# New developments in Charcot–Marie–Tooth neuropathy and related diseases

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**Purpose of review**
Charcot–Marie–Tooth disease (CMT) and related neuropathies represent a heterogeneous group of hereditary disorders. The present review will discuss the most recent advances in the field.

**Recent findings**
Knowledge of CMT epidemiology and frequency of the main associated genes is increasing, with an overall prevalence estimated at 10–28/100 000. In the last years, the huge number of newly uncovered genes, thanks to next-generation sequencing techniques, is challenging the current classification of CMT. During the last 18 months other genes have been associated with CMT, such as *PMP2*, *MORC2*, *NEFH*, *MME*, and *DGAT2*. For the most common forms of CMT, numerous promising compounds are under study in cellular and animal models, mainly targeting either the protein degradation pathway or the protein overexpression. Consequently, efforts are devoted to develop responsive outcome measures and biomarkers for this overall slowly progressive disorder, with quantitative muscle MRI resulting the most sensitive-to-change measure.

**Summary**
This is a rapidly evolving field where better understanding of pathophysiology is paving the way to develop potentially effective treatments, part of which will soon be tested in patients. Intense research is currently devoted to prepare clinical trials and develop responsive outcome measures.

**Keywords**
Charcot–Marie–Tooth disease, clinical trials, next-generation sequencing, prevalence, therapy

## INTRODUCTION

Charcot–Marie–Tooth disease (CMT) and related neuropathies represent a heterogeneous group of hereditary disorders, with over 80 associated genes, many uncovered in the last few years thanks to next-generation sequencing (NGS) technology. We are progressively shedding light on the several different pathomechanisms underlying CMT, almost all leading to a length-dependent degeneration of sensory and/or motor fibres. Numerous promising compounds are under study in cellular and animal models, making it necessary to develop responsive outcome measures for this overall slowly progressive disorder. We discuss the most recent advances in the field.

## Epidemiology

Although CMT prevalence is estimated to be about 1:2500, with a worldwide distribution and no ethnic predisposition, epidemiological studies are still scarce, and knowledge of CMT frequency in different parts of the world remains extremely limited. It is difficult to assess the exact CMT prevalence because of the wide variation of clinical symptoms and the different disease forms, accounting for the high variability in prevalence rates in epidemiological studies. A recent systematic review [1] revised 12 studies mainly from Europe and reported a prevalence range of 9.37–20.1/100 000. In line with these data and according to a recent study in Ireland [2], the European prevalence rate is believed to be 10–28/100 000.

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CMT1A, associated with the peripheral myelin protein-22 gene (*PMP22*) duplication, is the most common CMT subtype and accounts for 60–70% of demyelinating patients with CMT1 (around 40–50% of all CMT cases). Mutations in the gap junction beta-1 gene (*GJB1*) causing CMTX1 result in approximately 10–20% of CMT cases and CMT1B associated with myelin protein zero gene (*MPZ*) mutations accounts for <5%. Patients with axonal CMT2 are about 20% of all cases [3,4]. Many studies found that about 90% of patients with genetically confirmed CMT diagnosis had a mutation in one of these four genes, *PMP22*, *GJB1*, *MPZ*, and either *MFN2* or *GDAP1*, according to the geographic area. The former is more frequent in North America and Northern Europe [5,6], the latter in the Mediterranean area [7,8].

These data justify a step-wise diagnostic algorithm based on phenotype, inheritance pattern, nerve conduction velocities, frequency of subtypes, and ethnicity. Although this approach is reasonably successful, with over 60% of patients with CMT achieving a genetic diagnosis, it is gradually being replaced by NGS techniques [9▪,10]. Actually, a practical approach is based on the screening of few frequent genes first (*PMP22*, *GJB1*, *MPZ*, *MFN2*, *GDAP1*, *HSPB1*, *HSPB8*), taking into account the clinical data, and then requires the use of NGS techniques. However, multigene panel testing or whole exome sequencing (WES) can be considered first-line in many circumstances, after initial targeted testing for the *PMP22* duplication in patients with demyelinating CMT. With the advent of WES, that allows the identification of new genes and the diagnosis of patients with rare and atypical conditions, the number of genes known to be associated with CMT is continuing to grow. Gene discoveries within the past two decades have challenged the simplistic classification of CMT, as for example we have reached and overcome the alphabet letters for CMT2. A novel classification based on inheritance pattern, nerve conduction values, and mutated gene has been proposed but needs further discussion and widespread agreement [11].

## Novel genes

Mostly thanks to NGS technology, during the last 18 months other genes have been associated with CMT (Table 1).

Peripheral myelin protein-2 (PMP2), a major protein in nerve compact myelin, belongs to the family of fatty acid binding proteins and is likely involved in intracellular trafficking of lipids. Recent papers [12,13▪,14▪▪] identified dominant *PMP2* missense mutations (p.Ile43Asn, p.Ile52Thr, p.Thr51Pro) in a highly conserved domain in four demyelinating CMT1 families. The clinical and electrophysiological phenotype was similar to CMT1A, and nerve biopsies showed demyelination and onion bulbs. *PMP2*-Ile43Asn transgenic mice replicated these neuropathic features, as did to some extent also those overexpressing wt-PMP2. Prevalence of *PMP2* mutations is probably low as, when extending investigations, they were found in only 1/104 genetically undiagnosed European CMT1 families.

The frequency of mutations in another recently identified gene, *MORC2*, encoding the microrchidia family CW-type zinc finger-2 protein, is higher as they were found in multiple axonal CMT2 families and sporadic cases from Spain, France, Germany, Czech Republic, Australia, Korea, and China [15▪▪,16▪▪,17–21]. More than 75% de-novo occurrence rate suggests the presence of hot spots, particularly for p.Arg190Trp, the most frequent among the seven missense reported variants. They are associated with a peculiar clinical picture, characterized by autosomal dominant inheritance, sensory-motor deficits, early onset, sometimes congenital, and muscle weakness involving not only distal but also proximal muscles and limb girdles, requiring aids for walking or confining patients to a wheelchair in an asymmetric progression over decades. Some patients have developmental delay and a SMA-like presentation, whereas others have later onset, up to the third decade, and much milder course. Variably associated findings include hearing loss, facial dysmorphism, pyramidal features, learning difficulties, microcephaly, seizures, respiratory involvement, cataract, retinal changes, cerebellar atrophy. Electrophysiology shows an axonal sensory-motor neuropathy, fasciculations and myokymias. Myelinated fibre loss is patchy and uneven at nerve biopsy,

**Table 1.** Recently identified genes and related proteins associated to CMT, hypothesized function and clinical phenotypes

| Novel or candidate CMT genes (protein) | Cytogenetic location (OMIM #) | Protein function | CMT type Inheritance | Clinical phenotype | Families/sporadic cases |
|---|---|---|---|---|---|
| PMP2 (peripheral myelin protein-2, P2) | 8q21.13 (170715) | Major protein component of peripheral myelin. Transport of fatty acids and lipid homeostasis. | CMT1 AD | Similar to CMT1A. Onset in 1st or 2nd decade; pes cavus, distal muscle atrophy and weakness, mild sensory loss; CMTNS 9–20; slowed MCV (15–23 m/s); onion bulbs. | Four families |
| MORC2 Microrchidia family CW-type zinc finger-2 | 22q12.2 (616661) | Chromatin remodeling after DNA damage, gene transcription, lipid homeostasis | CMT2Z AD | Variable onset (congenital to third decade) and usually severe progressive course. Early: 'SMA-like' weakness; distal and later proximal limb weakness, asymmetric, sensory loss. CMTNS 11–32 Frequent associated findings (see text). | Several families and sporadic cases from different countries |
| NEFH (neurofilament protein, heavy polypeptide) | 22q12.2 (162230) | Neurofilament component; maintenance of axonal caliber; mutations causes prominent toxic protein aggregates | CMT2CC AD | Onset age 2–38; variable severity (two chair bound in the 70s); predominantly lower limbs weakness and atrophy; nonlength dependent pattern with proximal involvement; panmodal sensory loss; increased CK (myopathic changes at EMG and muscle biopsy in 1); neurophysiological evidence of pyramidal involvement in 1; hearing loss in 2. | Three families |

**Table 1** (*Continued*)

| Novel or candidate CMT genes (protein) | Cytogenetic location (OMIM #) | Protein function | CMT type Inheritance | Clinical phenotype | Families/sporadic cases |
|---|---|---|---|---|---|
| MME (membrane metalloendopeptidase-Neprilysin) | 3q25.2 (120520) | Degradation of various neuropeptides and peptide hormones. The most important enzyme for β-amyloid in the CNS | CMT2 AR, AD | Late onset (30–80 yrs in het, 36–56 in hmz). Late-onset and slowly progressive typical CMT2 in AR cases. Heterogeneous phenotype in AD cases, with late-onset sensory-motor or motor neuropathy of variable severity in most instances, but also sensory ataxia with dystonia, dHMN, atypical ALS. | Multiple families and sporadic cases |
| SPG11 (spatacsin) | 15q21.1 (610844) | Axonal maintenance and cargo trafficking | CMT2X AR | Onset 4–35 yrs; pes cavus, distal muscle weakness and atrophy with lower limbs predominance; distal sensory loss; gait disturbance; tremor; mild cognitive impairment, thin corpus callosum or pyramidal signs in some patients. | Twelve families |
| DGAT2 (Diacylglycerol-O acyltransferase-2) | 11q13.5 (606983) | Synthesis and storage of intracellular triglycerides | CMT2 AD | Onset 1st decade; sensory ataxia, low triglyceride level; CMTNS 10. | One family |
| Large interchromosomal insertion | Xq27.1 (–) | – | CMTX3 XLR | Early onset; lower limbs predominance; sensory loss. Intermediate MCV. Slightly milder phenotype in comparison to CMTX1. | Two families |

AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; CK, creatine kinase; CMTNS, Charcot–Marie–Tooth neuropathy score; CNS, central nervous system; dHMN, distal hereditary motor neuropathy; EMG, electromyography; het, heterozygosity; hmz, homozygosity; MCV, motor conduction velocity; SMA, spinal muscular atrophy; XLR, X-linked recessive; yrs, years.

confirming the multifocal nature of this neuropathy. Mutations occur preferentially but not exclusively in the GHL-ATPase domain of this nuclear protein, which is believed to regulate chromatin remodeling during DNA repair and repress gene transcription.

An interesting disease mechanism was identified by Rebelo *et al.* [22■■] in two families with autosomal dominant axonal CMT2, carrying distinct frameshift mutations (p.Asp1004Glnfs*58, p.Pro1008Alafs*56) of the neurofilament heavy chain gene (*NEFH*). Both variants lead to loss of the terminating codon and translation of additional forty amino-acids containing cryptic amyloidogenic elements (CAE) in the untranslated 3'UTR. Such mutants caused prominent toxic protein aggregation in transfected cells and shorter axon lengths in zebrafish. A de-novo 13-bp tandem duplication in the same region, predicting a similar frame shift, stop loss (p.Lys1010Glnfs*57), and CAE translation, has been very recently described in a Chinese family, suggesting that this is a mutational hot spot and possibly a frequent CMT causative mechanism [23]. The phenotype is characterized by nonlength-dependent sensory-motor neuropathy with prominent lower limb involvement and proximal weakness, progressively leading to loss of independent walking; creatine kinase levels are increased, sometimes considerably, and one affected member showed EMG and muscle biopsy evidence of myopathy. Thigh and leg MRI in two patients showed early proximal muscle abnormalities and relative sparing of tibialis anterior muscle.

Missense and nonsense bi-allelic mutations in membrane metalloendopeptidase (*MME*) were found in a series of 10 Japanese patients with late-onset autosomal recessive CMT2 [24]. Their phenotype was otherwise typical with slow progression of distal sensory-motor deficits; nerve conduction studies and two nerve biopsies were consistent with an axonal neuropathy. Interestingly, *MME* heterozygous mutations were also found in European and North-American familial and sporadic patients with late-onset axonal neuropathy [25]; in this population, the phenotype was broad and more heterogeneous, ranging from subclinical neuropathy to severe CMT2, but including also sensory ataxia with dystonia, distal hereditary motor neuropathy with bulbar involvement, and atypical amyotrophic lateral sclerosis. *MME* encodes the metalloprotease neprilysin degrading β-amyloid; neprilysin tissue concentrations were reduced and enzymatic activity impaired in some investigated cases, but no patient had amyloid deposition or evidence of dementia. Further studies are awaited to confirm and clarify the *MME* role in late-onset neuropathies.

Mutations in the *SPG11* gene encoding spatacsin cause autosomal recessive spastic paraplegia with thin *corpus callosum* (TCC). Peripheral neuropathy is frequent particularly in advanced disease [26]. Montecchiani *et al.* [27] reported 29 affected individuals from 12 families harboring recessive *SPG11* mutations and prominent or isolated sensory-motor axonal neuropathy; only some of them had TCC, mild cognitive impairment, or pyramidal involvement.

*DGAT2*, encoding diacylglycerol O-acyltransferase-2 which catalyzes the final step of triglyceride biosynthesis, is another candidate CMT-gene as the p.Tyr223His variant was found in a Korean family with early-onset AD-CMT2 with sensory ataxia and low triglyceride levels [28].

By performing WES in two distantly related families, Brewer *et al.* [29■] has recently found that a 78kb-insertion at chromosome Xq27.1 is the genetic basis of CMTX3, a rare X-linked recessive sensory-motor CMT with intermediate NCV.

## Therapeutic advances and perspectives

Many promising compounds are being tested in cellular and animal models, and may reach the phase of assessing tolerability and effectiveness in clinical trials (Table 2) [4,30,31].

A phase III trial in CMT1A is in progress employing PXT3003, a mixture of low-dose sorbitol, naltrexone and baclofen, after encouraging results in the animal model and a phase II trial [32]. The rationale for using these compounds is that they should synergistically lower PMP22 overexpression. Other treatments aimed at PMP22 downregulation proved effective in preclinical studies, including progesterone antagonists. Gene silencing is a promising approach also in CMT, by lowering expression of wt-PMP22 or gain-of-function mutants employing antisense oligonucleotides, and small interfering or hairpin RNA (siRNA, shRNA). Lee *et al.* [33■] designed allele-specific siRNAs aimed at silencing the L16P-*Pmp22* mutant characterizing the Trembler-J (Tr$^J$) mouse, a model of CMT1E; siRNA treatment reduced L16P-*Pmp22* mRNA in Schwann cells *in vitro* and in Tr$^J$ mice; intraperitoneally treated Tr$^J$ mice improved in rotarod performance, NCV, CMAP amplitudes, muscle volume at MRI, and sciatic nerve myelination as compared to the placebo group.

A gene therapy approach has been attempted by intrathecal injection of *GJB1*, with a lentiviral vector and the myelin-specific *MPZ* promoter, in *Gjb1*-knocked-out mice lacking Cx32 expression; gene delivery appeared to result in stable Cx32 expression not only in lumbar roots Schwann cells, but also in

**Table 2.** Compounds under study for CMT and related neuropathies

| CMT type | Compound | Rationale | Preclinical studies | Translability to patients |
|---|---|---|---|---|
| CMT1A | Progesterone antagonists/modulators: onapristone, lonaprisan, ulipristal acetate | Downregulation of PMP22 overexpression | Onapristone effective in CMT1A rats | Onapristone toxic, lonaprisan has hormonal action, need to reduce side effects; trial with ulipristal acetate ongoing (NCT02600286, EudraCT2015–001716–36) |
| CMT1A | Soluble neuregulin 1 | Balancing PI3K–Akt and Mek–Erk signaling pathways | Effective in CMT1A rats | Already used in other diseases, but risk of side effects |
| CMT1A | PXT3003 (combination of low-dose baclofen, naltrexone, D-sorbitol) | Down-regulation of PMP22 overexpression | Encouraging results in cellular models and in CMT1A rats | Phase II concluded; phase III trial ongoing (NCT03023540) |
| CMT1A | P2X7 purinoceptor inhibitors | Reduce abnormal calcium influx into Schwann cells, mediated by P2X7 overexpression | Improvement of limb strength, distal motor latency and myelinated fibre number in CMT1A rats | To be considered for clinical trials in CMT1A |
| CMT1A | GABA-B receptor modulators | Downregulation of PMP22 overexpression | Tested in CMT1A rats | Baclofen contained in PXT3003 (see above) |
| CMT1A | Neurotrophin 3 (NT3) | Adeno-associated virus delivery of NT3, neurotrophic action | Encouraging results in Trembler J mice | Subcutaneous NT3 already tested in a pilot trial |
| CMT1A-CMT1E | Gene silencing (ASO, siRNA, shRNA) | Partial silencing of overexpressed (CMT1A) or mutated (CMT1E) PMP22 gene | Allele specific L16P pmp22 siRNA effective in Trembler J mice | To be considered |
| CMT1A | Starvation, rapamycin | Action on endoplasmic reticulum stress and/or autophagy | Effective in Trembler J mice | Rapamycin too toxic |
| CMT1A-CMT1B | Sephin 1 | UPR inhibition, GADD34 phosphatase inhibitor | Effective for CMT1B models | Possible |
| CMT1B | Curcumin | UPR inhibition | Effective in mouse models of CMT1B and CMT1E | Possible |
| CMT1B | Lecithin and other lipids | Improve impaired lipid biogenesis in Schwann cells? | Studies on CMT1A rats ongoing | Possible |
| CMT1B | Selective $Na_v1.8$ blockers (C31, others) | Detrimental ectopic $Na_v1.8$ expression in motor nerves in CMT1B models | Acute treatment with C31 effective in Mpz −/− and +/− mice | Possible |
| Hypermyelinating CMT (CMT4B, CMT4J), HNPP | Niacin/Niaspan | Activation of TACE, secretase of neuregulin 1–III | Effective in mouse models of CMT4B1 and HNPP | Possible for hypermyelinating neuropathies (i.e. CMT4B1-2, HNPP) |
| CMT4B, CMT4J | Apilimod | Inhibition of PIK fyve, enzyme in phosphoinositides metabolism where MTMR2 and FIG4 are active | Under investigation | Already used in phase II clinical trials for Crohn disease and Rheumatoid Arthritis |

**Table 2** (*Continued*)

| CMT type | Compound | Rationale | Preclinical studies | Translability to patients |
|---|---|---|---|---|
| CMTX1 | Gene therapy, lentiviral-GJB1 intratechal delivery | Gene delivery substituting lacking GJB1 | Effective in Cx32 KO mice by intrathecal administration | To be assessed |
| CMT1A - CMTX1 (all CMT) | ACE-083 | Locally acting myostatin inhibitor | Dose dependent increase in muscle mass and strength in various animal models | A Phase I trial concluded; phase II trial in preparation on CMT1A and CMTX1, NCT03124459 |
| CMT2 - dHMN (all CMT2-dHMN?) | HDCA6 (Histone deacetylase) inhibitors | Acetylation of α-tubulin improving axonal transport | Effective in mutant HSPB1 mice and iPSCs from HSPB1 patients | Possible |
| CMT2D | VEGF (vascular endothelial growth factor) | Antagonised aberrant interaction of mutated Gars to Neuropilin 1 receptor | Enhanced expression of VEGF improved motor function in CMT2D mice | To be assessed |
| HSAN1 | L-serine supplementation | Overcome SPTLC1-2 defect | Improved measures of motor and sensory performance and decrease of deoxysphingolipids levels in transgenic HSAN1 mice | Pilot study performed with decrease of deoxysphingolipids levels; further trials in preparation, NCT01733407 |

CMT, Charcot–Marie–Tooth; dHMN, distal hereditary motor neuropathy; HSAN1, hereditary sensory-autonomic neuropathy-1; PMP22, peripheral myelin protein-22.

sciatic nerves and trigeminal nerves, suggesting effective diffusion through CSF and then in the endoneurium of peripheral nerves. Treated mice improved rotarod motor performances, quadriceps muscle contraction, sciatic NCV; they had less abnormally myelinated fibres and macrophages in anterior lumbar roots than untreated mice [34▪]. Whether this approach is valid also for missense *GJB1* mutations remains to be established: Kyriakoudi *et al.* [35] showed that certain Golgi-retained Cx32 mutants interfere with exogenously delivered Cx32.

Fundamental issues to be considered before undertaking any gene therapy in CMT are safety of vectors and effective targeting of the Schwann cells or neurons.

Increased calcium influx in Schwann cell caused by *PMP22*-mediated P2X7 purinoceptor overexpression is a possible pathomechanism in CMT1A. Sociali *et al.* [36▪] tested the commercial P2X7-inhibitor A438079 in CMT1A rats; treatment decreased $Ca^{++}$ concentration and restored myelinated segments density in organotypic DRG cultures and ameliorated limb strength, distal motor latencies (but not NCV, CMAPs, and SAPs), and myelinated axons numbers in CMT1A rats. Expression levels of c-Jun and Ki67 were significantly decreased in treated mice nerves, indicating Schwann cell differentiation.

Calcium ions release from Schwann cell mitochondria mediated by the voltage-dependent anion channel-1 (VDAC1) appears to be another important factor in pathophysiology of demyelinating neuropathies. CMT1A rats treated for 15 or 30 days with the VDAC1-inhibiting agent TRO19622 improved in several function tests, showed increased CMAPs and NCV, and decreased g-ratios and demyelinated fibre number [37▪].

The important role of ions in demyelinating neuropathies is confirmed by Rosberg *et al.* [38▪], who demonstrated that in mice with partial or complete absence of *Mpz* (*Mpz*+/− and −/−) $Na_V1.8$ channels, specific of sensory neurons, are ectopically expressed in motor nerves, causing abnormal electrophysiological properties and axonal loss; acute oral treatment with the novel selective $Na_V1.8$ blocker C31 at age 1 and 4 months in mice lacking *Mpz* improved rotarod test, CMAP amplitudes and motor nerve excitability. Whether chronic treatment with sodium-channel blockers induces a stable improvement and plays a neuroprotective role by preventing axonal damage remains to be tested.

Neuregulin-1 III activity determines myelin thickness and is regulated by the secretases BACE1, which enhances myelination, and TACE, inhibiting myelination. Bolino *et al.* [39▪▪] elegantly showed

that increasing TACE activity with the commercially available niacin-Niaspan is of benefit in different models of hypermyelinating neuropathies: CMT4B1 associated with *MTMR2* recessive mutations and HNPP caused by *PMP22* deletion. Niaspan was able to decrease myelin outfoldings in *Mtmr2−/−* co-cultures and in sciatic nerves from *Mtmr2−/−* treated animals, and this effect was mediated by TACE. Moreover, Niaspan treatment reduced tomacula in *Pmp22+/−* animals.

An important therapeutic target is axonal transport, which likely plays a key role in several CMT subtypes. Acetylation of α-tubulin is important for binding of kinesins and dynein, molecular motor proteins, to microtubules and decreased acetylation influences axonal transport dynamics. Histone deacetylase-6 (HDAC6) is the major deacetylating enzyme of α-tubulin and the HDAC6-inhibitor Tubastatin A reversed motor and sensory deficits in the CMT2 mouse model associated with *HSPB1* mutations. ACY-738, ACY-775, and ricolinostat proved to be more potent and selective HDAC6-inhibitors when testing candidates for their efficiency to: acetylate α-tubulin in a neuronal cell line, rescue mitochondrial axonal transport defects in cultured DRG neurons from mutant *HSPB1* mice, and restore motor and sensory problems in these mice *in vivo* [40▪]. Moreover, the newly developed HDAC6-inhibitors, CHEMICAL X4 and X9, increased α-tubulin acetylation and improved axonal movement defects of mitochondria in iPSCs differentiated into motoneurons from two patients with different *HPSB1* mutations [41].

While waiting for effective drug or gene therapies, the only treatment options are symptomatic drugs, physiotherapy, orthotics use, and surgery for skeletal deformities. A European Neuro-Muscular Centre workshop on foot surgery in CMT took place in 2016 and the report is in preparation. Pazzaglia *et al.* [42] tested a novel approach to improve balance in 14 patients with CMT1A, by means of a 3-day treatment with focal mechanical vibration, a selective stimulus for Ia spindle afferents, on quadriceps and triceps surae muscles. Single-blind assessment after 1 month showed significant amelioration in balance and gait scores; stabilometric variables in the eye-closed condition went better, whereas other measures of strength, walking ability and quality of life did not change. Such encouraging results need confirmation on larger series.

## Outcome measures

As novel treatments are approaching, a parallel research line is aimed at preparing clinical trials and finding responsive outcome measures to detect treatment efficacy in a reasonable time-period and with feasible sample sizes. Attempts at improving clinical outcome measures have been carried out during the last years, and CMTNS and CMTPedS are considered the composite outcome measures to be used for clinical trials in adults and children, respectively. The CMTPedS is designed according to Rasch methodology [30,43▪▪], whereas a Rasch version of the CMTNS has been later developed to obtain a more linear measure [30]; both scales are being tested in longitudinal studies. Wang and co-workers [44] also performed, in their patient series, a Rasch analysis of the CMTNS, which overall proved more suitable for moderate-to-severe forms, and suggested considering additional items and/or categories for mild-to-moderate patients. The Italian CMT network [45] tested novel outcome measures in 168 CMT patients and obtained encouraging results for the 6-min walking test, as a measure of walking ability and endurance, which highly correlated with other clinical measures, and the Step-Watch activity monitor, as indicator of motor activity in a prolonged time set, which correlated rather with quality of life. A major advance in this field has been brought by quantitative muscle MRI employing Dixon sequences, which enables to precisely and reliably measure thigh and leg muscle atrophy and denervation-related fatty substitution. Morrow *et al.* [46▪▪] showed that in 19 CMT1A patients fatty substitution correlated with clinical impairment and loss of strength, thus providing a measure of disease severity, and significantly progressed in the leg over a 1-year period; currently, this is the most responsive measure available for CMT.

Nerve [47,48] and muscle [49] ultrasound echography and MR neurography [50] are providing accurate images in different peripheral neuropathies including CMT and might become useful not only for diagnostics but also for follow-up studies. Meaningful biomarkers are actively searched for in skin biopsies, blood, and CSF and hopefully will be found in the next future [51]. An example of the importance of careful clinical trial preparation is provided by hereditary sensory-autonomic neuropathy-1 associated to serine palmitoyltransferase subunits-1 and serine palmitoyltransferase subunits-2 (*SPTCL1*, *SPTCL2*) mutations, where oral administration of L-serine overcomes the metabolic defect and lowers neurotoxic deoxysphingolipids (dSLs) levels; dSLs are suitable blood biomarkers, but it is also important to select appropriate clinical and paraclinical measures, based on natural history studies [52].

## CONCLUSION

CMT is a rapidly evolving field where better understanding of pathophysiology is paving the way to

develop potentially effective treatments, part of which will soon be tested in patients. Intense efforts of collaborative networks of researchers are currently devoted to prepare clinical trials and develop responsive outcome measures and thus far achieved results are promising.

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## Conflicts of interest

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