the transmembrane domain. GDAP1 plays an important role in mitochondrial dynamics and functioning[64,65]. Another important function of GDAP1 is due to the GST domains: it regulates glutathione metabolism, sensitivity to apoptosis, and reactive oxygen species levels[66]. *GDAP1* mutations are associated with CMT4A, the most frequent recessive demyelinating CMT phenotype, with AR-CMT2K, the recessive axonal CMT phenotype, with CMTRIA, the recessive intermediate CMT

with mixed features of demyelinating and axonal forms, and with AD-CMT2K, the rare dominant phenotype[67-70]. In 2003, Senderek *et al.* found two *GDAP1* mutations disrupting the reading frame in a Turkish inbred family and a German patient. These patients displayed severe, early childhood-onset CMT neuropathy with prominent pes cavus and claw hands, intermediate MNCVs ranging from 25 to 35 m/s, and axonal loss as well as demyelinating changes in the peripheral nerve pathology[71]. In 2006, Kabzinska *et al.* found a homozygous mutation (p.Leu239Phe) of *GDAP1* in a 39-year-old female with severe intermediate CMT[69]. This intermediate CMT caused by *GDAP1* mutation is defined as the RI-CMTA phenotype.

KARS and RI-CMTB Phenotype

KARS is located on chromosome 16q23.1 and spans ~20 kb[72]. It encodes Lysyl-tRNA synthetase, the enzyme responsible for charging $tRNA^{Lys}$ molecules. Importantly, *KARS* is the only locus in the human genome encoding an enzyme responsible for $tRNA^{Lys}$ charging and it is essential for protein translation in both the cytoplasm and mitochondria[72,73]. ARSs charge amino-acids onto their cognate tRNAs during protein translation. Three ARS genes (*GARS*, *YARS*, and *AARS*) are associated with the CMT2D, DI-CMTC, and CMT2N phenotypes[6,74,75]. In 2010, McLaughlin *et al.* discovered two *KARS* mutations in two intermediate CMT patients, and *KARS* became the fourth causative ARS gene for CMT[73]. One is a heterozygous mutation (p.Ile302Met) and the other is a compound heterozygous mutation (p.Leu133His; c.524_525insTT)[73]. The mMNCVs of these patients were mildly slowed, ranging from 30 to 40 m/s. In addition, they had dysmorphic features, developmental delay, and vestibular Schwannoma[73]. This intermediate CMT caused by *KARS* mutations is defined as the RI-CMTB phenotype.

PLEKHG5 and the RI-CMTC Phenotype

PLEKHG5 is located on chromosome 1p36.31, within a 1.5 Mb interval[76]. Pleckstrin homology domain-containing family G member 5 (*PLEKHG5)* is predominantly expressed in the peripheral nervous system, and it possesses a Dbl-homology - pleckstrin-homology motif, which is the minimal unit for the nucleotide exchange-promoting function of guanine nucleotide exchange factors (GEFs)[77]. Pleckstrin-homology domains also independently play an important role in the allosteric regulation of the RhoGEF domain. The RhoGEF domain can activate GTPases by stimulating the exchange of GDP and GTP, thereby many signaling mechanisms are initiated that regulate neuronal shape and plasticity, dendrite growth, synapse formation, and neuronal survival[76]. Different mutations in *PLEKHG5* have diverse clinical outcomes (intermediate CMT or lower motor neuron disease) affecting the functions of neurons and Schwann cells[78]. So far, three families with intermediate CMT caused by *PLEKHG5* mutations, from Korea, Portugal, and Morocco, have been reported (http://neuromuscular.wustl.edu/time/hmsn.html). *PLEKHG5* forms include compound heterozygous or homozygous, deletion, duplication (7 bp), and missense mutations. Kim *et al.* described a Korean RI-CMT family with childhood onset and identified novel compound heterozygous mutations of *PLEKHG5* (p.Thr663Met and p.Gly820Arg) in 2013[79]. Immunohistochemical studies revealed that the patients expressed a low level of PLEKHG5 in the distal sural nerve and *in vitro* assays suggested that the PLEKHG5 mutants are defective in activating the NF-κB signaling pathway[79]. The clinical features of this intermediate CMT are onset age from the 1st to 5th decades, a progressive course, mildly increased serum creatine kinase, and 24–39 m/s mMNCV. This intermediate CMT is defined as the RI-CMTC phenotype. The locations of the intermediate CMT proteins in the peripheral nerve are shown in Figure 1.

Diagnostic Procedure for Intermediate CMT

The genes *GJB1*, *MPZ*, *INF2*, *DNM2*, *YARS*, *GNB4*, *NEFL*, *GDAP1*, *KARS*, and *PLEKHG5* are associated with intermediate CMT. Braathen *et al.* reported that intermediate CMT accounted for 3.4% of a cohort of 232 Norwegian CMT cases and confirmed that *MFN2* gene mutation can also cause intermediate CMT[80]. Of these genes, some are also associated with the classical CMT1 or CMT2 phenotype, and some are only associated with the intermediate CMT phenotype. The relationships of these intermediate CMT genes and CMT1 and CMT2 are shown in Figure 2.

In a study by Miller *et al.*, patients with identified genetic causes of CMT who had intermediate MNCVs (35–45 m/s) had primarily CMT1X (52.8%) or CMT1B (27.8%)[81]. For patients with intermediate MNCVs (25–45 m/s), the first step is to determine whether there is evidence of male-to-

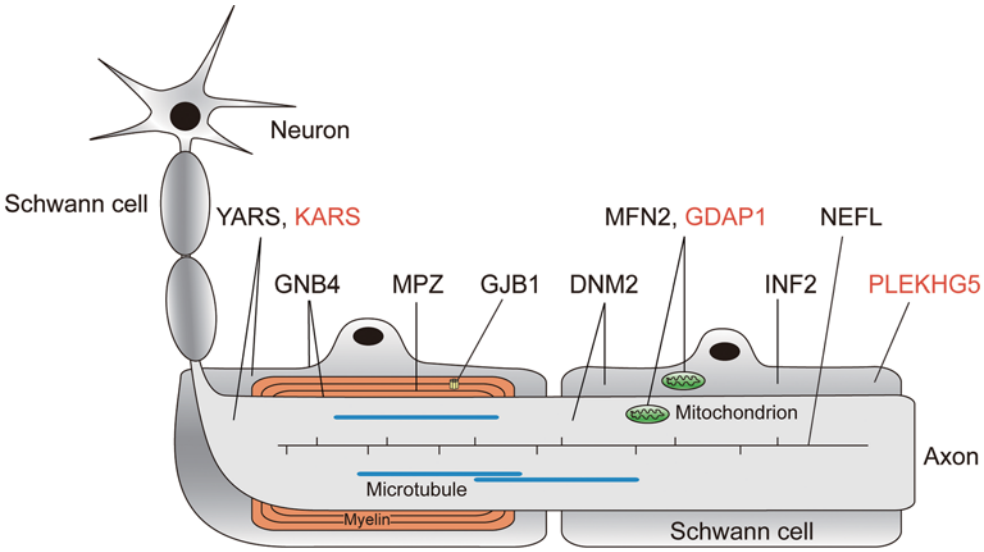

Fig. 1. Image of a myelinated nerve fiber. The mutated proteins that cause dominant intermediate CMT are shown in black and those that cause recessive intermediate CMT are shown in red.

male transmission. CMT patients with intermediate MNCVs and no male-to-male transmission should first be screened for *GJB1* mutations. If this is negative, testing should proceed to *MPZ* mutations. Alternatively, if there is male-to-male transmission, testing for *MPZ* mutations should occur first. If both tests are negative, whether there is an affected parent or child should be considered. If there is no affected parent or child, the genes associated with RI-CMT (*GDAP1*, *KARS*, and *PLEKHG5*) should be screened. If there is an affected parent or child, the remaining genes associated with DI-CMT (*DNM2*, *YARS*, *NEFL*, *GNB4*, and *INF2*) should be screened. If the patient has renal symptoms such as proteinuria and anuria, testing should proceed to *INF2* mutations. If the patient has no signs of renal failure or *INF2* mutation screening is negative, *DNM2* mutations should be screened. If both *INF2* and *DNM2* mutation screening is negative, *YARS*, *NEFL*, and *GNB4* should be screened one by one. If the above testing is all negative, advanced genetic technologies, such as exon sequencing of the whole genome, can be used for research purposes. Nevertheless, the procedure for intermediate CMT is not rigid, and appropriate adjustments can be made according to the specific situation of the patient. For instance, for an intermediate CMT patient with proteinuria, the *INF2* mutation should be tested first; for an intermediate CMT patient with cataract or neutropenia, the *DNM2* mutation

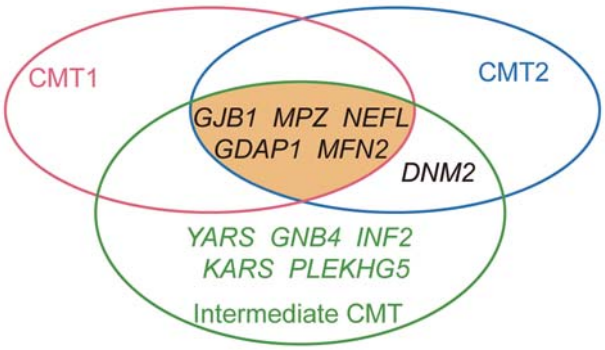

Fig. 2. Schematic showing the relationship of intermediate CMT genes with CMT1 and CMT2.

should be screened first; for an intermediate CMT patient of recessive hereditary mode with vestibular Schwannoma, *KARS* mutations should be tested first. The diagnostic procedure for intermediate CMT is shown in Figure 3.

Conclusion

In summary, the genes *GJB1*, *MPZ*, *INF2*, *DNM2*, *YARS*, *GNB4*, *NEFL*, *MFN2*, *GDAP1*, *KARS*, and *PLEKHG5* are associated with intermediate mMNCVs between 25 and 45 m/s. This article elaborates on the features of these genes, and further formulates a genetic diagnostic

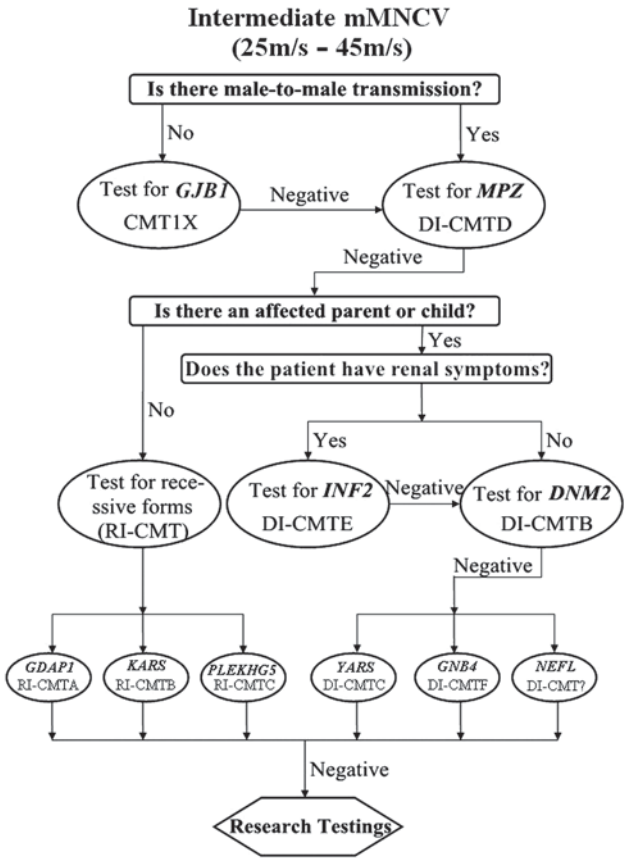

Fig. 3. Genetic diagnostic procedure for CMT patients with intermediate median motor nerve conduction velocity (mMNCV).

procedure for intermediate CMT based on the inheritance patterns, the frequencies of these genes, and the specific features.

Received date: 2014-04-15; Accepted date: 2014-06-19

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