Open access • Journal Article • DOI:10.1007/S00415-017-8474-3

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Published on: 31 Mar 2017 - Journal of Neurology (Springer Berlin Heidelberg)

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For: Journal of Neurology (Systematic review)

Article content: Text, 7,900 words; Abstract, 301 words; 156 References; 5 Figures; and 2 Tables

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Abstract

Charcot-Marie-Tooth disease (CMT) is the most frequent form of inherited neuropathy with great variety of phenotypes, inheritance patterns and causative genes. According to median motor nerve conduction velocity (MNCV), CMT is divided into demyelinating (CMT1) with MNCV below 38 m/s, axonal (CMT2) with MNCV above 38 m/s, and intermediate CMT with MNCV between 25 and 45 m/s. In each category, transmission may be autosomal dominant, autosomal recessive or X-linked. The nosology of intermediate CMT is controversial because of concerns about electrophysiological delimitation. A systematic computer-based literature search was conducted on PubMed, using the following MeSH: i/ intermediate Charcot-Marie-Tooth; ii/ X-linked intermediate Charcot-Marie-Tooth; and iii/ X-linked Charcot-Marie-Tooth and electrophysiology. We retrieved 225 articles reporting X-linked CMT or intermediate CMT with electrophysiological information. After eligibility, 156 papers were used for this review. In assessing median MNCV, compound muscle action potential (CMAP) amplitudes were taken into account. In cases with attenuated CMAP and wherever possible, proximal median MNCV was used for accurate definition of conduction slowing in the intermediate range. In the vast majority of males with X-linked CMT associated to *GJB1* mutation (CMTX1), median MNCV was intermediate. CMT associated with *DRP2* mutation is another well-documented X-linked intermediate disorder. Autosomal dominant intermediate CMT (DI-CMT) encompasses 11 different types, six of them with assigned phenotype MIM number, the remaining five being unnumbered. Based on available electrophysiological information, we wonder if DI-CMTA should be reclassified within CMT2. Autosomal recessive intermediate CMT (RI-CMT) covers four numbered MIM phenotypes though, in accordance with reported electrophysiology, two of them (RI-CMTB and RI-CMTD) should probably be

reclassified within AR-CMT2. We conclude that intermediate CMT is a complex inherited syndrome, whose characterization requires a specific electrophysiological protocol comprising evaluation of upper-limb proximal nerve trunks when distal CMAP amplitudes are reduced, and that an updated version of MIM phenotype numbering is needed.

Keywords: Autosomal dominant inheritance. Autosomal recessive inheritance. Axonal degeneration. Charcot-Marie-Tooth disease. Compound motor action potential. Demyelination. Electrophysiology. Intermediate Charcot-Marie-Tooth disease. Motor conduction velocity. Nerve biopsy. OMIM. PRISMA statement. Proximal motor nerve conduction velocity. Systematic review. X-linked inheritance.

Introduction

Charcot-Marie-Tooth disease (CMT) is the most frequent form of sensorimotor inherited neuropathy with a prevalence ratio of 28 cases/100,000 inhabitants [1]. CMT was initially classified according to the mode of transmission (autosomal dominant, autosomal recessive or X-linked), and electrophysiological or nerve biopsy features [2]. Characteristically, median motor nerve conduction velocity (MNCV) is below 38 m/s for the demyelinating form (CMT1) and above 38 m/s for the axonal form (CMT2) [3]. For want of a better name, Davis and colleagues introduced the term intermediate for designating dominant CMT (DI-CMT) patients with clinico-electrophysiological and pathological features not fitting into either CMT1 or CMT2 [4]. Their intermediate series comprised 49 affected individuals (31 male and 18 females) coming from seven kinships. It is worth noting that in four of their kinships "males tended to be more affected than females. The degree of sparing of females was illustrated by the presence of asymptomatic affected women in the seventh decade in kinship 22". Although these features pointed to an X-linked disorder, the authors did not consider this possibility in their paper. In short, DI-CMT was originally characterized by the following features: i/ absence of clinically observed nerve hypertrophy; ii/ median MNCV between 25 and 45 m/s (according to their table 1a, mean and standard deviation of 34.6±6.3); iii/ prolonged distal motor latencies (DML) (mean, 5.0±1.3 ms); iv/ preserved mean compound muscle action potential (CMAP) amplitude (mean, 4.6 mV; neither range nor standard deviation is given); and iv/ nerve biopsy showing axonal changes, clusters of regenerating myelinated fibres, loss of larger fibres noted from unimodal diameter histograms, and onion bulbs with fewer lamellae than in CMT1 [5].

The distinction of an intermediate CMT subtype was initially a controversial issue since Buchthal and Behse, in their seminal CMT study comprising 77 patients, did

not find any evidence of an intermediate type [6]. In fact, they reported that motor and sensory conduction velocities confirmed the existence of the two main types of CMT: hypertrophic with conduction velocity along the sural nerve (also superficial peroneal and median nerves) diminished to less than 60% of normal, and neuronal with conduction velocity preserved or slowed but never to less than 60% of normal.

In a series of 131 CMT patients, Bouché and colleagues found two main groups: 55 patients whose median MNCVs were below 30 m/s (mean, 19.47±5.46), and 64 whose corresponding MNCVs were above 40 m/s (mean, 50.72±5.80) [7]. It is worth noting that CMAP amplitude values are not given. There remained a third group, comprising 12 patients (6 dominant and 6 sporadic), that could not be classified since median MNCVs ranged between 30 and 40 m/s. Based upon these electrophysiological features and nerve biopsy findings [8], the authors suggested that this group might be included within intermediate CMT. Since then, the electrophysiological notion of intermediate CMT has widely been applied for patients showing either median MNCV between 25 and 45 m/s or between 30 and 40 m/s. More recently and starting from a comprehensive investigation of genetic testing strategies in CMT, Saportaand colleagues restricted the intermediate range of upper-limb MNCV from >35 to ≤45 m/s [9]. Furthermore, these authors established for ulnar nerve that if the CMAP is >0.5 mV, a conduction velocity of <38 m/s is considered demyelinating, and 38-45 m/s is intermediate [10]. As stated by Nicholson and Myers selection of nerve conduction values in different affected individuals in a family is challenging, because slow nerve conduction values can be found in axonal neuropathies as a result of the loss of large rapidly conduction fibres [11].

It is timely to remember the two basic patterns of motor conduction electrophysiological alteration occurring in axonal loss lesions [12]: i/ at one extreme,

there may be severe axonal loss with sparing of only a few of the fastest fibres remaining, which leads to CMAP amplitude reduction with relative preservation of MNCV and DML; and ii/ at the other extreme, as usually occurs in axonal CMT, if all axons are lost, except for a few of the normal slowly conducting fibres, the amplitude also falls dramatically, but conduction velocity can only drop as low as 35 m/s (=75% of the lower limit of normal [LLN]), and DML also prolong to 130% of the upper limit of normal. In axonal polyneuropathies, CMAP amplitude reduction is linearly correlated with decrease in median and peroneal MNCV, though in the long-term such nerve conduction alterations may be aggravated by the appearance of slow-regenerating fibres, and axonal atrophy with or without secondary de- or re-myelination [13]. However, the pattern and degree of axonal loss cannot be entirely determined from routine electrodiagnostic or strength testing due to collateral reinnervation, this calling for special techniques such as motor unit number estimation [14]. The common pathological hallmark of axonal CMT is length-dependent loss of large myelinated fibres with variable signs of regeneration and axonal atrophy[15-17] Applying a conversion factor of 6 [18] to sural nerve ultrathin images from a severe axonal CMT associated to homozygous E210X in the *NEFL* gene, we have estimated that conduction velocity of this nerve would be 18 m/s, namely, comparable to those found in median nerve, 22 m/s (CMAP, 1.2 mV), and ulnar nerve, 17 m/s (CMAP, 0.1 mV) [19, 20]. This conduction estimation could be even lower using a conversion factor of 4.3 reported in normal sural nerve [21].

At this point, we would like to emphasize that in any axonal CMT with severe amplitude reduction of distal CMAPs in upper-limb nerves, the corresponding MNCVs may fall down to the intermediate range or even below 25 m/s. In the seminal CMT2 series by Bienfaitand colleagues, comprising 61 patients from 18 kinships, median

MNCV was less than 38 m/s in nine patients from six families, all of them exhibiting severely reduced distal CMAPs (<1 mV) [22, 23]. Wisely the authors wrote that "nerve conduction velocities may be reduced because of loss of large-diameter fibres or secondary demyelination or both… Some authors adhere strictly to intermediate MCVs (between 20 and 45 m/s) in all family members, but others include families with wide ranges of MCV, making classification difficult… It remains a semantic discussion whether families with a wide range of MCVs should be included in a separate DI-CMT group or in CMT2". We addressed this question in six patients from two DI-CMT pedigrees associated with previously reported *NEFL* mutations [20, 24]. For the segment elbow-wrist, median MNCVs were systematically in the intermediate or even in the demyelinating range, both in patients with preserved CMAPs or attenuated distal CMAPs. Figure 1 illustrates that in case of severe distal CMAP attenuation with median MNCV in the demyelinating range, recording of proximal nerve segments (here axilla-elbow) might be a useful method for accurate detection of the intermediate conduction pattern.

CMT encompasses clinically and genetically heterogeneous syndromes, pathogenic mutations having been reported in over 80 genes with the incorporation of next-generation sequencing [25, 26]. Intermediate CMT has been associated with X-linked gene mutations (mainly *GBJ1* mutations), and gene mutations with either autosomal dominant (DI-CMT) or autosomal recessive (RI-CMT) transmission [11, 26-28]. DI-CMT comprises six loci with five cloned genes (*DNM2*, *YARS*, *MPZ*, *INF2*, and *GNB4*), whereas mutations in four genes (*GDAP1*, *KARS*, *PLEKHG5* and *COX6A1*) have been associated with RI-CMT. Frequencies of intermediate CMT have ranged between 2.9% and 15.6% (median, 6.8%) in comparison with the total CMT rate, its

prevalence in the Republic of Ireland being 0.61 cases/100,000 (95% IC, 0.35-0.87) [9, 29-34].

Given that the notion on intermediate CMT is to some degree controversial, an up-to-date overview of syndromes included under such designation is currently needed. In light to this, we aimed to systematically review the nosology of syndromes enclosed under the umbrella of intermediate CMT, taking into account reappraised motor nerve conduction parameters in upper-limb nerves.

Methods

Literature search strategy

A systematic computer-based literature search was conducted in October 27, 2016 on PubMed database. We used three Medical Subject Headings (MeSH) terms in title: i/ intermediate Charcot-Marie-Tooth; ii/ X-linked intermediate Charcot-Marie-Tooth; and iii/ X-linked Charcot-Marie-Tooth and electrophysiology. We selected papers published in English, French or Spanish languages. We also screened the database Online Mendelian Inheritance in Man (OMIM; https://www.omim.org/), Inherited Peripheral Neuropathies Mutation Database (http://www.molgen.ua.ac.be/CMTMutations/), and Neuromuscular Home Page (http://neuromuscular.wustl.edu/time/hmsn.html#). A manual search of relevant review papers was also performed. Our search methodology was done following the standard guidelines for systematic literature reviews outlined in the PRISMA statement [35].

Quality assessment

Given that intermediate CMT is an electrophysiological notion, we considered that re-assessment of electrophysiological parameters in each publication is a crucial issue.

Following the original description of intermediate CMT [4], we focused on median nerve motor conduction parameters, and alternatively on those of ulnar nerve, accepting that intermediate median MNCV fits in between 25 and 45 m/s with relative preservation of distal CMAP. For comparison purposes, control MNCV values were those used in our laboratory, reported elsewhere [20, 24, 36]. Starting from nerve biopsy features, it is a well-established histological fact that both in CMT2 and in intermediate CMT loss of myelinated fibres mainly involves the largest peak (≥7μm) [5, 6, 8, 16, 17]. With a cutoff of 7 μm, in normal nerves 32% to 45% of myelinated fibres fall above, and 55% to 68% fall below this value [37]. If we assume a selective loss of large myelinated fibres, distal median nerve CMAP amplitude could fall into values between 2.2 and 2.7 mV (LLN, ≥4). Accepting this corollary and applying conversion factors of 4.3 or 6 (see above), such large myelinated fibre loss could account for a decline of median MNCV between 26 and 36 m/s. Under such circumstances, when confronted with nerve conduction values below 38 m/s, characteristic of CMT1 [3], we should bear in mind that this could merely be accounted for by axonal loss. Therefore, in assessing median/ulnar MNCV in the forearm segment we invariably analyzed distal CMAP amplitude; in cases with CMAP reduction by half of the normal LLN or more, we also analyzed if proximal conduction values, axilla-elbow segment, were available (see figure 1). In short, in our view an accurate definition of intermediate CMT requires availability of nerve segments with normal or relatively preserved CMAP amplitudes.

The results of nerve biopsy in intermediate CMT, particularly in CMTX1 males, have been a matter of some controversy, given that they were initially interpreted as primary axonal pathology [38], demyelinating [39] or mixed pathology [40]. To clarify the issue we have paid particular attention to papers with detailed nerve histological

descriptions including fibre teasing or longitudinal thin sections, and fibre morphometry [2, 41].

Results

We retrieved 225 articles reporting X-linked CMT or intermediate CMT, although those containing electrophysiological information were selected for this review (see below). We will present our review results according to appearance order in table 1.

X-linked Charcot-Marie-Tooth disease (CMTX)

CMTX accounts for around 10% of all CMT forms, five phenotypes having been recognized [26]. By far, CMTX1 (MIM#302800) is the most frequent phenotype, which is associated with a plethora of point *GJB1* mutations, including a deletion of the entire coding sequence of the gene [42]. It has been established that most *GJB1* mutations cause a simple loss of function and that no particular mutation appeared more severe than deletion of the entire gene [43].

The CMTX1 phenotype is characterized by progressive muscle atrophy and weakness, areflexia and variable sensory abnormalities; central nervous system (CNS) involvement may also occur. Males have moderate to severe symptoms, whereas heterozygous females are usually less affected [42, 44, 45]. Although the CMTX1 electrophysiological pattern in males is currently accepted as a prototypic example of intermediate disorder, the issue is not without controversy. With heterogeneity between affected males and females, the results of nerve conduction studies differed in the three initial studies (table 2) [38, 39, 46, 47]. Phillips and colleagues found that ulnar MNCVs ranged between 33 and 42 m/s (mean, 38) in males, and between 44 and 56 m/s (mean, 49) in females; MNCV slowing was most prominent in individuals who had CMAP

amplitude reduction [46]. They considered that the observed phenotype was similar to that of intermediate CMT and, despite not having performed nerve biopsy, wisely proposed that electrophysiology indicates a mixed axonal and demyelinating disorder. In the pedigree reported by Rozear and colleagues median MNCV ranged between 28 and 37 m/s (mean, 34) in males, and between 38 and 53 m/s (mean, 50) in affected females [39]. Neither CMAP values nor DML values are given. Sural nerve biopsy in a male patient showed axonal loss, variation of myelin thickness, onion bulbs, and, on fibre teasing, shortened internodal lengths and variation in myelin thickness. These electrophysiological and pathological data in male patients were interpreted as characteristic of a demyelinating hypertrophic neuropathy. In a large Canadian kindred comprising 13 male patients and 19 affected females examined, Hahn and colleagues recorded peroneal MNCVs distally to extensor digitorum brevis muscle and proximally to anterolateral leg muscle compartment [38]. Where CMAP amplitudes were preserved, MNCVs were either normal or slightly reduced (30 to 39 m/s). Nerve biopsies in two cases showed loss of myelinated and unmyelinated fibres, regenerative sprouting and secondary demyelination. The authors conclude that this CMTX variant is the result of primary axonal degeneration.

Given the complex nosology of CMTX1, it is timely to make a brief comment on animal models of the disease. Initial characterization of *GJB1*-null mice showed that demyelination preceded axonal loss [48]. Indeed, connexin32-null mice developed, by three months of age, demyelinating peripheral neuropathy with widened incisures and accumulations of adaxonal cytoplasm (“periaxonal collars”), though main electrophysiological findings were significant decrease of M responses and slight conduction slowing indicative of mixed axonal and demyelinating pathology [49, 50]. Re-investigation of the issue has demonstrated that axonal abnormalities, including

impaired cytoskeletal organization and defects in axonal transport, preceded demyelination in this mouse model [51]. Prominent periaxonal collars have also been reported in nerve biopsies of CMTX1 patients [40, 52-55], but not in other inherited neuropathies [50]. The disorganization of the inner Schwann cell compartment might reflect poor communication between outer and inner cytoplasmic aspects of myelinating Schwann cells, resulting from altered gap junctions, which eventually leads to cytoplasmic abnormalities [49, 50]. Alternatively and accepting the relevant role of early axonal dysfunction, such periaxonal collars could represent a non-specific mechanism by which the Schwann cells clear debris and help maintaining the integrity of the axon under normal and pathologic conditions [56, 57]. Be that as it may, the corollary of these experimental studies is that both primary axonal dysfunction evolving into demyelination or primary demyelination evolving into axonal degeneration may occur in CMTX1.

Table 2 summarizes 40 papers describing motor conduction parameters in median nerve (alternatively ulnar nerve or peroneal nerve) reported between 1985 and 2016 [38-40, 43, 45, 46, 52, 53, 55, 58-88]. In the five largest CMTX1 series, mean median MNCV in males ranged from 33.2 to 36.0 m/s, mean median CMAP amplitudes varying between 2.0 and 3.7 mV [40, 45, 63, 69, 70]. Furthermore, the distribution of median MNCVs was unimodal peaking at 33 m/s [70]. In one series, 90% of CMTX1 males showed intermediate median MNCV (30-40 m/s), just one out of 21 cases exhibiting normal MNCV [63]; in another series, however, male median MNCV was normal in 20% (≥49 m/s), intermediate (33-41 m/s) in 40%, and slow (<32 m/s) in the remaining 20% [45]. In case report or family report papers, median MNCVs and CMAP amplitudes in males were almost always abnormal (≤48 m/s and ≤3.9 mV); exceptions are as follows (see Table 2): i/ two affected boys, aged 3 and 6 years, were normal

clinically and electrophysiologically thus indicating that CMTX1 phenotype is age dependent [38, 43, 63]; ii/ occasionally, male patients had normal MNCV with reduced CMAP amplitude, a feature giving support to the notion of a primary axonal involvement [38, 63]; and iii/ conversely, there were a few patients with intermediate median or ulnar MNCV and preserved CMAP amplitude, a binomial pointing to a demyelinating disorder [55, 67, 71, 73, 74]. A significant positive correlation was found between median, ulnar and peroneal MNCV slowing and CMAP amplitude reduction [63]. Where reported, DML and F-wave latencies were usually and harmonically delayed with the degree of MNCV slowing (see table 2). In no case exhibiting median or ulnar MNCV slowing with CMAP attenuation was proximal (axilla-elbow segment) MNCV evaluated.

Nerve biopsy in CMTX1 male patients has been reported in 17 papers (table 2) [38, 40, 47, 52, 53, 55, 63-65, 68, 70, 71, 74, 75, 80, 83, 85]. Leaving the initial controversy over pure demyelination versus pure axonal pathology into aside (see above), the common denominator of histological studies is a variable combination of demyelination and axonal degeneration, which can be summarized as follows: i/ on semithin sections, reduction of larger myelinated fibres and clusters of regeneration, with a shift of the fibre histogram to the smaller fibre range; ii/ variable presence of onion bulb formation; iii/ on fibre teasing and longitudinal semithin sections, paranodal demyelination, widening of the nodal gap, and to a lesser degree internodal de/remyelination; and iv/ on ultrastructural study, periaxonal collars (see above), thinly myelinated fibres, clusters of regeneration, and onion bulbs. The observed mixed pathology indicate that mutation and loss of function of connexin 32 gap junctions lead to profound alterations in the Schwann cell-axon unit with deleterious effects on both Schwann cells and myelin, as well as on the functions and integrity of axons [40]. In

short, these descriptions corroborate the former pathological hallmark reported in intermediate CMT [5, 8].

CMT associated with mutation in X-linked *DRP2*

In a unique pedigree, Brennan and colleagues described a 60-year-old man presenting with a 10-year history of length-dependent sensorimotor neuropathy [89]. A maternal grand-father had been diagnosed of peripheral neuropathy; his mother and two maternal aunts presented hammertoes. On three serial electrophysiological studies performed over a 4-year period, ulnar MNCVs ranged between 43 and 45 m/s, CMAP amplitudes being normal (13.4-8.2 mV); worthy of note is the fact that motor conduction study included two recordings from axilla that showed velocities of 36 and 35 m/s with preserved CMAP amplitudes. DML were normal or minimally prolonged, whereas F-latencies were delayed (37.6 ms in median nerve and 37.4 ms in ulnar nerve; normal, ≤31 and ≤32 ms, respectively). Whole exome sequencing revealed a stop codon mutation in *DRP2*, c805C>T (Q269*). Skin biopsies revealed a lack of DRP2 in myelinated nerves that exhibited no distinct Cajal bands and periaxonal collars (see above). DRP2 interacts with periaxin and dystroglican to form the periaxin-DRP2-dystroglican complex, which plays a role in the maintenance of the Cajal bands on myelinating Schwann cells. In short, this case is an emblematic example of intermediate CMT with motor conduction slowing in the range formerly reported by Davis and colleagues [4], here being more marked in proximal nerve segments.

DI-CMTA (MIM#606483)

Rossi and colleagues reported a 5-generation CMT pedigree with male-to-male transmission and 21 affected members, 15 of them having been studied clinically and

electrophysiologically [90]. Sensorimotor semeiology appeared in the second decade and stabilized by the fourth decade. Median MNCV ranged between 25 and 45 m/s, but data on CMAP amplitudes are not given. Ulnar MNCV varied from 27 to 61 m/s (mean, 37.8), but again CMAP values are lacking. Nerve biopsy studies showed signs of axonal degeneration and demyelination [91]. The authors concluded that their electrophysiological and histological data support the proposal for an intermediate form of CMT. After discarding mutations of *PMP22*, *MPZ* and *NEFL*, linkage analysis demonstrated linkage to chromosome 10q24.1-q25.1 [92, 93].

DI-CMTB (MIM#606482)

By linkage analysis in two large Australian and US intermediate CMT pedigrees, a second locus was mapped to chromosome 19p [94-96]. Concise electrophysiological descriptions indicate that conduction velocities ranged from 24 to 54 m/s in the Australian pedigree, and that median nerve conduction velocities in two patients from the DUK118 pedigree were 36 and 46 m/s. Sural-nerve biopsy showed similar findings to those reported in DI-CMTA. In these two pedigrees and in one additional originating from Belgium, Züchner and colleagues identified unique mutations in the pleckstrin homology domain of dynamin 2 (*DNM2*) [97]. DNM2 belongs to the family of large GTPases and is part of the cellular fusion-fission apparatus.

Claeys and colleagues described detailed clinical, haematological, electrophysiological and sural nerve biopsy findings in 34 patients belonging to six independent families in whom a *DNM2* mutation had been identified [98], including those reported by Züchner and colleagues [97] and by us [99]. Patients presented with a classical CMT phenotype, which was mild to moderately severe. The disease could cosegregate with neutropenia or cataracts. The mean age of onset was 16 years, varying

between 2 and 50 years. In 27 out the 34 patients electrophysiologically evaluated, median MNCVs ranged from 26 m/s to normal values indicating that the phenotypic spectrum of *DNM2* mutations encompasses both intermediate and axonal patterns. Distinction of such patterns is not always an easy task. This question is illustrated in figures 2 and 3 corresponding to a pedigree associated with *DNM2* G358R mutation [99]. Briefly, all three patients (proband and two affected daughters) showed marked lower-leg muscle atrophy and inexcitability of lower-limb nerves, a fact indicative of severe axonal degeneration. Starting from median MNCV in the proband, who had marked hand atrophy and even after examining the axilla-elbow segment, a diagnosis of DI-CMT could have been entertained, though widespread median and ulnar nerve CMAP attenuation pointed to an axonal disorder. It is worth remembering that a single conduction velocity value is not definitive of intermediate CMT, a term that should only be applied to the form and not the nerve conduction value [11]. This notion is applicable to the current family as affected proband's daughters had preserved median motor conduction parameters, indicative that in both we are confronted with a length- and age-dependent axonal disorder of lower-limb nerves, not yet involving upper-limb nerves. Therefore, in addition to DI-CMT, *DNM2* mutations may be associated with axonal CMT [100, 101], currently classified as CMT2M [26]. DI-CMTB and CMT2M are included under the same "Phenotype MIM number", 606482; in our view, this calls for future revision.

DI-CMTC (MIM#608323)

Jordanova and colleagues reported two large unrelated families with DI-CMT linked to a novel locus on chromosome 1p35 [102]. Three years later, they identified two heterogeneous missense mutations (G41R and E196K) and one *de novo* deletion (15-

156delVKQV) in tyrosil-tRNA synthetase (YARS) in three unrelated families with DI-CMTC [103]. Aminoacyl-tRNA synthetases (ARSs) are ubiquitously expressed and essential enzymes responsible for charging tRNA with cognate amino-acids. Mutations in 28 of the 37 nuclear-encoded human ARS genes have been linked to a variety of recessive or dominant tissue-specific disorders [104]. YARS (also known as TyrRS) mutations causing DI-CMTC alter the normal function and distribution of YARS in neuronal endings, which in turn could affect synaptic plasticity resulting in axonal degeneration [103]. *Drosophila* model of the three DI-CMTC associated *YARS* mutations, which recapitulates several hallmarks of the human disease, suggests that the resulting phenotype is most likely due to gain-of-function alteration of the mutant TyRS or to an interference of unknown function of wild type protein [105].

Thomas and colleagues have reported detailed clinical, electrophysiological and morphological features in the original Bulgarian and US families [106], upon whom the disease locus was mapped [102]. Twenty-one affected individuals from the US family and 27 from the Bulgarian family were evaluated. Essentially, the clinical picture is a sensorimotor neuropathy, predominating in lower limbs with pes cavus, hammertoes and atrophy of extensor digitorum brevis. Mean ages of onset were 9 years in US patients and 20 years in Bulgarian patients. Median MNCV ranged from 29.5 to 45.6 m/s (mean, 34.1) in US family, and from 24.7 to 57.8 m/s (mean, 41.5) in Bulgarian family, CMAP amplitudes varying from 3.0 to 13.6 mV (mean, 7.7) and from 1.1 to 8.1 mV (mean, 5.1), respectively. It is worth noting that median CMAP amplitudes were abnormal 7 out of 41 studies; median DML were prolonged in ~50% of studies. Median MNCV against age suggested progressive slowing with stabilization in the fourth decade. Since CMAP amplitudes remained stable, axon loss probably plays a lesser role in the declining of nerve conduction velocities. Sural nerve biopsies of five patients

revealed age-dependent axonal degeneration, reduced number of large myelinated fibres, some remyelinated axons, clusters of regeneration, and no onion bulbs. In short, the reported DI-CMTC phenotype is in line with the first description of intermediate CMT [4, 5].

DI-CMTD (MIM#607791)

Under the rubric of intermediate hereditary motor and sensory neuropathy, Mastaglia and colleagues reported a 4-generation CMT pedigree associated with *MPZ* D6Y mutation [107]. Electrophysiology in eight patients showed median MNCV ranging between 24 and 41 m/s (mean, 35), and ulnar MNCV ranging between 33 and 48 m/s (mean, 42.3). No data on CMAP are given, though sensory nerve potentials at the wrist were markedly delayed and attenuated in all affected members. Sural nerve biopsy in two cases showed advanced fibre loss, some thinly myelinated internodes, and absence of onion bulbs. Without knowing CMAP amplitude values, it does not seem possible to us to discard that the observed motor conduction slowing could merely be due to axonal loss (see above). In any case, three definite DI-CMT pedigrees associated with *MPZ* mutation have been reported [108-110], briefly analyzed below.

In a late-onset CMT family associated with *MPZ* K236del mutation, comprising two symptomatic and two sub-clinical heterozygous mutation carriers, median MNCV ranged from 29 to 56 m/s with normal CMAP amplitudes, and prolonged DML in two cases [108].

In a 4-generation family with a classic CMT phenotype harbouring *MPZ* K214M mutation, median MNCV in seven patients ranged from 34.2 to 40.1 m/s, CMAP amplitudes varying between 2.1 and 9.6 mV (≥4 in four patients) [109]. Intriguingly, examination of the axilla-elbow segment, performed in five patients, also showed motor

conduction slowing in the intermediate range. Median nerve DML ranged from 4.4 and 7.1 ms; F-latencies were prolonged in all three evaluated cases (37.5, 38 and 38.4 ms). As stated by the authors, these features cannot be accounted for by axonal degeneration and therefore unequivocally indicate that nerve conduction parameters are in the intermediate range.

A pedigree with *MPZ* W101X mutation, comprising 9 patients across 4 generations, showed a unique phenotype consisting of debilitating pain [110]. In the four patients electrophysiologically evaluated, median MNCV ranged from 38 to 43 m/s, CMAP amplitudes being always normal. DMLs were prolonged (4.5 to 4.9 ms) in three patients, whereas prolongation of F-latencies (32.1 and 35.6 ms) was noted in two out the three patients tested.

DI-CMTE (MIM#610982)

Inverted forming-2 (*INF2*) mutations are associated with 75% of patients suffering from DI-CMT and focal segmental glomerulosclerosis (FSGS) [111]. INF2 belongs to the group of proteins that regulates nucleation and elongation of actin filaments; when mutated, INF2 dysregulates actin-dependent processes, ultimately interfering with myelination and mitochondrial dynamics [112]. So far, 21 proband cases of CMT-FSGS have been reported associated with 15 different heterozygous point *INF2* mutations in the DID domain, which may appear either in dominant kinships or in sporadic cases (*de novo* mutation) [111-116]. Clinical phenotypes were similar to those seen in other CMT syndromes, onset usually occurring in the first or second decade, before the end-stage renal disease. There may be manifestations of CNS involvement. Median MNCVs, recorded in 18 patients, were as follows: i/ in nine patients ranged from 13 to 42 m/s (mean, 32), CMAP amplitudes not being reported [111, 112]; and ii/ as is characteristic

of intermediate CMT (see above), in the remaining nine patients ranged from 27 to 45 m/s (mean, 39), CMAP amplitudes being systematically normal [114-116]. Nerve biopsy studies have revealed a uniform pattern consisting of chronic demyelination associated with progressive axonal loss, accumulation of β-actin in the cytoplasm of Schwann cells, and cytoplasmic supernumerary elongated extensions in unmyelinating Schwann cells. These morphological features suggest that DI-CMTE is the first peripheral nerve disorder associated with Schwann cell actinopathy [113].

DI-CMTF (MIM#615185)

Lee and colleagues reported a dominant CMT pedigree with 11 affected members, six of them being symptomatic with onset between 10 and 45 years [117]. Clinical severity varied from no symptoms to being wheelchair-bound. Median nerve electrophysiology showed the characteristic intermediate CMT: MNCVs ranged from 16.5 to 45.7 m/s (mean, 32), and CMAP amplitudes from 3.2 to 9.6 mV (mean, 6.3). Sural biopsy in two patients revealed reduced myelinated fibres, clusters of regeneration and onion bulbs. The disease was mapped to 3q28-q29. Subsequent exome sequencing revealed a heterozygous mutation (G53D) in *GNB4,* encoding guanine-nucleotide-binding protein subunit beta-4 ($G\beta_4$), to cosegregate with the CMT phenotype in this family [118]. Therefore, the study identified *GNB4* mutation as a novel cause of CMT highlighting the importance of $G\beta_4$-related G-protein-coupled-receptor signalling in peripheral-nerve function in humans. Another pathogenic heterozygous *GNB4* mutation (K57E) has been reported in a severe CMT1 patient [119].

DI-CMT and *NEFL* mutation

Neurofilament triplet proteins (heavy, medium and light) are the major intermediate filaments present in adult neurons and their expression is restricted to neuronal cell types of CNS and peripheral nervous system (PNS). Their main roles are to increase the axonal calibre of myelinated axons and consequently their conduction velocity, and contribute to the dynamic properties of the axonal cytoskeleton during neuronal differentiation, axon outgrowth, regeneration and guidance [120, 121]. CMT has mainly been associated with *NEFL* dominant missense mutations causing disruption of neurofilament assembly and aggregate formation with interference of axonal transport [122]. Resulting phenotypes have being classified as CMT1F (MIM#607734) and CMT2E (MIM#607684) [26]. There have been several descriptions of *NEFL* mutations associated with an intermediate electrophysiological pattern systematically accompanied by variably reduced CMAP amplitudes, making it difficult to establish whether nerve conduction slowing is attributable to reduction of axonal diameters [122-125]. We have reported intermediate electrophysiological patterns in two dominant CMT pedigrees associated with either *NEFL* E396K mutation [20] or *NEFL* N98S mutation [24]. Our first pedigree comprised four patients over two generations, aged between 35 and 59 years. The clinical picture was characterized by pes cavus, sensorimotor neuropathy and spastic gait. Both older patients showed ascending leg weakness to involve pelvic musculature. Figure 1 illustrates median MNCV study in the oldest patient; the remaining three showed intermediate median MNCVs with normal or minimally attenuated CMAPs. Median F waves and DML were moderately delayed. Our second pedigree comprised two patients, the proband and her son, aged 38 and 5 years. The proband showed delayed motor milestones that, as of the second decade, evolved into severe phenotype consisting of sensorimotor neuropathy, pes cavus,

clawing hands, gait and kinetic cerebellar ataxia, nystagmus and dysarthria, she becoming wheelchair bound. Her median MNCV study is illustrated in figure 4. As a whole, electrophysiological recordings indicate that accurate detection of intermediate motor conduction slowing may require exploration of proximal nerve segments in upper-limb nerves. Intriguingly, another *NEFL* N98S mutation with intermediate phenotype has recently been reported [126]. DI-CMT associated with *NEFL* mutations is a distinct and complex syndrome implying a simultaneous involvement of the PNS and CNS, which also calls for specific OMIM numbering.

DI-CMT associated with mutation in other genes

Mutations in the *mitofusin 2* (*MFN2*) are the most frequent cause of CMT2A (MIM# 609260) [127, 128]. In a Norwegian genetic survey of 232 consecutive unselected CMT pedigrees, *MFN2* point mutations were identified in 8 (3.4%) of them [129]. Pedigree 8 in this study, with two male patients, aged 23 and 50 years, harbouring heterozygous *MFN2* A716T mutation, showed median/ulnar MNCVs that ranged from 37/39 to 44/43 m/s with normal or slightly reduced CMAP amplitudes. This is probably the only autosomal dominant *MFN2* pedigree so far reported fulfilling electrophysiological criteria of intermediate CMT.

Point *AARS* mutations are associated with CMT2N (MIM#613287) [130]. In a cohort of six families with dominant *AARS*-related neuropathies, median MNCVs, obtained in eight patients, ranged from 26 to 47.6 m/s (mean, 36), CMAP amplitudes varying between 2 and 18 mV (mean, 7.2) [131]. Therefore, the electrophysiological pattern in these families fits well into that of intermediate CMT.

Spaans and colleagues reported a unique dominant phenotype combining myotonic dystrophy type 1, CMT, encephalopathic attacks, and sensoneurial hearing

loss [131-134]. Median MNCV in 14 patients ranged from 23 to 48 m/s (mean, 37); although individual CMAP values are not indicated, it is specifically stated that in four patients the negative peak of the relevant CMAP was lower than 0.5 mV, which means that the corresponding MNCV value does not reliably represent the degree of demyelination [131]. Thus, it seems that intermediate CMT is an integral part of this exceptional pedigree.

Dynactin 2 (DCTN2) is a subunit of dynactin, a multiprotein complex associated with dynein. Mice over-expressing dynamitin, the p50 subunit of DCNT2, demonstrate a late-onset progressive motor unit degenerative syndrome [135]. Braathen and colleagues have identified a unique *DCNT2* H113Y DI-CMT pedigree with late onset phenotype [136]. In four electrophysiologically evaluated patients, median MNCV ranged from 35.5 to 50 m/s (mean, 46), and CMAP amplitudes from 1.5 to 6.8 mV (mean, 4). So far, no other intermediate CMT syndromes associated with *DCNT2* mutation have been reported.

Finally, in axonal dominant CMT pedigrees associated with *EGR2* R409Q mutation [137] or *HSP27* R17W mutation [138], there were isolated patients exhibiting intermediate median MNCV with normal or minimally attenuated CMAP amplitudes. We entirely agree in classifying both pedigrees within CMT2 given that axonal conduction pattern occurred in the majority of patients.

RI-CMTA (MIM#608340)

Mutations in ganglioside-induced differentiation associated protein (GDAP1), a new subfamily of the glutation-S-transferases, were initially described in autosomal recessive families showing a severe CMT phenotype sometimes with vocal cord paralysis, which are currently classified as CMT4A (MIM#214400) or AR-CMT2

(MIM#607706) [139, 140]. Heterozygous *GDAP1* mutations cause CMT2K, usually characterized by later onset, milder clinical picture, and axonal electrophysiology (MIM#607831) [141-144].

Baxter and colleagues found that motor conduction velocities ranged between 27 and 35 m/s; regrettably, reference to CMAP amplitudes is lacking [139]. Looking at their "Web Figure A", the outstanding histological feature in nerve biopsy is a decrease of large myelinated fibres without clear evidence to frequent onion bulbs, as mentioned in the paper. Although conduction values are in the intermediate CMT range, without knowing CMAP values this diagnostic label cannot be established with certainty.

In the series by Cuesta and colleagues [140], detailed electrophysiological and nerve biopsy studies showed the characteristic findings of a severe axonal CMT [145, 146]. Intriguingly, where peripheral nerves were inexcitable on distal stimulation, the motor latency of the axillary nerve was not delayed, as would have been expected in any intermediate CMT, but normal.

In another series of seven AR-CMT families associated with *GDAP1* mutations, the range of MNCV was variable: some patients having normal or near normal MNCV, whereas in other showing severe slowed MNCV [147]. The peripheral nerve biopsy findings were equally variable and showed features of demyelination or axonal degeneration.

The eponym RI-CMTA is used in OMIM in relation to the Polish pedigree reported by Kabzińska and colleagues, comprising two severely affected sisters, aged 29 and 21 years, harbouring homozygous *GDAP1* G327D mutation [148]. In elder patient ulnar MNCV was 32.7 m/s with CMAP of 0.2 mV and DML of 6.0 ms. In her sister ulnar and median nerves were inexcitable, whereas the axillary nerve exhibited attenuated CMAP (0.28 mV) with normal latency. Sural nerve biopsy showed marked

loss of large myelinated fibres, clusters of regeneration, and a few onion bulbs. We concur with the authors that the current pedigree is a prototypic example of axonal CMT with autosomal recessive transmission.

Senderek and colleagues introduced the term “intermediate” in the title of a paper describing two Turkish families with mutations in *GDAP1* gene in autosomal recessive CMT neuropathy [149]. In one patient of each family, aged 7 and 6 years, median MNCV were 31 and 26.4 m/s with CMAP amplitudes of 0.5 and 0.3 mV, respectively. Sural nerve biopsies showed similar findings consisting of marked loss of large myelinated axons, active axonal degeneration, numerous clusters of regeneration, and occasional onion bulbs. As previously discussed, both electrophysiological and histological features may be explained by severe, primary axonal pathology.

Under the rubric of recessive intermediate CMT, Chung and colleagues reported two unrelated patients harbouring homozygous H256R or compound heterozygous P111H and V219G in *GDAP1* [150]. Patients were aged 5 (patient 1) and 8 (patient 2) years, and showed a severe CMT phenotype. In patient 1, at ages 4 and 5, median MNCVs were 38.7 and 37.5 m/s with CMAP amplitudes of 0.1 and 0.3 ms, respectively. In patient 2, at ages 7, 7.5 and 8 years, MNCVs were 44.8, 43.8 and 44.1 m/s with CMAP amplitudes of 4.5, 2.7 and 3.9 mV, respectively. Sural nerve biopsies showed decreased number of large myelinated fibres with unimodal histogram, clusters of regeneration, and onion- or pseudo-onion bulb formations. Starting from electrophysiological parameters and considering normal median MNCV and CMAP values at ages 1-6 and 6-12 years [35], only patient 2 could be included within the RI-CMTA.

RI-CMTB (MIM#613641)

To further explore the role of ARSs in CMT disease (see above), McLaughlin and colleagues carried out a large-scale mutation screening of the 37 human ARS genes in a cohort of 355 patients with a phenotype consistent with CMT [151]. They identified two variants (L133H and Y173SfsX7) in the lysyl-tRNA synthesase (*KARS*) gene in a CMT patient showing developmental delay, self-abuse behaviour, dysmorphic features, and vestibular Schwannoma. Median/ulnar MNCVs were 39.5/30.6 m/s with CMAP amplitude of 0.5 mV. Although these MNCV values are in the intermediate range, severe CMAP attenuation, indicative of axonal loss, may account for the observed conduction slowing, and therefore the case should probably be reclassified as AR-CMT2.

RI-CMTC (MIM#615376)

Mutation in *Pleckstrin homology domain-containing family G member 5* (*PLEKHG5*) gene has been reported in severe childhood onset lower motor neuron disease, and in three well-defined RI-CMTC pedigrees [152-154]. PH domain of PLEKHG5 contributes to the allosteric regulation of the RhoGEF domain, activating GTPases by stimulating the exchange of GDP to GTP, thereby initiating various signalling mechanisms that regulate neuronal shape and plasticity, dendrite growth, synapse formation, and neuronal survival.

Azzedine and colleagues reported two consanguineous pedigrees originated from Portugal and Morocco harbouring two different homozygous truncating mutations in the *PLEKHG5* gene [153]. The Portuguese family comprised two affected siblings, aged 43 and 53 years, and four unaffected siblings; in the Moroccan family four out of eight siblings were affected, aged between 42 and 51 years. With onset from the first to

the fifth decade of life, the clinical picture consisted of distal lower- and upper-limb amyotrophy, areflexia, distal sensory loss, and foot deformities. There was no CNS semeiology. Median MNCV in four cases ranged from 35 to 39 m/s, CMAP amplitudes being normal in three patients or minimally reduced (3.7 mV) in the remaining one. Sural nerve biopsy showed reduced fibre density to about 70% of normal, with predominant loss of larger fibres and remyelinated fibres.

In a 19-year-old Korean patient, Kim and colleagues identified a novel compound heterozygous (T663M and G820R) mutations in the *PLEKHG5* gene [154]. The patient showed early onset CMT with moderate CMT neuropathy score (15 for a maximum of 36). At ages 14, 15 16 and 19 years, median MNCV were 25.3, 24.7, 25.6 and 29.2 m/s, CMAP amplitudes being always normal (between 5.5 and 9.5 mV). Median DMLs were prolonged (between 5.4 and 7 ms). Histological findings in sural nerve included complete loss of large- and medium-size myelinated fibres to 297/mm^2, rare regeneration clusters, and abnormal PLEKHG5 reaction pattern on immuno-histochemical study.

RI-CMTD (MIM#616039)

To identify the genetic background of Japanese CMT, Tamiya and colleagues analyzed the disease-causing mutation in 350 affected individuals, pathogenic mutations being found in 50% of demyelinating CMT and 20% of axonal CMT [155]. Using whole-genome sequencing, the authors found *COX6A1* mutation (c.247-10_247-6del CACTC) in two families with affected members from consanguineous marriages at different sites in Japan. The affected member in family 2, aged 39 years, had minimally reduced or normal median MNCVs (right median nerve, 45.3 m/s; left median nerve, 49.6 m/s) with preserved CMAP amplitudes, and inexcitable tibial nerve. We entirely agree with

the authors that, as a whole, such electrophysiology is indicative of axonal CMT almost selectively involving lower-limb nerves. The affected siblings of family 1 (V-1 and V-2), aged 30-39 years, showed similar clinical findings consisting of early and severe CMT phenotype. Electrophysiological and pathological data are tabulated in their tables S1 and S2. At 8 years of age, patient V-1 showed median/ulnar MNCV of 43.4/45.0 m/s with undetectable sensory nerve action potentials. Sural nerve biopsy showed decrease of myelinated fibres and onion bulbs. At ages 30-39 electrophysiological features were as follows: i/ patient V-1, median/ulnar MNCVs of 35.7/40.7 m/s with CMAP amplitudes of 0.7/1.3 mV, respectively; and ii/ patient V-2, median/ulnar MNCVs of 34.8/37.3 m/s with CMAP amplitudes of 0.08/0.05 mV, respectively. In our view, these electrophysiological data are indicative of a severe axonal neuropathy.

In a recent report, Laššuthová and colleagues confirmed that *COX6A1* homozygous mutation (according to the authors, the correct nomenclature for the deletion should be c.247-7_247-3del) may cause severe, early-onset axonal CMT [156]. In their 37-year-old patient, median/ulnar MNCV were 44 and 46 m/s, CMAP amplitudes being 1.2/1.1 mV, respectively

As said before, exploration of proximal nerve segments of median or ulnar nerves would have been very useful to demonstrate a possible pattern of intermediate motor nerve conduction (see figures 1 and 4). In any case, we are persuaded that up to now the reported COX6A1-related disorders should be re-classified under the rubric of AR-CMT2, thus RI-CMTD becoming vacant.

Discussion

To the best of our knowledge, this is the first systematic review of intermediate CMT. The notion of intermediate CMT initially emerged as a particular pattern of median MNCV slowing, different from that of CMT1 (usually <25 m/s) and CMT2 (usually >45 m/s) [4]. So, MNCV limits were firstly established between 25 and 45 m/s, soon after being narrowed down to 30-40 m/s [7]. Although these electrophysiological limits have widely been accepted in the literature, one important drawback is that the contribution of CMAP amplitude reduction, reflecting axonal loss, to conduction slowing was not specifically taken into account. It is important to note that nerve biopsies in original intermediate CMT series showed similar pathological features consisting of loss of large myelinated fibres, clusters of regeneration, and a few onion bulbs [5, 8], that is, a combination of axonal and demyelinating changes.

As reported for sural nerve in patients with either CMT or acquired neuropathy, the velocity is predictable from the largest fibres present in the biopsy [11, 21]. Overall, nerve biopsy and autopsy studies in CMT2 have demonstrated length-dependent loss of large myelinated fibres (≥7µm) and clusters of regeneration [15-17]. In regards to median MNCV at the elbow-wrist segment and applying sural nerve conversion factor of 3.85 reported in axonal neuropathy [21], such pathologic framework could imply a secondary slowing down of motor conduction into the intermediate range or even into the demyelinating range. Under such circumstances is not an easy task to determine whether median MNCV slowing in intermediate CMT is accounted for by loss of rapidly conducting fibres or by demyelination [11]. Based on our experience with intermediate CMT [20, 24] and previous electrophysiological and pathological considerations (see above), figure 5 provides a proposed algorithm of the

electrophysiological approach when investigating a patient with presumptive intermediate CMT.

Having in mind all these aspects, we proceeded to review PubMed descriptions published under the rubric of intermediate CMT or X-linked CMT. We adhered to the classification proposed in updated review CMT papers [26, 27] (see table 1).

CMTX1 in males is the most frequent cause of intermediate CMT. We found 40 papers describing median MNCV study (alternatively ulnar or peroneal nerves) (see table 2). The great majority of male CMTX1 patients showed median MNCV in the intermediate range, though distal CMAP amplitudes were often attenuated, making it difficult to establish the contribution of axonal loss to conduction slowing.

DRP2 mutation causes an exceptional, well-defined form of X-linked intermediate CMT [89], whose phenotype MIM number has not yet been assigned.

DI-CMT encompasses six numbered MIM phenotypes (see table 1). DI-CMTA is assigned to the pedigree reported by Rossi and colleagues [90]. Its locus mapped to chromosome 10q [92, 93], but the responsible gene has not yet been cloned. Certainly, in this pedigree ulnar MNCVs were within the intermediate range (see above), but considering that CMAP amplitudes are lacking, the question arises as to whether this family could merely represent an example of CMT2 with slowed motor conduction due to potentially severe loss of large axons [22, 23]. DI-CMTB is associated with *DNM2* gene mutations covering a wide phenotypic expression, which includes axonal and intermediate CMT phenotypes sometimes associated with neutropenia or cataracts [98]. Our nerve conduction study in a *DNM2* G359R CMT2 pedigree illustrates that deciphering of the electrophysiological hallmark may require not only investigation of the proband, but also of other less affected secondary cases (see figures 2, 3 and 5) [99], thus emphasizing the notion that the term intermediate should only be applied to the

form and not to nerve conduction values [11]. *YARS* mutations are associated with DI-CMTC, a well-defined electrophysiological intermediate pattern in the reported pedigrees [102, 103, 106]. DI-CMTD was initially reported in a family harbouring *MPZ* D6Y mutation, whose eight patients showed median MNCV in the intermediate range, CMAP amplitudes being lacking [107]. Afterwards, there have been three family reports conforming to the characteristic hallmark of intermediate CMT [108-110]. The relevant diagnostic role of investigating proximal median MNCV for accurate detection of intermediate conduction slowing is demonstrated in one of these pedigrees [110]. DI-CMTE is due to *INF2* mutations, manifesting with intermediate CMT and FSGS [111]. DI-CMTF caused by *GNB4* mutations is another well-defined intermediate phenotype [117, 118], though this gene mutation may also cause CMT1 with severe nerve conduction slowing [119].

Additional DI-CMT forms, not yet numbered in OMIM, are as follows: i/ that associated with *NEFL* mutations, usually showing CNS involvement [20, 24]; ii/ that associated with *MFN2* mutations, just one kinship having been reported [129]; iii/ that associated with *AARS* mutations [131]; iv/ the exceptional phenotype combining myotonic dystrophy type 1 and CMT [132-134]; and v/ that associated with *DCNT2* mutation [136].

Currently, RI-CMT comprises four phenotypes (see table 1). RI-CMTA is associated with *GDAP1* mutation. Most *GDAP1* mutations cause AR-CMT2 and less frequently CMT2K [140, 141, 143-146]. Median MNCVs in the intermediate range have been reported in several pedigrees [136, 145, 146], but their correct electrophysiological classification is not possible given that CMAP amplitudes were severely reduced or not specified. In fact, just one the patients reported by Chung and colleagues could be included within RI-CMTA [150]. Individualization of RI-CMTB

has been based upon a single pedigree associated with *KARS* mutation [151]. Our review indicates that electrophysiology is compatible with a severe axonal neuropathy, so this pedigree should be reclassified within AR-CMT2 phenotypes, RI-CMTB becoming a vacant rubric. RI-CMTC is a well-documented intermediate CMT phenotype caused by *PELKHG5* mutation [153, 154]. RI-CMTD was individualized in two Japanese pedigrees with *COX6A1* mutation, showing either median MNCV over 45 m/s, or median MNCV in the intermediate range with severe CMAP attenuation [155]. Therefore, we propose that this disorder should be reclassified within AR-CMT2, RI-CMTC being another vacant rubric.

We conclude that intermediate CMT is a complex inherited syndrome, whose characterization requires a specific electrophysiological protocol comprising evaluation of upper-limb proximal nerve trunks when distal CMAP amplitudes are reduced or even complementary evaluation of other affected kinship members. An updated version of MIM phenotype numbering is needed.

Acknowledgements. The authors are grateful to Mr Mario Corral (Director of "Marquesa de Pelayo" Library, Santander, Spain) for his invaluable help in literature search. The authors also thank Drs Mario Reviriego and Antonio Murciano (Smart Intelligence Services, SL, Madrid, Spain) for technical support, Mrs Marta de la Fuente for secretarial work, and Miss Mar Ruiz for her help in contacting our patients.

Compliance with ethical standards.

Conflicts of interests. None.

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FIGURE LEGENDS

Figure 1. Median MNCV study in a 59-year-old patient belonging to a DI-CMT family associated with *NEFL* E396K mutation [24]. (**A**) The recorded MCV value of 36 m/s (elbow-wrist segment), within the intermediate range, could be attributed to severe CMAP amplitude reduction (to 15% of the LLN, ≥4 mV), namely, to loss of large myelinated fibres. This question is clarified here analysing the segment axilla-elbow, with stimulation at axilla and elbow, and recording from flexor digitorum sublimis (FDS) (**B**). Note that despite CMAP amplitudes being preserved, the recorded MCV, 44 m/s (normal, ≥54), is in the intermediate range.

Figure 2. Clinical pictures of the proband (**A-C**), aged 55 years, and her younger affected daughter (**D, E**), aged 23 years, suffering from axonal CMT2 associated with *DNM2* Gly358Arg mutation [99]. These patients are identified as CMT-103/I.2 and CMT-103/II.3 in the series by Claeys and colleagues [98]. In the proband note marked lower-leg amyotrophy (**A**) and hand wasting involving first dorsal interossei (**B**) and thenar eminences (**C**). Her daughter shows preserved hand musculature (**D**), but evident lower-leg amyotrophy. MNCV study revealed absence of responses in tibial and peroneal nerves, median MNCV study being illustrated in figure 3.

Figure 3. Median MNCV study in three patients from a pedigree with CMT2 associated with *DNM2* Gly358Arg mutation (see figure 2). (**A**) In the 55-year-old proband patient showing marked hand amyotrophy, CMAPs are severely attenuated in APB muscles, elbow-wrist conduction velocity being 33 m/s. As argued in figure 1, one may wonder whether so marked motor conduction slowing could be accounted for by axonal loss or

demyelination. To clarify the issue we recorded median MNCV in the segment axilla-elbow with recording in FDS. The recorded CMAPs are now of greater amplitude but still clearly attenuated, so the recorded MNCV, 44 m/s, might be interpreted either intermediate or secondary to axonal loss. Electrophysiological evaluation of her two affected daughters, not showing hand amyotrophy, is crucial in this regard. Indeed, median MNCV study in elder affected daughter (**B**), aged 32 years, and younger affected daughter (**C**; see figure 2D, E) shows almost normal conduction parameters, the usual electrophysiologic hallmark of uninvolved nerves in axonal CMT.

Figure 4. Median MNCV study in a 31-year-old patient belonging to a DI-CMT family associated to *NEFL* N98S mutation [20]. (**A**) The recorded MNCV value of 18.3 m/s (elbow-wrist segment; normal, ≥49), within the demyelinating range, could be attributed greatly to severe CMAP amplitude reduction in abductor pollicis brevis (APB) (to 5% of the LLN), namely, to presumptive complete loss of large myelinated fibres. (**B**) This question is again clarified analyzing the segment axilla-elbow, with stimulation at axilla and elbow, and recording in flexor digitorum sublimis (FDS). Note that despite CMAP amplitudes being relatively preserved, recorded MNCV, 47.5 m/s (normal, ≥55), is in the intermediate range. As argued in the text, proximal MNCV study might be essential for accurately defining the CMT pattern of conduction slowing.

Figure 5. An algorithm for electrophysiological approach in CMT focused on the intermediate form of the disease. For further details, see also figures 1, 3 and 4. For abbreviations see text.

Figure

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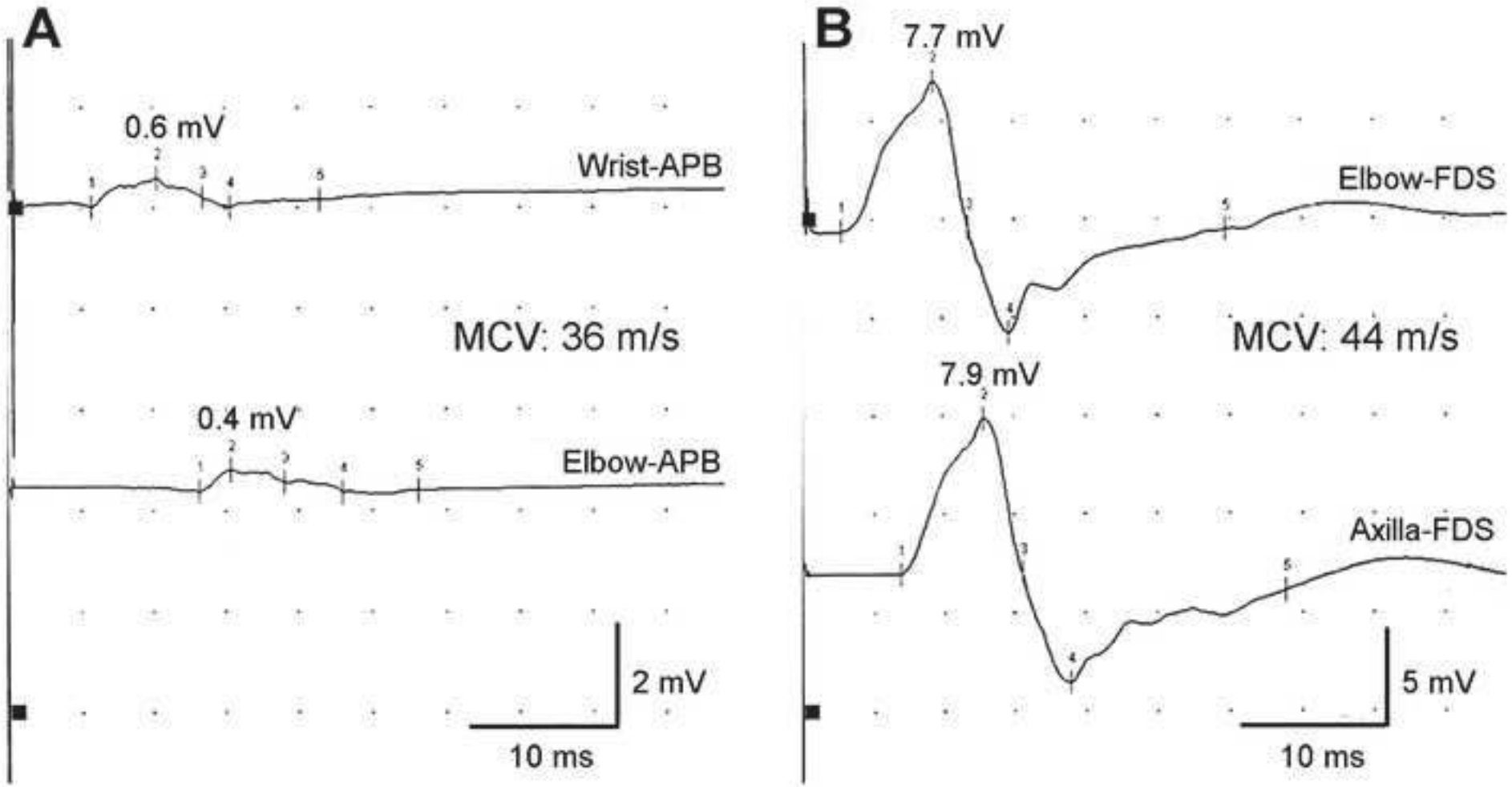

Figure

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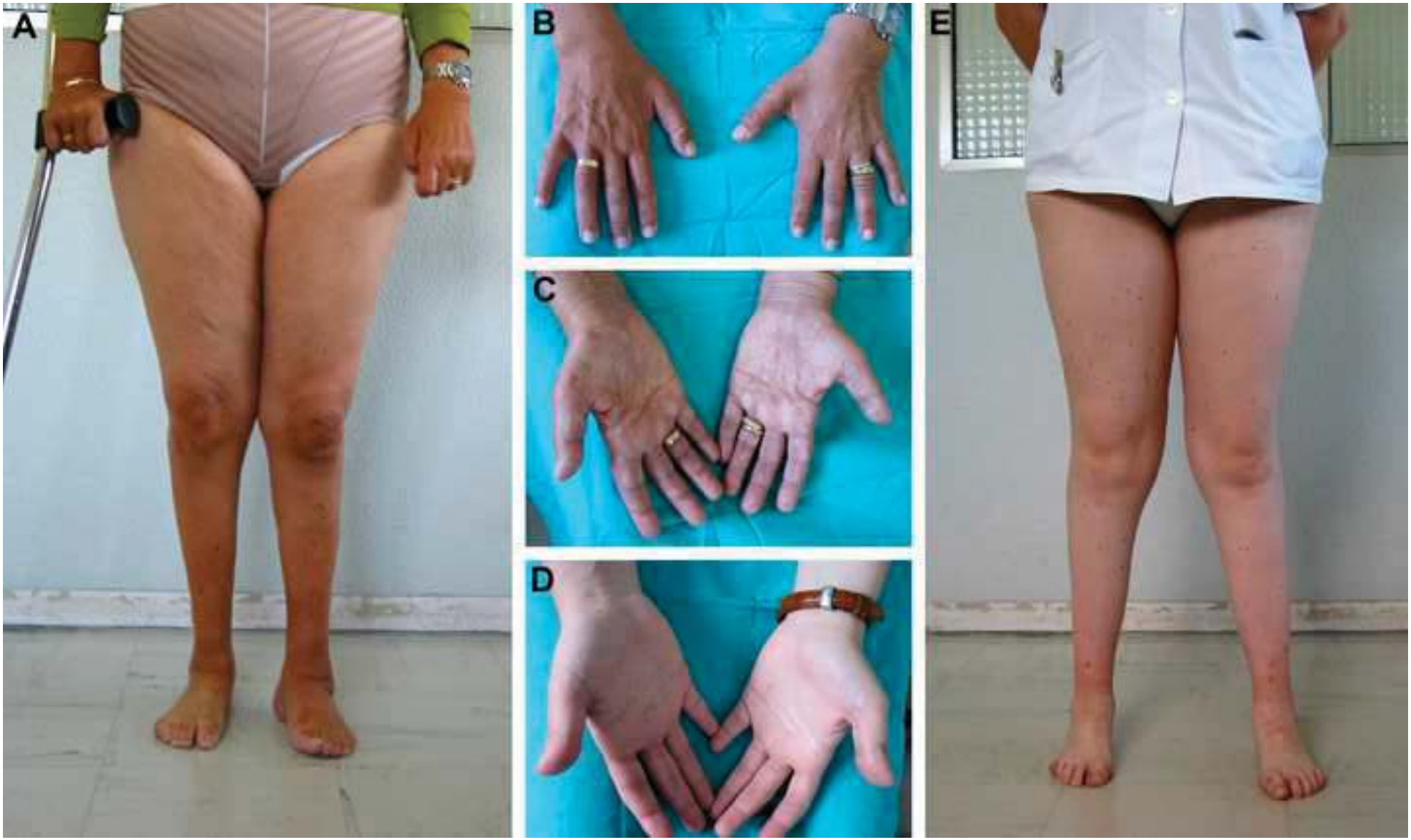

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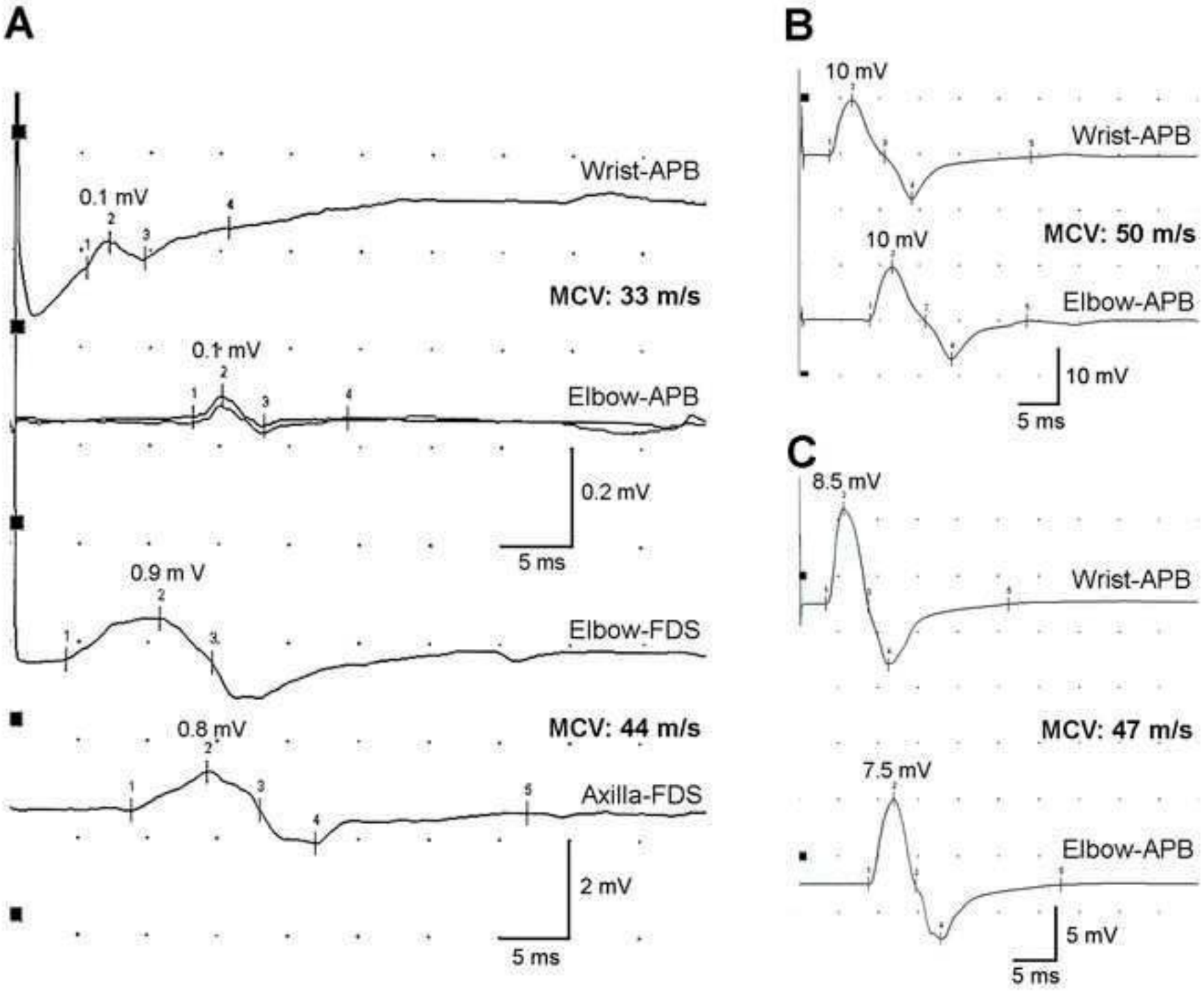

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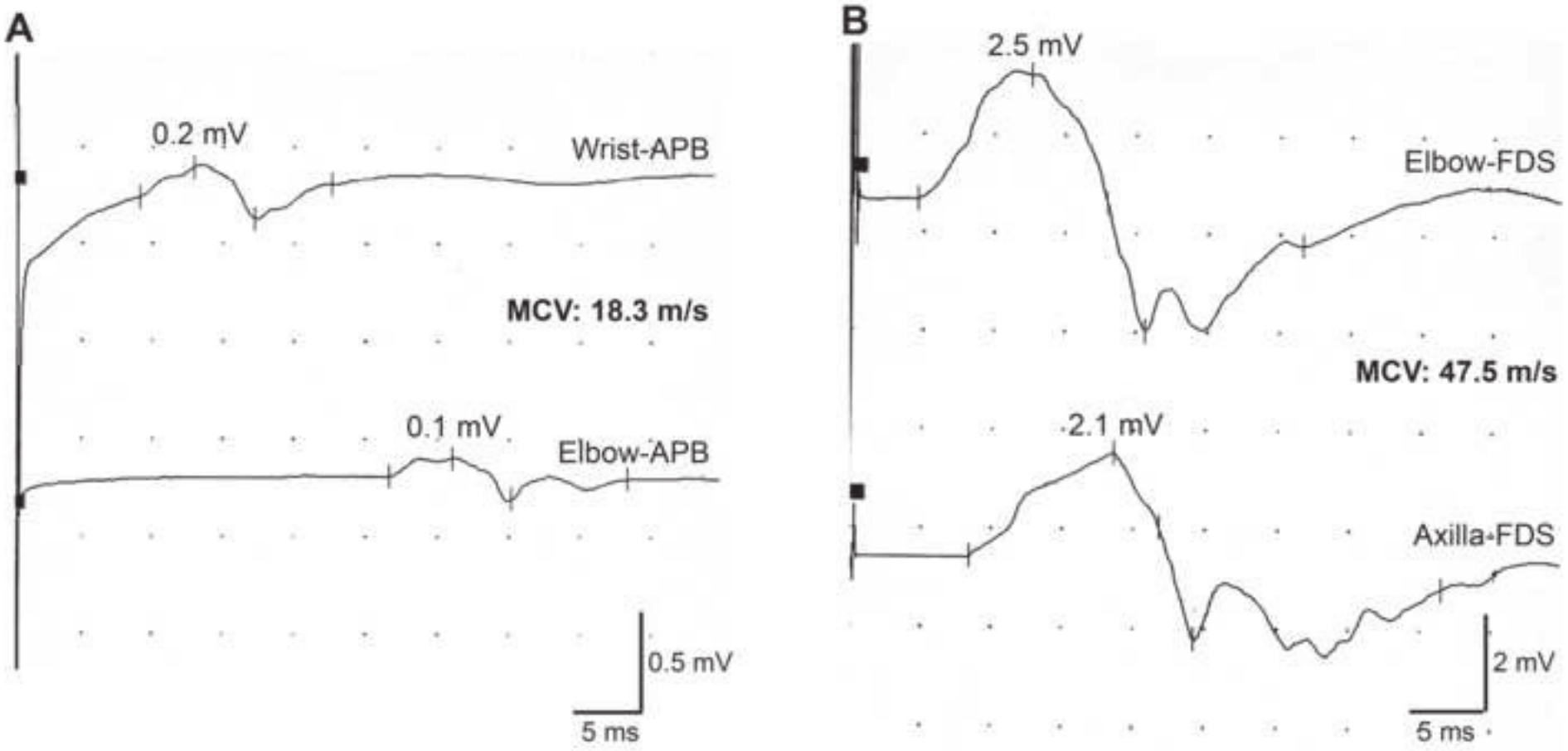

Figure

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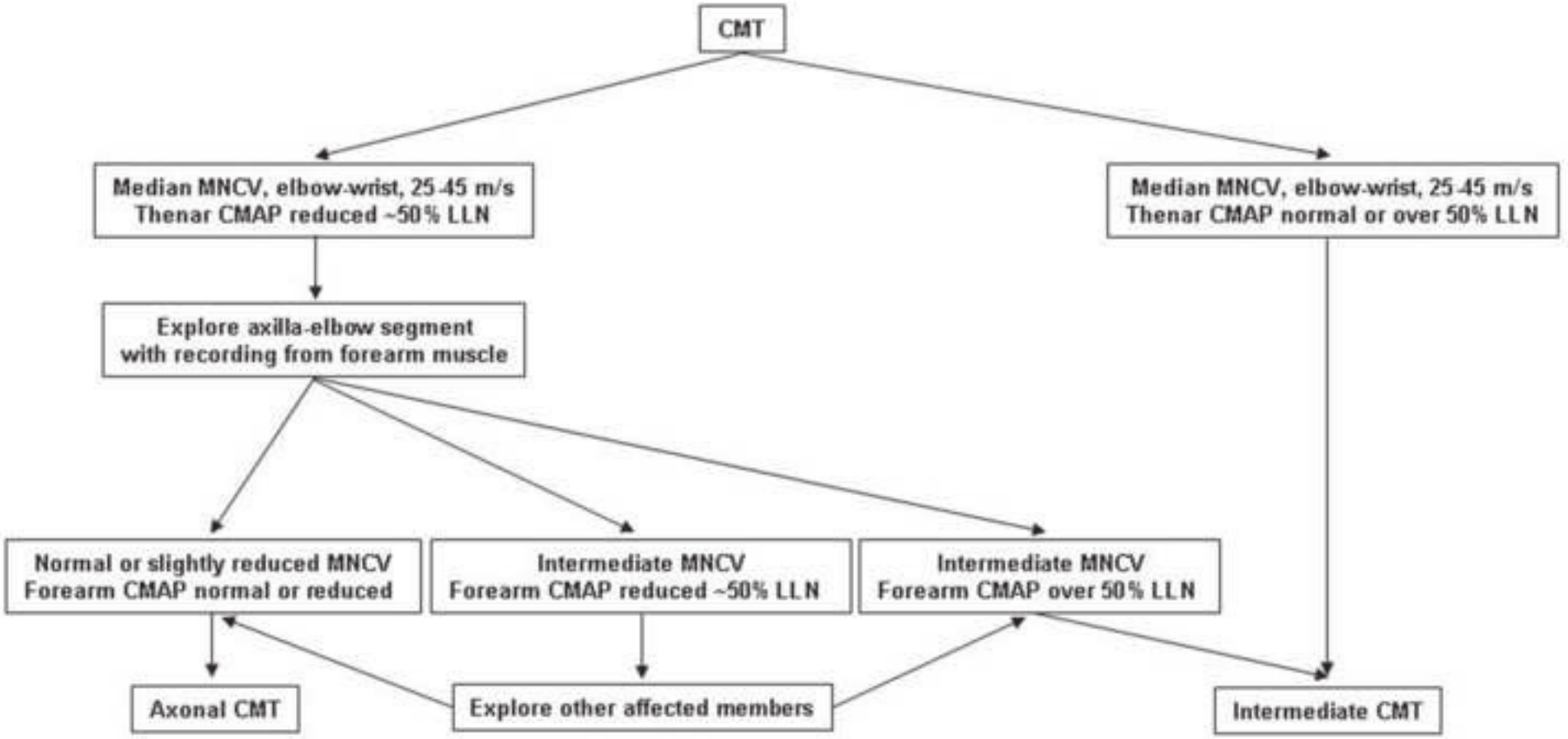

Table

Table 1. Classification of intermediate CMT

Transmission and types	Gene	Loci	OMIM number
X-linked			
CMTX1	*GJB1*	Xq13.1	302800
DRP2-disorder	*DRP2*	Xq22.1	Not yet assigned
Autosomal dominant			
DI-CMTA*	Not cloned	10q24.1	Not cloned
DI-CMTB	*DNM2*	19p13.2	606482
DI-CMTC	*YARS*	1p35.1	608323
DI-CMTD	*MPZ*	1q23.3	607791
DI-CMTE	*INF2*	14q32.33	614455
DI-CMTF	*GNB4*	3q26.33	615185
Other dominant types			
DI-CMT/NEFL	*NEFL*	8p21.2	Not yet assigned
DI-CMT/MNF2	*MNF2*	1p36.22	Not yet assigned
DI-CMT/AARS	*AARS*	16q22.1	Not yet assigned
DI-CMT/MD1	Not cloned	19q13.32	Not yet assigned
DI-CMT/DCTN2	*DCTN2*	12q13.3	Not yet assigned
Autosomal recessive			
RI-CMTA	*GDAP1*	8q21.11	608340
RI-CMTB*	*KARS*	16q23.1	613641
RI-CMTC	*PLEKHG5*	1p36.31	615376
RI-CMTD*	*COX6M*	12q24.31	616039

(*) Phenotypes that could be classified within CMT2 or AR-CMT2

Table

Table 2. Median nerve motor conduction parameters in CMTX1 males

Reference	No. patients electrophysiologically evaluated	MNCV m/s Range (Mean ± SD)	CMAP mV Range (Mean ± SD)	DML ms Range (Mean ± SD)	Comments
Phillips *et al*[44]	6	NR	NR	NR	Ulnar MNCV ranged from 33.1 to 44.7 (mean 38). Slowing was in parallel with loss of CMAP amplitude. Ulnar MNCV in 6 obligate heterozygous women ranged from 43.7 to 56.4 (mean 49.4)
Rozear *et al*[37]	4	28 – 37 (34.5)	NR	NR	Median MNCV in 6 obligate heterozygous women ranged from 38 to 53 (mean 50)
Hahn *et al*[38]	See comments	NR	NR	NR	Peroneal MNCV showed abnormalities in 28/31 cases (males and females). Modest reduction in MNCV paralleled changes in CMAP amplitudes for EDB and anterolateral leg muscles. Electrophysiology was normal in one affected male at age 3, who later on developed signs of the disease
Mostacciuolo *et al*[58]	41	NR	NR	NR	Mean ulnar MNCVs were 26 ± 5.9 in males and 38 m/s in females
Ionasescu *et al*[59]	9	NR	NR	NR	Electrophysiologic findings were consistent with both demyelination (slowing) and axonal denervation in all male patients
Nicholson and Nash[60]	15	(30.8 ± 5.9)	NR	NR	In comparison with CMT1A, mean median MNCVs were faster with an average of 10 m/s for males and 20 m/s for females
Oterino *et al*[61]	7	0 – 40 (24.2)	NR	NR	Only 2 out of 7 male patients remained asymptomatic but areflexic
Ionasescu *et al*[62]	9	NR	NR	NR	Ibid Ionasescu (1992). Prolonged DMLs (6-7 ms) were present. The CMT obligate female carriers showed normal electrophysiology
Birouk *et al*[63]	21	31 – 60 (36.2 ± 6.7)	0.3 – 6.3 (2.6 ± 1.8)	2.3 – 7 4.7 ± 1.1)	In 90% of CMTX1 males, median MNCV was intermediate ranging from 30 to 40 m/s. Only one male had normal MNCV. There was significant correlation between the decrease in CMAP amplitude and MNCV in the median, ulnar and peroneal nerves. In 27 obligate female carriers, median MNCVs ranged between 31 and 55 m/s (44 ± 7.3). Two children with the mutation, a 6-year-old boy and a 8-year-old girl, were normal clinically and electrophysiologically
Ainsworth *et al*[64]	1	NR	NR	NR	Ulnar MNCV was 34.8 m/s; CMAP was 3.83 mV
Sander *et al*[65]	4	25 – 40 (32)	NR	NR	Median MNCV in a female patient was 30 m/s
Senderek *et al*[52]	6	27.7 – 33.3 (31)	Reduced	NR	Median MNCV in 9 female patients ranged from 35 to 49 m/s (mean, 40)

Table 2. Median nerve motor conduction parameters in CMTX1 males (cont. 1)

Reference	No. patients electrophysiologically evaluated	MNCV m/s Range (Mean ± SD)	CMAP mV Range (Mean ± SD)	DML ms Range (Mean ± SD)	Comments
Hahn *et al*[40]	53	23 – 50 (34.5 ± 6.1)	0 – 12 (3.7 ± 3.7)	3 – 12 (5.4 ± 1.8)	Ten (34%) male patients showed CMAP amplitude ≤ 0.5 mV. F-wave latencies ranged between 38 and 53 ms (mean, 47). In 63 affected females, median MNCV ranged between 33 and 60 m/s (mean 45.8 ± 7.3); CMAP amplitudes ranged from 2 to 17 mV (7.8 ± 3.4); DML ranged from 3 to 5 ms (4.1 ± 0.6); and F-waves ranged from 30 to 36 ms (mean, 33)
Hahn *et al*[53]	1	27.9	4.9	4.8	This pedigree comprises two male patients. Ulnar MNCVs were 32.3 and 38.0 m/s; CMAPs were 3.8 and 6.6 mV; and DML were 4.5 and 5.0 ms. F-wave in one case was 42.8 ms
Gutiérrez *et al*[66]	3	25 – 54	0.045 – 2.2	3.8 – 6	F-wave latency recorded in one case was 32.1 ms. Terminal latency index was preserved. Ulnar MNCV ranged between 27 and 39 m/s
Dupré *et al*[67]	6	22 – 33 (29.0 ± 4.8)	0.3 – 6 (2.0 ± 2.2)	6.6 – 7.7 (7.0 ± 0.9)	One male patient showed no response. In six female patients median MNCV ranged from 41 to 57 m/s (49 ± 6). Three patients with reduced median MNCV had normal CMAP amplitudes
Nakagawa *et al*[68]	2	26.5 ; 30.1	0.1 ; 3.6	5.7 ; 5.5	F-wave latency, recorded in one case, was 48 ms. Ulnar MNCVs were 33.3 / 33.2; CMAP amplitudes were 4.0 / 3.4; DMLs were 4.5 / 5.0; and F-wave latencies were 48.2 / 47.8
Dubourg *et al*[69]	41	20 – 60 (34.5 ± 6.8)	0 – 8.6 (2.2 ± 2.1)	2.3 – 8.8 (4.9 ± 1.2)	In five male patients there was no response. In this comprehensive study the authors analyzed ulnar and peroneal MNCV, and included 52 female patients. In males mean MNCV in all three nerves ranged between 30 to 40 m/s, but were slightly below normal values in females. MNCV below 30 m/s were found in 18% of men but only in 5% of women
Hattori *et al*[70]	42	22.8 – 46.6 (33.2 ± 5.7)	0 – 8.1 (2.0 ± 1.8)	NR (5.3 ± 1.7)	Median MNCVs were discordant in terms of the 38 m/s cut-off value among siblings in the six families examined. CMAP amplitude significantly decreased with age and duration of illness
Capasso *et al*[71]	2	23; 31	0.8 ; 1.4	5.7; 6.2	The authors also studied ulnar MNCVs that were 36 m/s and 30 m/s; CMAP amplitudes were 5.3 and 5.2 mV; and DML were 3.0 and 3.7 ms. They considered that ulnar MNCV slowing with normal or relatively preserved CMAP amplitudes point to a demyelinating disorder
Houlden *et al*[72]	See comments	See comments	See comments	See comments	In male patients (5 according to their figure 1), ulnar MNCV ranged from 28 to 36 m/s (mean 32 ± 4.0), whereas in affected females (11) ranged from 34 to 55 m/s (46 ± 2.9). No data on CMAP amplitudes or DML are given

Table 2. Median nerve motor conduction parameters in CMTX1 males (cont. 2)

Reference	No. patients electrophysiologically evaluated	MNCV m/s Range (Mean ± SD)	CMAP mV Range (Mean ± SD)	DML ms Range (Mean ± SD)	Comments
Ryan and Jones[73]	1	38	3.87	5.6	Tabulated data are those of right median nerve. Left median MNCV was 41 m/s; CMAP was 7.55 mV, and DML was 3.9 ms, In this 13 year-old patient, there was response to immunoglobulin therapy
Vondracek *et al*[74]	1	33	5.9	4.8	Median MNCVs in the intermediate range were also found in two affected women
Liang *et al*[75]	0	0	0	0	The authors describe a 13-year-old girl with severe Dejerine-Sottas phenotype and MNCV of 18-20 m/s
Zhang *et al*[76]	20	10.7 – 45	NR	NR	In 18 affected females median MNCV ranged between 23 and 61 m/s
Huttner *et al*[77]	11	25 – 57 (42.1 ± 9.4)	0.5 – 11 (4.1 ± 3.1)	3.0 – 6.6 (4.9 ± 9.4)	There were no responses in one patient
Kleopa *et al*[55]	3	23; 38	0.19 ; 5.7	7.2 ,4.2	In one male patient median nerve showed no responses. Ulnar MNCV in all three patients were 32, 38, 38 m/s with CMAP of 0.87, 2.31 and 6.5 mV, respectively
Beauvais *et al*[78]	1	34	5.3	NR	One affected woman showed median MNCV of 34 m/s and CMAP of 5.3 mV
Vazza *et al*[79]	2	36 ; 31	0.3 ; 1.3	NR ; 5.9	One affected woman showed median MNCV of 38 m/s and CMAP of 8 mV
Shy *et al*[43]	73	See comments	See comments	See comments	This comprehensive study demonstrates important issues: i/ in all patients disability increased with age and loss of motor units; ii/ ulnar MNCVs (wrist-elbow) were predominantly in the intermediate range (30-50 m/s); iii/ occasional patients had forearm ulnar MNCV < 30 m/s, which was always associated with markedly reduced CMAP amplitudes; and iv/ most *GJB1* mutations cause neuropathty by a loss of normal connexin 32 function
Kim *et al*[80]	1	See comments	See comments	See comments	Ulnar MNCV was 34.8 m/s; CMAP was 4.1 mV; and DML was 3.9 ms
Vrancken *et al*[81]	2	33 ; 33	4.8 ; 7.9	4.7 ; 4.5	Ulnar MNCV showed similar parameters. In an affected woman median MNCV was 53 m/s; CMAP amplitude was 7.3 mV; and DML was 4.0 ms
Sakaguchi *et al*[82]	1	36.9	0.59	5.8	Ulnar nerve MNCV was 37 m/s with CMAP of 2.1 mV, and DML of 3.8 m/s. There was response to immunoglobulin therapy

Table 2. Median nerve motor conduction parameters in CMTX1 males (cont. 3)

Reference	No. patients electrophysiologically evaluated	MNCV m/s Range (Mean ± SD)	CMAP mV Range (Mean ± SD)	DML ms Range (Mean ± SD)	Comments
Yiu *et al*[83]	3 See comment	30 ; 40	NR	4.5	Electrophysiology is complemented by ulnar, peroneal and tibial MNCV. Intermediate MNCV, particularly in combination with axonal features, should raise the possibility of CMTX
Murphy *et al*[84]	3	32 – 42 (34.7)	0.5 – 0.9	NR	Median MNCV in three females ranged from 32 to 53.7 m/s (mean 44.9). Ulnar nerve was also evaluated
Chen *et al*[85]	2	32.8 ,28.5	3.5 ; 0.2	4.7 ; 3.5	F-wave latency in one patient was 43.8 ms. Ulnar nerve was also evaluated. In a female patient, median MNCV was 43.9 m/s with CMAP of 0.9 mV
Borgulová *et al*[86]	1	43.9	2.2	4.85	In another male patient peroneal MNCV was 39.8 m/s with CMAP of 3.3 mV. In three female patients median MNCV ranged between 40 and 49 m/s, CMAPs being normal or slightly reduced
Zhao *et al*[87]	1	33	7.7	2.7	In this 15-year-old patient contralateral median MNCV was 40 m/s with CMAP of 1.7 mV
Jerath *et al*[45]	20	21 – 44 (35 ± 1.5)	3 ± 0.6	5 ± 0.3	Median MNCV were normal in 4 (20%) male patients, intermediate (33-41 m/s) in 8 (40%), and slow (< 32 m/s) in 8 (40%)
Liu *et al*[88]	5	29.1 – 37.2	0.1 – 17.2	NR	Median MNCV were from 29.1 to 40 m/s in 5 out of the 31 proband male patients. CMAP amplitudes ranging from 0.1 to 17.2 mV. This comprehensive study comprises 226 CMT pedigrees, 31 (13.7%) being categorized as CMTX1, representing 65% of all intermediate CMT

NR= not recorded; SD= standard deviation; for the remaining abbreviations see text.