REVIEW ARTICLE

Guidelines for molecular diagnosis of Charcot-Marie-Tooth disease

J. Berciano a, *, T. Sevilla b, C. Casasnovas c, R. Sivera b, J.J. Vilchez b, J. Infante a, C. Ramón d, A.L. Pelayo-Negro a, I. Illa e, Programme 3 (Neuromuscular Diseases), Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III

a Servicio de Neurología, Hospital Universitario Marqués de Valdecilla (IFIMA), Universidad de Cantabria, CIBERNED, Santander, Spain
b Servicio de Neurología, Hospital Universitari la Fe, CIBERNED, Valencia, Spain
c Servicio de Neurología, Centre per el Diagnòstic Genètic i Molecular de Malalties Hereditaries, Institut de Investigacions Biomèdiques de Bellvitge (IDIBELL), Barcelona, Spain
d Servicio de Neurología, Hospital Universitario Central de Asturias, Oviedo, Spain
e Servicio de Neurología, Hospital de la Santa Creu i Sant Pau, CIBERNED, Barcelona, Spain

Received 13 April 2011; accepted 14 April 2011

KEYWORDS
Axon; Charcot-Marie-Tooth disease; Clinical guideline; Dejerine–Sottas disease; Genetic counselling; Gene mutation; Genetic neuropathy; Molecular diagnosis; Myelin; Motor nerve conduction velocity

Abstract

Introduction: Charcot-Marie-Tooth disease (CMT) is the most frequent form of inherited neuropathy. In accordance with the inheritance pattern and degree of slowing of motor conduction velocity (MCV) of the median nerve, CMT encompasses five main forms: CMT1 (autosomal dominant [AD] or X-linked transmission and MCV < 38 m/s); CMT2 (AD or X-linked transmission and MCV > 38 m/s); CMT4 (autosomal recessive [AR] and severe slowing of MCV); AR-CMT2 (AR transmission and MCV > 38 m/s); and DI-CMT (intermediate form with AD transmission and MCV between 30 and 40 m/s). In spite of its stereotyped semiological repertoire (basically, symptoms and signs of sensory-motor polyneuropathy and pes cavus), CMT seems to be one of the most complex hereditary neurodegenerative syndromes, 31 causative genes having been cloned.

Development: This paper is aimed at performing a nosological review of the disease, emphasising the guidelines for its molecular diagnosis. Genetic epidemiological studies and genotypes reported in Spanish patients are revised.

Conclusions: In the great majority of CMT cases, mutations involve a reduced number of genes, namely: for CMT1, PMP22, GJB1 and MPZ; for CMT2, MFN2 and GJB1; for CMT4, GDAP1, and NDRG1, HK1 and SH3TC2 (gypsies); for AR-CMT2, GDAP1; and for DI-CMT, GJB1 and MPZ. Given their low prevalence, mutations in other pathogenic genes should be investigated after discarding the previous ones. There is no place for the indiscriminate use of diagnostic CMT genetic panels.

© 2011 Sociedad Española de Neurología. Published by Elsevier España, S.L. All rights reserved.
Guía diagnóstica en el paciente con enfermedad de Charcot-Marie-Tooth

Introducción: La enfermedad de Charcot-Marie-Tooth (CMT) es la neuropatía hereditaria más frecuente. Clásicamente dividida según su patrón de herencia y de alteración de la velocidad de conducción motora (VCM) del nervio mediano, CMT incluye cinco grandes categorías: CMT1 (herencia autosómica dominante [AD] o ligada al sexo, y VCM < 38 m/s); CMT2 (herencia AD o ligada al sexo y VCM > 38 m/s); CMT4 (herencia autosómica recesiva [AR] y VCM muy lentificada); AR-CMT2 (forma recesiva con VCM > 38 m/s), y DI-CMT (forma intermedia con herencia AD y VCM entre 30 y 40 m/s). Pese a su estereotipado cuadro clínico (básicamente, semiólogía polineuropática sensitivo-motora y pie cavo), CMT ha resultado ser uno de los síndromes neurodegenerativos genéticamente más complejos, con 31 genes patogénicos clonados.

Desarrollo: El objetivo de esta guía es efectuar una revisión nosológica de la enfermedad de CMT, con énfasis en las directrices para llevar a cabo el diagnóstico molecular. A tal fin, revisamos los estudios de epidemiología y genética, y los genotipos descritos en España.

Conclusiones: En la inmensa mayoría de los pacientes con CMT, las mutaciones recaen en un reducido número de genes: para CMT1, PMP22, GJB1 y MPZ; para CMT2, MFN2 y GJB1; para CMT4, GDAP1, y NDRG1, HK1 y SH3TC2 (sujetos de etnia gitana); para AR-CMT2, GDAP1, y para DI-CMT, GJB1 y MPZ. Por su baja prevalencia, las mutaciones en otros genes sólo deberían investigarse cuando las anteriores han sido descartadas. Se desaconseja el uso indiscriminado de paneles de múltiples genes para el diagnóstico molecular de la enfermedad.

© 2011 Sociedad Española de Neurología. Publicado por Elsevier España, S.L. Todos los derechos reservados.
It is somewhat ironic that, despite the apparent simplicity of its semiotic repertoire, CMT has emerged as one of the most genetically complex neurological syndromes. **Table 1**, adapted from references 4, 7 and 8, shows an updated clinical-genetic classification of CMT, which is tentative since there is no unanimous view on the use of its types and subtypes. There is universal agreement in accepting CMT1 as a header for demyelinating phenotypes with AD inheritance. Meanwhile, some authors include axonal forms with AD or AR inheritance in CMT2, while others include only AD forms, thus creating the acronym AR-CMT2 for axonal forms with AR transmission. We have followed this approach. The acronym CMT3, applied in the Dyck classification to syndromes similar to that described by Dejerine and Sottas, disappears and is replaced by CMT4, which encompasses all demyelinating syndromes with AR inheritance. In short, the acronym DI-CMT is introduced for intermediate forms with AD transmission.

**Table 1** illustrates the location of the mutated proteins, which was predictable for those known components of the PNS, such as proteins PMP22 and MPZ (P0) of compact myelin. However, in other cases the discovery of the mutated pathogenic protein proved unexpected. Example is illustrated by the case of GDAP1, whose function in the PNS was unknown until the identification of CMT4A took place. From an educational point of view and according to Niemann et al, the aetiopathogenetic mechanisms of the mutated proteins are summarised as follows: a) alteration of the development and maintenance of myelin; b) alteration of the biosynthesis and degradation of proteins; c) alteration of the endocytosis and dynamics of membranes, including the mitochondrial membrane; d) alteration of the axonal cytoskeleton; e) seipinopathies, and f) channelopathies by mutation of TRPV4. We will briefly review these sections. In CMT1/CMT4 forms by mutation of certain myelin components, it is assumed that the Schwann cell defect causes demyelination/dysmyelination with secondary axonopathy, which is ultimately responsible for the clinical semiology. The most common syndrome in this section is CMT1A, which accounts for 55% of all CMT cases and 66.8% of CMT cases, and which is usually caused by an allelic trisomy of 17p11.2 of 1.5 Mb, containing the gene PMP22. This trisomy causes an excessive gene dosage, leading to overproduction of PMP22 and its accumulation in Schwann cells, inducing endoplasmic reticulum stress, resulting in programmed cell death. Death occurs by reducing the expression of PMP22, producing unstable myelin which is manifested as hereditary neuropathy with liability to pressure palsies (HNPP) (**Table 1**). In a small percentage of cases, duplication/deletion can occur as a *de novo* phenomenon. Other point mutations (e.g. single-base substitutions) of the PMP22 gene are rare and cause severe phenotypes, either AD (probably through a function gain mechanism) or AR (loss of function due to failure in the synthesis of PMP22). Protein MPZ is quantitatively the most abundant within compact myelin and an essential element for its compaction. In 10% of cases, CMT is caused by point mutations of MPZ resulting in either a demyelinating, early-onset AD phenotype (CMT1B) or, exceptionally, an AR phenotype, or else in an axonal, late-onset phenotype (CMT2 and CMT2J). Thus, the molecular pathology of PMP22/MPZ unveiled that their mutations can be inherited through both AD and AR transmission, and that, in the case of MPZ, its mutations cause both a demyelinating and axonal phenotype. This only highlights the fact that communication between Schwann cells and accompanying axons within the PNS is continuous. Such phenomena are applicable to mutations causing CMT in other genes (**Table 1**). GB11 (Cx32) is a gap-type protein of paroxial myelin, whose gene is located on chromosome X. Point mutations in the GB11 gene are the second most common cause of CMT and cause dysfunction in the radial transit of small molecules between Schwann cells and axons. Probably by a haploinsufficiency mechanism, such mutations cause a more severe phenotype in males than in females, which neurophysiologically can be demyelinating, axonal or intermediate. Other, rarer causes of CMT1/CMT4 include mutations in the EGR2 gene (which encodes a transcription factor involved in regulating myelin genes) and the PRX gene (which encodes a cytoskeletal anchoring protein of Schwann cells).

The correct composition and maintenance of the membranous compartments of Schwann cells and PNS neurons depend on a perfect balance between the synthesis of structural and signalling components and their degradation processes. Among the mutated proteins involved in endocytosis processes are the following (**Table 1**): a) phosphatases (MTMR2, MTMR13 and FIG4), which cause severe AR phenotypes (CMT4B1, CMT4B2 and CMT4J) with focal myelin folds (CMT4B1 and CMT4B2); b) GTPases: DNM2 with an AD phenotype that can be either intermediate (DI-CMTB) or axonal, RAB7 which causes CMTZB (a phenotype similar to that of HSNA1), and FRABIN which is associated with CMT4H. Mutations of NDRG1, a regulatory gene whose function is poorly known, cause a severe syndrome (CMT4D) in subjects from the Romani ethnic group. With regard to the components involved in the synthesis, classification and degradation of proteins, the mutations affect the following components: a) LITAF/SIMPLE, an ubiquitin ligase, which causes CMT1C, and b) GARS, YARS and AARS, proteins involved in the loading of tRNA with glycine, tyrosine and alanine, which originate CMT2D/dHMN-V, DI-CMT and CMT2N, respectively (**Table 1**).

PNS neurons, both sensory and motor, must move proteins, vesicles and organelles through the long axonal stretches ranging from the soma to their terminals, and so require a complex and efficient transport system. Thus, the growing number of axonal CMT forms caused by mutations in proteins related to the cytoskeleton and to protein, vesicle and organelle transport (**Table 1**) should not be surprising. Mutations in the neurofilament light chain (NFL) cause CMT2E and, exceptionally, CMT1F. Heat shock proteins (HSP) are ubiquitous macromolecules which, in the PNS, control the assembly of neurofilaments. Mutations of the HSP27 gene cause CMTZF/dHMN-II, while mutations in the HSP22 gene are associated with CMT2L/dHMN-II. Recently, a CMT line associated with the mutation HSP27 R127W, comprised by 10 patients who had been examined clinically and neurophysiologically, presented some cases with CMT2 phenotype and others with HMN phenotype. This highlights the fact that both syndromes may be a single nosological entity. Kinesins are a family of motor proteins which mediate in anterograde axonal transport on microtubules, while dyneins mediate retrograde transport. Mutations of the KIF1B gene are
<table>
<thead>
<tr>
<th>Type</th>
<th>Gene or locus</th>
<th>Specific phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMT1A</td>
<td>PMP22 duplication</td>
<td>Classical form of CMT1</td>
</tr>
<tr>
<td>CMT1B</td>
<td>MPZ (P0)</td>
<td>Classical CMT1/DSD/CHN/Intermediate/CMT2</td>
</tr>
<tr>
<td>CMT1C</td>
<td>LITAF</td>
<td>Classical CMT1</td>
</tr>
<tr>
<td>CMT1D</td>
<td>EGR2</td>
<td>CMT1/DSD/CHN</td>
</tr>
<tr>
<td>CMT1 (no letter assigned)</td>
<td>NEFL</td>
<td>Usually CMT2, but a severe form with low MCV has been described</td>
</tr>
<tr>
<td>HNPP</td>
<td>PMP22 deletion</td>
<td>Hereditary neuropathy with liability to pressure palsies</td>
</tr>
<tr>
<td>CMT2A1</td>
<td>KIF1Bβ</td>
<td>Classical CMT2 (without nerve thickening)</td>
</tr>
<tr>
<td>CMT2A2</td>
<td>MFN2</td>
<td>CMT2 with optic atrophy</td>
</tr>
<tr>
<td>CMT2B</td>
<td>RAB7</td>
<td>CMT2 with sensory predominance</td>
</tr>
<tr>
<td>CMT2C</td>
<td>TPRV4</td>
<td>CMT2 with motor predominance/distal SMA/scapulo-peroneal atrophy</td>
</tr>
<tr>
<td>CMT2D</td>
<td>GARS</td>
<td>CMT2 with predominant involvement of hands (dHMN-V)</td>
</tr>
<tr>
<td>CMT2E</td>
<td>NEFL</td>
<td>Classical CMT2 (exceptionally CMT1)</td>
</tr>
<tr>
<td>CMT2F</td>
<td>HSP27 (HSPB1)</td>
<td>Classical CMT2 or dHMN-II</td>
</tr>
<tr>
<td>CMT2G</td>
<td>12q13.2</td>
<td>Classical CMT2</td>
</tr>
<tr>
<td>CMT2J/CMT2J</td>
<td>MPZ</td>
<td>Late onset, classical CMT2 with Adie pupil/intermediate</td>
</tr>
<tr>
<td>CMT2K</td>
<td>GADAP1</td>
<td>Usually CMT4A or AR-CMT2K</td>
</tr>
<tr>
<td>CMT2L</td>
<td>HSP22 (HSPB8)</td>
<td>Classical CMT2 or dHMN-II</td>
</tr>
<tr>
<td>CMT2N</td>
<td>DNAM2</td>
<td>CMT2 with classical/intermediate CMT2</td>
</tr>
<tr>
<td>CMT2N</td>
<td>AARS</td>
<td>Classical CMT2</td>
</tr>
<tr>
<td>CMT2 (HMSNP)</td>
<td>3q</td>
<td>Classical CMT2 with proximal weakness</td>
</tr>
<tr>
<td>CMT4A</td>
<td>GDAP1</td>
<td>Severe CMT1 phenotype with diaphragm and vocal cord paralysis</td>
</tr>
<tr>
<td>CMT4B</td>
<td>MTMR2</td>
<td>Severe CMT1 phenotype with bulbar paralysis and focal myelin folding</td>
</tr>
<tr>
<td>CMT4B2</td>
<td>MTMR13</td>
<td>Severe CMT1 phenotype with glaucoma and focal myelin folding</td>
</tr>
<tr>
<td>CMT4C</td>
<td>KIAA1985 (SH3TC2)</td>
<td>Severe CMT1 phenotype with scoliosis (Romani ethnic group)</td>
</tr>
<tr>
<td>CMT4D (HMSNL)</td>
<td>NDRG1</td>
<td>Severe CMT1 phenotype with hearing loss and lingual atrophy (Romani ethnic group)</td>
</tr>
<tr>
<td>CMT4E</td>
<td>EGR2</td>
<td>Classical CMT1/DSD/CHN</td>
</tr>
<tr>
<td>CMT4F</td>
<td>PRX</td>
<td>CMT1 with prominent sensory semiology and focal myelin folding</td>
</tr>
<tr>
<td>CMT4H</td>
<td>FGD4</td>
<td>Classical CMT1</td>
</tr>
<tr>
<td>CMT4I</td>
<td>FIG4</td>
<td>Classical CMT1</td>
</tr>
<tr>
<td>HMSN-Russe</td>
<td>CTDP1</td>
<td>CMT1 with dysmorphic signs in the Romani ethnic group</td>
</tr>
<tr>
<td>CMT4 (no letter assigned)</td>
<td>HK1</td>
<td>Classical CMT1/DSD/CMT2/Intermediate</td>
</tr>
<tr>
<td>CMT4 (no letter assigned)</td>
<td>Other PMP22 point mutation</td>
<td>Classical CMT1/DSD/CMT2/Intermediate</td>
</tr>
<tr>
<td>AR-CMT2A</td>
<td>LMNA</td>
<td>Severe CMT2 with proximal musculature involvement</td>
</tr>
<tr>
<td>AR-CMT2B</td>
<td>19q13.1-13.3</td>
<td>Classical CMT2</td>
</tr>
<tr>
<td>AR-CMT2 (CMT2K)</td>
<td>GADAP1</td>
<td>Similar to CMT4A</td>
</tr>
<tr>
<td>AR-CMT2 (no letter assigned)</td>
<td>NEFL</td>
<td>Severe CMT2</td>
</tr>
<tr>
<td>CMT5 linked to chromosome X</td>
<td>GJB1 (Cx32)</td>
<td>CMT1/CMT2/Intermediate (subclinical involvement in females)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 additional loci identified (CMT2X-5)</td>
</tr>
</tbody>
</table>

Intermediate CMT with AD inheritance

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene or locus</th>
<th>Specific phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-CMT7A</td>
<td>1q24.1-25.1</td>
<td>Classical CMT1 (without nerve thickening)</td>
</tr>
<tr>
<td>Di-CMTB</td>
<td>1q24.1-25.1</td>
<td>Classical CMT1 with cataract and neutropenia</td>
</tr>
<tr>
<td>Di-CMTD</td>
<td>YARS</td>
<td>Classical CMT1</td>
</tr>
<tr>
<td>Di-CMTD</td>
<td>MPZ</td>
<td>Classical CMT1</td>
</tr>
</tbody>
</table>

AARS: alanyl tRNA synthetase; CHN: congenital hypomyelinating neuropathy; CMT: Charcot-Marie-Tooth disease; CTDP1: CTD phosphatase subunit 1; DNAM2: dynamin 2; DSD: Dejerine–Sottas disease; EGR2: early growth response 2; FGD4: RhoGEF; FIG4: PtdIns(3,5)P2 5-phosphatase; GARS: glycy l tRNA synthetase; GJB1: gap junction protein beta 1; GDAP1: ganglioside induced differentiation associated protein 1; HK1: hexokinase 1; HMSNL: hereditary motor and sensory neuropathy Lom; HNPP: hereditary susceptibility pressure palsy; HSP22: heat shock 22kDa protein; HSP27: heat shock 27kDa protein; KIF1Bβ: kinesin family member 1-Bβ; LITAF: lipopolysaccharide induced tumour necrosis factor; LMNA: lamin A/C; MFN2: mitofusin 2; MTMR2: myotubularin related protein 2; MTMR13: myotubularin related protein 13; NDRG1: N-myc downstream regulated gene; NEFL: neurofilament light polypeptide 68kDa; PMP22: peripheral myelin protein 22; P0: myelin protein zero; PRX: peroxin; RAB7: member RAS engulfment family; SH3TC2: SH3 domain and tetraticopeptide repeats; SMA: spinal muscular atrophy; TPRV4: transient receptor potential vallinoid 4; YARS: tyrosyl tRNA synthetase.

See http://neuromuscular.wustl.edu/time/hmsn.html.
associated with CMT2A1 and mutations of the RAB7 gene, encoding a GTPase which regulates dynein function, cause CMT2B. Mitochondrial morphology is determined by a balance between fusion and fission processes of the organelle. MFN2 is a GTPase of the mitochondrial outer wall, where it acts as a regulator of mitochondrial fusion. Point mutations in the MFN2 gene cause CMT2A2, which is currently the most common form of CMT2 (20%), with one fifth of cases appearing as de novo mutations. MFN2 mutations participate in mitochondrial fission processes. Homozygous mutations of GADD1 are cause either CMT4A or AR-CMT2. Exceptionally, certain mutations in this gene cause disease in a heterozygous state (CMT2K). The LMNA gene encodes a nuclear membrane protein whose mutation is associated with AR-CMT2A; it is interesting to note that mutations in this gene can cause Emery-Dreifuss myopathy. The KIAA1985/SH3TC2 gene encodes an adapter protein and its mutations cause a severe phenotype (CMT4C) (Table 1). BSCL2 is an acronym derived from Berardinelli-Seip congenital lipodystrophy 2, a syndrome originally described in strains with lipoatrophy, insulin resistance, hypertriglyceridemia, mental retardation and AD inheritance. BSCL2 or seipin is a glycosylated protein of the endoplasmic reticulum, whose mutations activate the UPR pathway (unfolded protein response), inducing endoplasmic reticulum stress and programmed cell death. Seipinopathies are considered a new model of disease by alteration of protein conformation. Point mutations of BSCL2 cause a continuous spectrum of neurodegenerative syndromes with AD transmission, including dHMN-V, Silver syndrome (spastic paraparesis and hand amyotrophy), CMT2 and hereditary spastic paraparesis. In a considerable percentage of cases, the mutation displays incomplete penetrance.

TRPV4 is a nonselective cation channel involved in the detection of physical and chemical stimuli which take part in multiple physiological functions. Heterozygous mutations of the TRPV4 gene have been associated with bone dysplasias. Genetic linkage analysis has shown that CMT2C, the scapulo-peroneal form of spinal muscular atrophy (SMA) and the congenital, distal form of SMA could be allelic syndromes (12q21-q24). Indeed, recent studies have shown that these syndromes are associated with different, heterozygous, point mutations in the ankyrin domain of TRPV4, sometimes with incomplete penetrance. The mechanism by which these mutations cause degeneration of the PNS is unknown. In any case, the disease is a prototypical example of variable inter- and intrafamilial expression.

Clinical diagnosis of Charcot-Marie-Tooth disease

The first step is to establish whether the patient suffers a hereditary neuropathy. The answer may be obvious when the family survey shows a lineage with affected ancestors, suggesting AD or gender-linked inheritance (when there...
is no male-to-male transmission). The occurrence of disease among siblings and parental consanguinity suggest an AR inheritance. However, sometimes the family survey is negative, in which case there are a number of factors which may point towards genetic neuropathies, namely: a) onset during childhood; b) prolonged and slowly progressive clinical course; c) presence of pes cavanus, and d) unlike in acquired neuropathies, absence of positive sensory symptoms (paraesthesias or dysaesthesias) despite a clear semiology of sensory deficit. Since affected individuals often present subtle symptoms or are even asymptomatic, it is also important to test the maximum possible number of subjects at risk within the lineage (secondary cases). This will enable detection of minimal signs of disease (e.g. arreflexia or pes cavanus) in subclinical cases and, thus, help define the hereditary pattern more accurately. Examination of the musculature of the lower limbs using MRI has helped to detect early signs of fat atrophy in the intrinsic muscles of the feet.

The next step should be a neurophysiological examination, including determination of the MCV and SCV (sensory conduction velocity) in at least 3 nerves. Following the guidelines outlined by the Charcot-Marie-Tooth Neuropathy Score (CMTNS) is advisable. The amplitude of compound motor action potentials (CMAP) should be taken into consideration when interpreting the degree of slowness in the MCV. A sharp drop in distal CMAP amplitude implies a loss of thick fibres depending on the distance, and this may involve a proportional reduction in the MCV. Examination of proximal nerve segments is recommended, in order to discern between MCV drops due to axonopathy or to myelopathy. Conduction at these points will be similarly slowed down in cases of demyelinating CMT and less slowed down or even preserved in cases of axonal CMT. In intermediate forms, the MCV is usually preserved, in contrast to cases of acquired inflammatory neuropathy. In some authors) in nerve trunks with both reduced CMAP (usually distal segments) and preserved CMAP (usually proximal segments). In other words, the causative gene mutation acts by originating a dysfunction of both the axon and the Schwann cell.

Currently, nerve biopsy is reserved for cases where there are problems in the differential diagnosis with other hereditary neuropathies (e.g. amyloidosis) or with acquired neuropathies.

**Molecular diagnosis of Charcot-Marie-Tooth disease**

Out of over 30 pathogenic genes identified so far (Table 1), only a small and variable proportion of them are available in Spain for study in molecular genetics laboratories, either from public hospitals or private entities. The cost of molecular diagnosis in our environment is covered by the Ministry of Health through the Cohesion Fund Information System (SIFCO). Given the enormous and growing number of pathogenic genes involved in CMT and the considerable cost of molecular studies, it is clear that genetic testing must be specifically targeted. A recent communication from the American Academy of Neurology recommends that the molecular diagnosis of CMT be carried out based on the clinical phenotype, inheritance pattern and neurophysiological findings, starting with the analysis of PMP22 duplication/deletion in the case of demyelinating phenotypes with AD inheritance or GJB1 or MFN2 mutations in the case of axonal phenotypes with vertical inheritance.

Given the enormous molecular complexity of CMT, genetic epidemiology studies are scarce. We will refer to the work of Saporta et al, as it is the most recent, extensive, and detailed.

These authors reviewed cases referred to their monographic CMT consultation between 1997 and 2007. Their results included 1024 patients, of whom 787 were diagnosed with CMT. A total of 527 patients (67%) were categorised genetically and the remaining 260 were not categorised as CMT. Among the 527 cases defined genetically, the most common subtypes were: CMT1A (55%; PMP22 duplication), CMT1X (15.2%; GJB1 mutation), HNPP (9.1%; PMP22 deletion), CMT1B (8.5%; MPZ mutation) and CMT2A (4%; MFN2 mutation). Other mutations were detected in 23 patients (4.4% of total CMT cases defined genetically). These affected CMT1 or CMT2 patients and were distributed as follows: CMT1C (LITAF), 5 cases; CMT1D (EGR2), 1 case; CMT1E (PMP22 point mutation), 5 cases; CMT2D (GARS), 3 cases; CMT2E (NEFL), 4 cases, and CMT2K (GDPAP1), 5 cases. In this study, only 1.8% of patients with CMT1 were not genetically categorised, while the percentage of such cases for CMT2 was 65%. Only 7 patients (1.4%) presented demyelinating, autosomal recessive forms (CMT4), with the following distribution: CMT4A (GDPAP1), 1 case; CMT4C (SH3TC2), 3 cases; CMT4F (PRX), 1 case, and CMT4J (FIG4), 2 cases.

The work of Saporta et al also helps establish the percentages of success for each mutation studied. These range between 80% for PMP22 and 13% for MFN2. Based on certain phenotypic traits and the neurophysiological study, the authors provide several algorithms for the selection of molecular analysis, with the intention of enhancing diagnostic confidence. On the basis of these algorithms and our own experience, we designed a comprehensive diagnostic algorithm for all forms of CMT (Fig. 2). Below, we discuss some peculiarities which facilitate molecular diagnosis in CMT with vertical transmission:

- Given that 89% of patients with classical phenotype, without delay in the onset of walking (<15 months), AD inheritance and MCV in arms between 15 and 35 m/s present CMT1A, the first and only genetic test to be performed in case of a CMT1 syndrome should be PMP22 duplication. If negative, then GJB1 and MPZ should be analysed; mutations in other genes which cause CMT1 are exceptional (see above and Fig. 2).
- In patients with similar characteristics to the previous paragraph but with very slow MCV (<15 m/s), molecular analysis may start with PMP22 or MPZ, although delay in the onset of walking occurs more often in CMT1B that in CMT1A.
- Mutation of GJB1 (CMT1X) may be associated with MCV within the limits established for demyelinating CMT,
Guidelines for molecular diagnosis of CMT

Figure 2  Diagnostic algorithm for patients with CMT. Genetic mutations described both in the work of Saporta et al and in Spanish patients are shown in bold (see text for details, especially in relation to the priorities of molecular study).

AD: autosomal dominant; AR: autosomal recessive; ♂→♂: male—male transmission; ♂→♀: no evidence of male—male transmission.

axonal CMT or intermediate CMT. Due to their high prevalence, connectopathies should be considered in all CMT syndromes with apparent vertical inheritance but no evidence of male—male transmission (Fig. 2).

— The molecular study of patients with axonal CMT and AD inheritance should start with MFN2, MPZ and GJB1.

— The association with optic atrophy is characteristic of early onset CMT2A (Table 1). A late onset phenotype with Adie pupil should point towards CMT2J. Although the probability of detecting mutations in the remaining 11 genes is low (Fig. 2), there have been reports of Spanish CMT2 strains with mutations in DNM2, GDAP1 and TRPV4. Moreover, in our laboratories we have detected occasional, unpublished cases of CMT2 associated with mutations in NEFL, HSP27/HSPB1 and HSP22/HSPB22.

CMT with intermediate MCV and AD transmission (DICMT in Table 1) includes 4 different forms. The first has no definite molecular basis and the remaining 3 are linked to mutations in DNM2, YARS and MPZ. In our environment, there have been reports of intermediate phenotypes caused by mutations in MPZ. As previously noted, mutations in GJB1 should be included here. It is worth noting that in the series described by Saporta et al, the 2 causes of DI-CMT were mutations in GJB1 and MPZ.

CMT forms with AR transmission represent about 4% of the European CMT population, although in countries with high consanguinity, such as those in the Mediterranean and Middle East regions, the percentages of AR-CMT can reach between 30% and 50% of all CMT cases.

Information on the genetic epidemiology of these regions is essential to prioritise the molecular study of the 14 genes underlying AR forms, whether demyelinating (CMT4) or axonal (AR-CMT2). In general (Table 1), recessive forms have a childhood onset and are more severe than dominant phenotypes, whilst CMT4 forms present a marked decrease in MCV (usually <15 m/s). The eponyms Dejerine—Sottas disease and congenital hypomyelinating neuropathy probably apply to these recessive demyelinating forms. The founding mutations of NDRG1, SH3TC2 and HK1, all 3 identified in Spain, must be considered in patients from the Romani ethnic group. In our environment, GDAP1 mutation is the most frequent cause of recessive CMT, whether CMT4A or AR-CMT2 (CMT2K). This is a severe, childhood-onset phenotype, with proximal muscle involvement and diaphragmatic and vocal cord paralysis. It is interesting that GDAP1 heterozygous mutations can also be associated...
with a less severe axonal phenotype with AD transmission. The same phenomenon, co-participation of AR and AD inheritance patterns, has been exceptionally described for mutations of NEFL, HSP22 and MFN2. 

Table 1 and Fig. 2 show other mutations associated with CMT with an AR transmission.

Conclusion

The diagnosis of CMT should be based on an adequate clinical, family and neurophysiological study. This will enable the CMT phenotype (CMT1, CMT2, CMT4, AR-CMT2 or DI-CMT) to be established with some certainty. With this clinical background, and for the vast majority of patients, genetic analysis can be directed towards the search for mutations within a reduced group of genes, out of over thirty which have been pathogenically associated with CMT. There should be no place for the indiscriminate use of multiple gene panels in the molecular diagnosis of disease.

Conflict of interests

The authors have no conflicts of interest to declare.

References


