Manuscript ID: NEUROLOGY/2013/525345

Charcot-Marie-Tooth disease: genetic and clinical spectrum in a Spanish clinical series

Rafael Sivera MD[1*], Teresa Sevilla MD, PhD[1,2,3*], Juan Jesús Vílchez MD, PhD[1,2,3], Dolores Martínez-Rubio MBChB[4,5,6], María José Chumillas MD[2,7], Juan Francisco Vázquez MD[1], Nuria Muelas MD, PhD[1,2], Luis Bataller MD, PhD[1,2,7], José María Millán PhD[5,8], Francesc Palau MD, PhD[4,5,6,9], Carmen Espinós PhD[4,5,6,10].

Departments of [1]Neurology, [7]Clinical Neurophysiology and [8]Genetics, Hospital Univesitari i Politècnic La Fe, Valencia, Spain.
[2]Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Valencia, Spain.
[3]Department of Medicine and [10]Department of Genetics, University of Valencia, Valencia, Spain.
[4]Program in Rare and Genetic Diseases, Centro de Investigación Príncipe Felipe (CIPF), Valencia, Spain.
[5]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Valencia, Spain.
[6]CIPF Associated Unit to the IBV-CSIC, Valencia, Spain.
[9]School of Medicine, University of Castilla-La Mancha, Ciudad Real, Spain.

[*]These authors contributed equally to this manuscript.

Correspondence should be addressed to:

Teresa Sevilla.

Hospital Universitari i Politècnic La Fe. Dept. Neurology.

Bulevar Sur s/n, 46024-Valencia, Spain.

Tel. +3496-3862761, FAX. +3496-1973290, E-mail: sevilla_ter@gva.es

Key words: Charcot-Marie-Tooth disease; genetic distribution; *GDAP1*.

Total number of words in the main text: 2833

Total number of words in the abstract: 166

Total number character count for the title: 87

Number of references: 36

Number of tables: 4

Number of figures: 1

Study funding: This collaborative joint project is awarded by IRDiRC and funded by the Instituto de Salud Carlos III (ISCIII)- Subdirección General de Evaluación y Fomento de la Investigación within the framework of the National R+D+I Plan [Grants no IR11/TREAT-CMT, PI08/90857, PI08/0889, CP08/00053 and PI12/00453], co-funded with FEDER funds, the Fundación para la Investigación del Hospital Universitari La Fe [CM06/00154], and the Generalitat Valenciana [grant no. Prometeo/2009/051]. The Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED) and the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) are initiatives from the ISCIII.

Disclosures

Mr. Sivera reports no disclosures.

Dr. Sevilla is funded by grants from the ISCIII (PI08/0889), IRDiRC (IR11/TREAT-CMT) and the Fundación para la Investigación del Hospital Universitari La Fe [CM06/00154].

Dr. Vílchez received research support from the CIBERNED.

Ms. Martínez-Rubio reports no disclosures.

Ms. Chumillas reports no disclosures.

Mr. Vázquez reports no disclosures.

Dr. Muelas reports no disclosures.

Dr. Bataller reports no disclosures.

Dr. Millán reports no disclosures.

Dr. Palau is funded by grants from Fondo de Investigación Sanitaria (PI08/90857), the Generalitat Valenciana (Prometeo/2009/051), the IRDiRC (IR11/TREAT-CMT) and the CIBERER.

Dr. Espinós is funded by a grant from the ISCIII (PI12/00453) and IRDiRC (IR11/TREAT-CMT). Dr. Espinós has a "Miguel Servet" contract funded by the ISCIII and the CIBERER (CP08/00053).

Author contributions:

Dr. Sivera: acquisition of data, analysis and interpretation, manuscript initial elaboration.

Dr. Sevilla: Study concept and design, manuscript initial elaboration.

Dr. Vílchez: Critical revision of the manuscript for important intellectual content.

Ms. Martínez-Rubio: Genetic studies, acquisition of data.

Dr. Chumillas: Nerve conduction studies, acquisition of data.

Dr. Vázquez: Acquisition of data, analysis and interpretation.

Dr. Muelas: Critical revision of the manuscript for important intellectual content.

Dr. Bataller: Critical revision of the manuscript for important intellectual content.

Dr. Millán: Genetic studies (CMT1A duplication), acquisition of data.

Dr. Palau: Study concept and design, critical revision of the manuscript for important intellectual content.

Dr. Espinós: Study concept and design, study supervision, genetic screening.

ABSTRACT

Objectives: To determine the genetic distribution and the phenotypic correlation of an extensive series of Charcot Marie Tooth disease patients in a geographically well-defined Mediterranean area.

Methods: A thorough genetic screening, including most of the known genes involved in this disease, was performed and analyzed in this longitudinal descriptive study. Clinical data were analyzed and compared among the genetic subgroups.

Results: Molecular diagnosis was accomplished in 365/438 patients (83.3%), with a higher success rate in demyelinating forms of the disease. The CMT1A duplication (*PMP22* gene) was the most frequent genetic diagnosis (50.4%), followed by mutations in the *GJB1* gene (15.3%), and in the *GDAP1* gene (11.5%). Mutations in 13 other genes were identified, but were much less frequent. Sixteen novel mutations were detected and characterized phenotypically.

Conclusions: The relatively high frequency of *GDAP1* mutations, coupled with the scarceness of *MFN2* mutations (1.1%) and the high proportion of recessive inheritance (11.6%) in this series exemplify the particularity of the genetic distribution of Charcot-Marie-Tooth disease in this region.

INTRODUCTION

Charcot-Marie-Tooth disease (CMT) refers to the genetically heterogeneous group of hereditary motor and sensory neuropathies. It is one of the most common inherited neurological disorders, with a prevalence of 15.2-40 cases/100.000[1-3]. Molecular studies have provided an ever-growing list of more than 40 involved genes and loci (http://www.molgen.ua.ac.be/CMTMutations/, accessed 24 June 2013; http://neuromuscular.wustl.edu/, accessed 24 June 2013). Most of the patients with CMT have autosomal dominant (AD) inheritance, but many have X-linked, or autosomal recessive (AR) inheritance. CMT can be classified according to clinical, electrophysiological and nerve pathology findings into demyelinating (CMT1, CMT4) forms, with a median motor nerve conduction velocity (MMNCV) of < 38 m/s and pathologic evidence of nerve fiber demyelination; and axonal forms (CMT2), with preserved conduction velocities (MMNCV> 38 m/s) and pathological signs of axonal degeneration and regeneration[4]. An intermediate type (CMT-I) is accepted in which MMNCV lies between 25 and 45 m/s and nerve pathology shows axonal and/or demyelinating features[5].

Clinically, the most frequent CMT phenotype is characterized by progressive distal weakness and sensory loss appearing towards the second decade, with foot deformities, and absent reflexes. However, other patients develop a much more severe form with onset in infancy or early childhood and great disability in few years, or a milder course with few symptoms until adulthood. This clinical heterogeneity, coupled with the expanding genetic diversity is the complex scenario of the inherited neuropathies. Comprehensive clinical series, in which at least the most frequent genes have been studied, are needed to shed light upon the populational genetic distribution and genotype-phenotype correlation in CMT[6-7]. Here we present the genetic

distribution and phenotypic characterization of an extensive series of CMT after an exhaustive genetic screening in the Region of Valencia, a geographically well-defined Mediterranean area.

PATIENTS AND METHODS

Subjects

This is a longitudinal descriptive study which includes all of the patients with the diagnosis of CMT and evaluated at the inherited neuropathy clinic of Hospital Universitari i Politècnic La Fe in Valencia from 2000-2012. Patients with sensory-motor neuropathy were considered to be CMT if: a) a causative genetic defect was determined; b) family members with similar characteristics were detected; or c) sporadic cases were included if their medical history, examination and neurophysiology were compatible with CMT, and other known causes of acquired neuropathy were reasonably discarded. Patients with inherited neuropathies with exclusive motor (distal hereditary motor neuropathies, dHMNs) or sensory and autonomic (hereditary sensory and autonomic neuropathies, HSANs) signs were excluded from this study, as well as those with hereditary neuropathy with liability to pressure palsies (HNPP), and those with complex disorders in which neuropathy was not the most predominant phenotypic feature. Patients were subclassified as demyelinating or axonal CMT according to MMNCVs of the proband, except when the amplitudes of median compound motor action potentials (CMAPs) were reduced > 90%. In those cases the conduction velocities to nerves innervating proximal muscles were measured (palmaris longus for the median nerve, flexor carpi ulnaris for the ulnar nerve, etc.), and occasionally latencies of other proximal nerves like the axillary nerve, or pathologic evidence were taken into account.

Standard Protocol Approvals, Registrations, and Patient Consents

This study protocol was approved by the Institutional Review Board of the Hospital U. i P. La Fe. Written informed consents were obtained from all the members included in this study.

Clinical assessments

The clinical assessment included strength, muscular atrophy, sensory loss, reflexes, foot deformities as well as a general and neurologic examination. Muscle strength was graded using the standard Medical Research Council (MRC) scale. CMT neuropathy score (CMTNS) was recorded in all patients followed since 2006[8], and the functional disability scale (FDS) in those after 2000[9]; previous clinical data was extrapolated to CMTNS and FDS scores when possible. Comprehensive electrophysiological studies were carried out in 401/438 (91.6%) of the patients, not being performed only when the genetic diagnosis of another family member was already available. Lower limb muscle magnetic resonance imaging (MRI), and sural nerve biopsy were only performed when there were reasonable doubts regarding the clinical diagnosis or for investigational purposes, and followed the protocols described previously[10].

Mutational analysis

Blood samples were drawn and genomic DNA was obtained by standard methods from peripheral white blood cells. In all the probands, the CMT1A duplication was analyzed by MLPA (Multiplex Ligation-dependent Probe Amplification, SALSA kit P033 CMT1, MRC-Holland, Amsterdam, The Netherlands) in a genetic analyzer ABI Prism 3130xl (Applied Biosystem, Foster City, CA, USA). Once the CMT1A

duplication was discarded, a mutational screening of genes involved in CMT was performed taking into account the ethnicity of the proband and the phenotype. In patients with Gypsy ethnicity, the genetic testing strategy was planned as described previously[11]. In Caucasian patients the mutational screening was clinically oriented, and included the genes detailed in table 1 until the causative mutation was identified or all the genes had been analyzed.

The mutational screening was performed by amplification of all exons and their intronic flanking sequences, except in the *GJB1* gene in which the promoter sequence has also been analyzed. The Gene Runner vs 3.05 software was used for designing primers (available on request). The PCR products were analyzed by denaturing high performance liquid chromatography (DHPLC, WAVE® System, Transgenomic Inc., Omaha, NE, USA) and the anomalous patterns were investigated by Sanger sequencing (ABI Prism 3130xl). Finally, in both the *MPZ* and the *GJB1* genes large deletions and/or duplications were investigated by MPLA using the SALSA kits P143 and P129 (MRC-Holland, Amsterdam, The Netherlands) in an ABI Prism 3130xl autoanalyzer. We did not screen *MT-ATP6, PDK3, DHTKD1, GNB4* or *TRIM2* genes as they had not been described when this project was concluded[12-16].

When possible, segregation analyses within the families were performed, and novel mutations were analyzed in 200 chromosomes from healthy controls of Spanish ancestry. The biological relevance of the amino acid changes was studied using both SIFT (http://blocks.fhcrc.org/sift/SIFT.html, accessed 24 June 2013) and PolyPhen (http://genetics.bwh.harvard.edu/pph, accessed 24 June 2013) programs. When the detected alteration modified a splicing sequence, we used the NNSPLICE (http://fruitfly.org:9005/seq_tools/splice.html, accessed 24 June 2013) and the Splice View (http:// http://zeus2.itb.cnr.it/~webgene/wwwspliceview_ex.html, accessed 24

June 2013) softwares.

RESULTS

A total of 1009 patients were evaluated at our inherited neuropathy clinic during the timeframe 2000-2012, 438 of them were considered CMT and met our inclusion criteria. All were Spanish, and 401 of them (91.6%) were currently living or had ancestral roots in the Region of Valencia, in the Western Mediterranean area. Initially 275 (62.8%) were classified as demyelinating CMT, and 163 (37.2%) as axonal CMT. Regarding the inheritance pattern, 242 (55.3%) were considered as AD, 51 (11.6%) were AR, 52 (11.9%) were X-linked, and 93 (21.2%) were considered sporadic. Genetic diagnosis was achieved in 365/438 patients (83.3%), with a higher success rate in the demyelinating forms (263/275; 95.6%) over the axonal forms (102/163; 62.6%). The causative mutations were detected in 214/242 (88.4%) patients with AD inheritance, 45/51 (88.2%) with AR inheritance, 52/52 (100%) with X-linked inheritance and only in 54/93 (58.1%) with a sporadic presentation. In table 2 the detailed genetic diagnosis can be analyzed, and can be compared to the latest published data, and in figure 1 the distribution according to CMT subtype is exposed. All of the genetic and clinical information has also been recorded in a readily accessible mutation database (http://www.treat-cmt.es/db, accessed 24 June 2013).

Demyelinating CMT patients

Of the 275 demyelinating CMT patients, 241 were of Caucasian ethnicity, and 34 were of Gypsy origin. Regarding the demyelinating Caucasian patients, 184 (76.3%) carried the CMT1A duplication, being the most frequent cause of CMT. In the

remaining 57 Caucasian patients the disease causing mutation was identified in 45 with the following distribution: 25 mutations in *GJB1*, 9 in *MPZ*, 4 in *PRX*, 2 point mutations in *PMP22*, 2 in *FGD4*, 2 in *SH3TC2* and 1 in *NEFL*. Six novel mutations were detected in demyelinating CMT (table 3). Once the genetic screening was performed, the causative change remained unknown in 12 (4.9%) patients. No mutations were identified in any of the following genes: *LITAF, EGR2, GDAP1, MTMR2, MTMR13, FIG4, PRPS1, DNM2, YARS* and *SOX10*. In the Gypsy population, the disease-causing mutation was identified in all the cases, and consisted exclusively in founder mutations related to CMT in Gypsy population[11].

Table 4 shows the relevant clinical features associated with AR forms of demyelinating CMT (CMT4). These forms have certain common characteristics like early onset, delayed motor development, severe disability, etc., but other features differ between the CMT4 subtypes.

Axonal CMT patients

The mutational screening detailed in table 1 led to identify the disease causing mutation in 102/163 axonal CMT patients (62.6%). In this set of patients, there is a marked genetic heterogeneity, being the two most frequent causes of axonal CMT mutations in the *GDAP1* and *GJB1* genes. The mutations in the *GDAP1* gene, correspond to 24 patients (14.7% of CMT2) with AD inheritance (caused by the p.R120W mutation in all cases except one) and 18 patients (11.0%) with AR inheritance and diverse genotype. All our patients with *GDAP1* mutations were defined as CMT2, as the neurophysiologic findings were clearly axonal; although the pathology included both axonal features (fiber loss, axonal degeneration, few regenerative clusters, etc.) and myelin abnormalities (thin myelin sheaths, abnormal

myelin folding, occasional onion bulb-like formations). Patients with AR inheritance developed a severe phenotype with important disability, vocal cord and diaphragmatic palsies while patients with dominant GDAP1 mutations presented with a mild to moderate phenotype with certain clinical and MRI particularities reported previously[10].

Regarding mutations in the *GJB1* gene, they were detected in 31 (19.0%) axonal CMT patients. It is interesting to note that although the patients were classified as demyelinating or axonal CMT according to the MMNCVs of the proband, over 80% of these families would be classified as intermediate forms of CMT.

The remaining mutations were actually quite rare, accounting for only 29 cases (17.8%) and are distributed among several genes: 10 patients with mutations in *MPZ*, 7 in *HSPB1*, 4 in *MFN2*, 3 in *HSPB8*, 3 in *NEFL*, 1 in *GARS* and 1 in *KARS*. In the aggregate 25 different mutations were identified in the CMT2 series and 10 of them were novel (table 3). Once the mutational screening was performed, the disease causing mutation remains unknown in 61 (37.4%) patients. No change was identified in the following genes: *RAB7*, *DNM2*, *YARS*, *AARS*, *LRSAM1*, *TRPV4* nor the founder mutations *MED25* p.A335V or *LMNA* p.R298C.

DISCUSSION

A thorough genetic screening has been performed in an extensive clinical series of patients with CMT in a Western Mediterranean area. Overall, a molecular diagnosis was achieved in 83.3%, with a higher success rate in demyelinating than in axonal CMT. In demyelinating patients these rates are comparable to the other series in which a comprehensive genetic screening has been performed (table 2)[6,7,17], suggesting that few genes involved in this form of CMT remain undiscovered. However in CMT2,

although the success rate is higher than in other series, there are still 37.4% of patients who remain without genetic diagnosis. The mutational distribution described confirms the extensive heterogeneity intrinsic to this disease; 56 different mutations have been detected in this series, and 16 had not been described previously. This comprehensive study depicts the genetic distribution of a large CMT series in the Mediterranean basin, and there are certain distinctive features compared to other geographic areas. The CMT1A duplication is by far the most common mutation detected, and all patients were classified as demyelinating CMT, in fact none had MMNCV greater than 30 m/s. CMT1A accounts for 66.9% of the demyelinating forms, which is slightly lower than other series that report just over 70%[18]. Actually, these results are biased by the presence of 34 Gypsy patients affected by demyelinating CMT who harbored the previously described founder mutations associated with Gypsy population as we have previously reported[11,19] These 34 Gypsy patients and 8 other of Caucasian ethnicity (4 with mutations in *PRX*, 2 in *SH3TC2*, and 2 in *FGD4;* table 4) comprise the 11.6% of demyelinating CMT with an AR inheritance (CMT4). The percentage of patients with AR or sporadic presentation is in fact greater than other series[6] and may reflect certain populational peculiarities, as the Region of Valencia hosts a numerous Gypsy population (over 50 thousand) and certain isolated areas have a high consanguinity rate.

Mutations in *GJB1* were the 2nd most common genetic diagnosis after CMT1A, accounting for 12.8% of the CMT series. These patients were classified according to the MMNCV of the proband, but clinically all patients had a consistent phenotype which was not so much influenced by conduction velocities, as by gender[20,21]. Only 5 patients (9%), had signs of central nervous system involvement (brisk reflexes and Babinski sign in two of them) with normal encephalic and spinal MRI. It is worth

noting that in two of these patients after a long follow-up (>20 ys), the pyramidal signs became less prominent as the neuropathy progressed becoming overshadowed by the neuropathic signs. More than 300 mutations have been described in the *GJB1* gene, throughout the coding region and exceptionally, in the 5'-UTR (untranslated region). A very extensive family of our series was found to be carriers of a novel c.-540C>G mutation in this region. Its pathogenicity was demonstrated by a luciferase assay (data not shown).

Mutations in *MPZ* were detected in only 4.3% of the series; 9 were classified as demyelinating CMT and 10 as axonal CMT. In this case, there was important phenotypical variability, as has been reported in this gene[22,23]. Except for one family, demyelinating patients were more severely affected, with earlier disease onset (1st decade), prominent sensory loss and moderate to severe disability with progression. One of these patients, carrier of the *MPZ* p.S121F mutation, developed a severe congenital hypomyelinating neuropathy[24]. Other genes were actually quite scarcely affected in our CMT1 series (*NEFL*, point mutations in *PMP22, PRX*, *SH3TC2* and *FGD4*).

Regarding axonal forms of CMT, there is a great genetic diversity, as 25 different mutations were detected in 9 genes. The success rate of our series in these patients (62.6%) is one of the highest that has been published, probably due to the ample genetic screening that has been performed, the high relative frequency of *GDAP1*. The genetic distribution in CMT2 shows that the two most frequent causes of axonal CMT were mutations in the *GDAP1* and *GJB1* genes, which combined accounted for 44.8% of patients who suffer from axonal CMT. However, 37.4% of the patients with CMT2 still remain undiagnosed, and this constitutes a great challenge for the near future.

Our series of 42 patients with mutations in the *GDAP1* gene is to date the most

extensive one published and all of them presented neurophysiological features of axonal CMT. Patients with apparently demyelinating or intermediate nerve conduction studies have been reported[25,26] but in our patients, the only ones with slow conduction velocities were those in which CMAP was < 0.5mV, and nerve conduction velocity was clearly normal if measured to nerves innervating proximal muscles. Although the neurophysiologic findings in these patients were unequivocally axonal, the pathology included both axonal degeneration and myelin abnormalities[10,27]. Eighteen patients with recessive *GDAP1* mutations were detected, with an early disease onset and rapid progression, being wheelchair-bound in the second or third decade in all cases except two (associated with p.L344R/p.Q163X compound heterozygote, and p.R282C/p.R282C homozygote genotypes) that had a relatively milder phenotype[27]. Regarding the patients with dominant *GDAP1* mutations, 24 out of 25 patients carry the p.R120W substitution, which is up to date the most frequent dominant mutation detected in the *GDAP1* gene. Despite of this mutation has been described in families with different geographic origin[28-30], the *GDAP1* p.R120W has probably a founder effect in our population, and presents with a mild to moderate phenotype[10].

Apart from the high prevalence of *GDAP1* mutations, the other notable factor in the axonal CMT series is the low number of cases with mutations in the *MFN2* gene (2.5%). *MFN2* has been identified as the most common gene in axonal CMT in many series[7,8] accounting consistently for 10-33%[31-33] of this CMT form, even in other Spanish Mediterranean areas[34]. Certain other European series have described even lower frequencies[35] than our own, suggesting that the distribution of *MFN2* mutations may be quite heterogeneous within Europe. The remaining mutations identified in axonal patients were even less frequent, including *MPZ, HSPB1, NEFL, GARS, HSPB8,* and *YARS* genes (15.3% of the CMT2 series).

The knowledge derived from thoroughly screened CMT series is essential to comprehend the global picture of this disease, as there may be relevant changes in the genetic distribution of different areas. A clear example of this is the relatively high prevalence of recessive forms and the predominance of *GDAP1* over *MFN2* in this clinical series. More information about the genetic distribution in other Spanish or Mediterranean areas is needed to discern whether this is only a local characteristic, or can be extrapolated to other areas.

ACKNOWLEDGEMENTS

We are grateful to Paula Sancho for helping us to generate the data base of CMT mutations in Spanish population, and to Susana Rovira for performing the study of the *GJB1* gene promoter region.

REFERENCES

1. Combarros O, Calleja J, Polo JM, Berciano J. Prevalence of hereditary motor and sensory neuropathy in Cantabria. Acta Neurol Scand. 1987;75:9-12.
2. Foley C, Schofield I, Eglon G, Bailey G, Chinnery PF, Horvath R. Charcot-Marie-Tooth disease in Northern England. J Neurol Neurosurg Psychiatry 2012;83:572-573.
3. Skre H. Genetic and clinical aspects of Charcot Marie Tooth's disease. Clin Genet 1974;6:98-118.
4. Dyck PJ, Lambert EH. Lower motor and primary sensory neuron diseases with peroneal muscular atrophy. I. Neurologic, genetic, and electrophysiologic findings in hereditary polyneuropathies. Arch Neurol 1968;18:603–618.
5. Payreson D, Marchesi C. Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. Lancet Neurol 2009;8:654-667.
6. Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME. Charcot-Marie-Tooth disease subtypes and genetic testing strategies. Ann Neurol 2011;69:22-33.
7. Murphy SM, Laura M, Fawcett K, et al. Charcot-Marie-Tooth disease: frequency of genetic subtypes and guidelines for genetic testing. J Neurol Neurosurg Psychiatry 2012;83:706-710.
8. Shy ME, Blake J, Krajewski K, et al. Reliability and validity of the CMT neuropathy score as a measure of disability. Neurology 2005;64:1209-1214.
9. Birouk N, Gouider R, Le Guern E, et al. Charcot–Marie–Tooth disease type 1A with 17p11.2 duplication. Clinical and electrophysiological phenotype study and factors influencing disease severity in 119 cases. Brain 1997;120:813–823.

10. Sivera R, Espinós C, Vílchez JJ, et al. Phenotypical features of the p.R120W mutation in the GDAP1 gene causing autosomal dominant Charcot-Marie-Tooth disease. J Peripher Nerv Syst. 2010 Dec;15:334-344.
11. Sevilla T, Martínez-Rubio D, Márquez C, et al. Genetics of the Charcot-Marie-Tooth disease in the Spanish Gypsy population: the hereditary motor and sensory neuropathy-Russe in depth. Clin Genet 2013; 83:565-570.
12. Pitceathly R, Murphy SM, Cottenie E, et al. Genetic dysfunction of MT-ATP6 causes axonal Charcot-Marie-Tooth disease. Neurology 2012;79:1145-1154.
13. Kennerson ML, Yiu EM, Chuang DT, et al. A new locus for X-linked dominant Charcot-Marie-Tooth disease (CMTX6) is caused by mutations in the pyruvate dehydrogenase kinase isoenzyme 3 (PDK3) gene. Hum Mol Genet 2013;22:1404-1416.
14. Xu W, Gu MM, Sun LH et al. A nonsense mutation in DHTKD1 causes Charcot-Marie-Tooth disease type 2 in a large Chinese pedigree. Am J Hum Genet 2012; 91:1088-1094.
15. Soong B-W, Huang YH, Tsai PC, et al. Exome sequencing identifies GNB4 mutations as a cause of dominant intermediate Charcot-Marie-Tooth disease. Am J Hum Genet 2013; 92:422-430.
16. Ylikallio E, Pöyhönen R, Zimon M, et al. Deficiency of the E3 ubiquitin ligase TRIM2 in early-onset axonal neuropathy. Hum Mol Genet 2013; doi: 10.1093/hmg/ddt149.
17. Lin KP, Soong BW, Yang CC, et al. The Mutational Spectrum in a Cohort of Charcot-Marie-Tooth Disease Type 2 among the Han Chinese in Taiwan. PLoS ONE 2011;6(12):e29393.

18. Nelis E, Van Broeckhoven C, De Jonghe P, et al. Estimation of the mutation frequencies in Charcot-Marie-Tooth disease type 1 and hereditary neuropathy with liability to pressure palsies: a European collaborative study. Eur J Hum Genet 1996;4:25-33.
19. Claramunt R, Sevilla T, Lupo V, et al. The p.R1109X mutation in SH3TC2 gene is predominant in Spanish Gypsies with Charcot-Marie-Tooth disease type 4. Clin Genet 2007;71:343-349.
20. Shy ME, Siskind C, Swan ER, et al. CMT1X phenotypes represent loss of GJB1 gene function. Neurology 2007;68:849–855.
21. Siskind CE, Murphy SM, Ovens R, Polke J, Reilly MM, Shy ME. Phenotype expression in women with CMT1X. J Peripher Nerv Syst 2011;16:102–107.
22. Warner L, Hilz M, Appel S, et al. Clinical phenotypes of different MPZ (P0) Mutations may include Charcot–Marie–Tooth type 1B, Dejerine–Sottas, and congenital hypomyelination. Neuron 1996; 17:451–460.
23. Shy ME, Jani A, Krajewski K, et al. Phenotypic clustering in MPZ mutations. Brain 2004;127:371–384.
24. Sevilla T, Lupo V, Sivera R, et al. Congenital hypomyelinating neuropathy due to a novel MPZ mutation. J Peripher Nerv Syst. 2011;16:347-352.
25. Baxter RV, Ben Othmane K, Rochelle JM, et al. Ganglioside-induced differentiation-associated protein-1 is mutant in Charcot-Marie-Tooth disease type 4A/8q21. Nat Genet. 2002;30:21-22.
26. Senderek J, Bergmann C, Ramaekers VT, et al. Mutations in the ganglioside-induced-differentiation-associated protein-1 (GDAP1) gene in intermediate type autosomal recessive Charcot-Marie-Tooth neuropathy. Brain 2003;126:642-649.

27. Sevilla T, Jaijo T, Nauffal D, et al. Vocal cord paresis and diaphragmatic dysfunction are severe and frequent symptoms of GDAP1-associated neuropathy. Brain 2008;131:3051-3061.

28. Claramunt R, Pedrola L, Sevilla T, et al. Genetics of Charcot-Marie-Tooth disease type 4A: mutations, inheritance, phenotypic variability, and founder effect. J Med Genet 2005; 42:358–365.

29. Zimoń M, Baets J, Fabrizi GM, Jaakkola E, et al. Dominant GDAP1 mutations cause predominantly mild CMT phenotypes. Neurology 2011;77:540-548.

30. Ammar N, Nelis E, Merlini L, Barisic N, et al. Identification of novel GDAP mutations causing autosomal recessive Charcot-Marie-Tooth disease. Neuromuscul Disord 2003;13:720-728.

31. Verhoeven K, Claeys KG, Züchner S, et al. MFN2 mutation distribution and genotype/phenotype correlation in Charcot-Marie-Tooth type 2. Brain. 2006; 129:2093-2102.

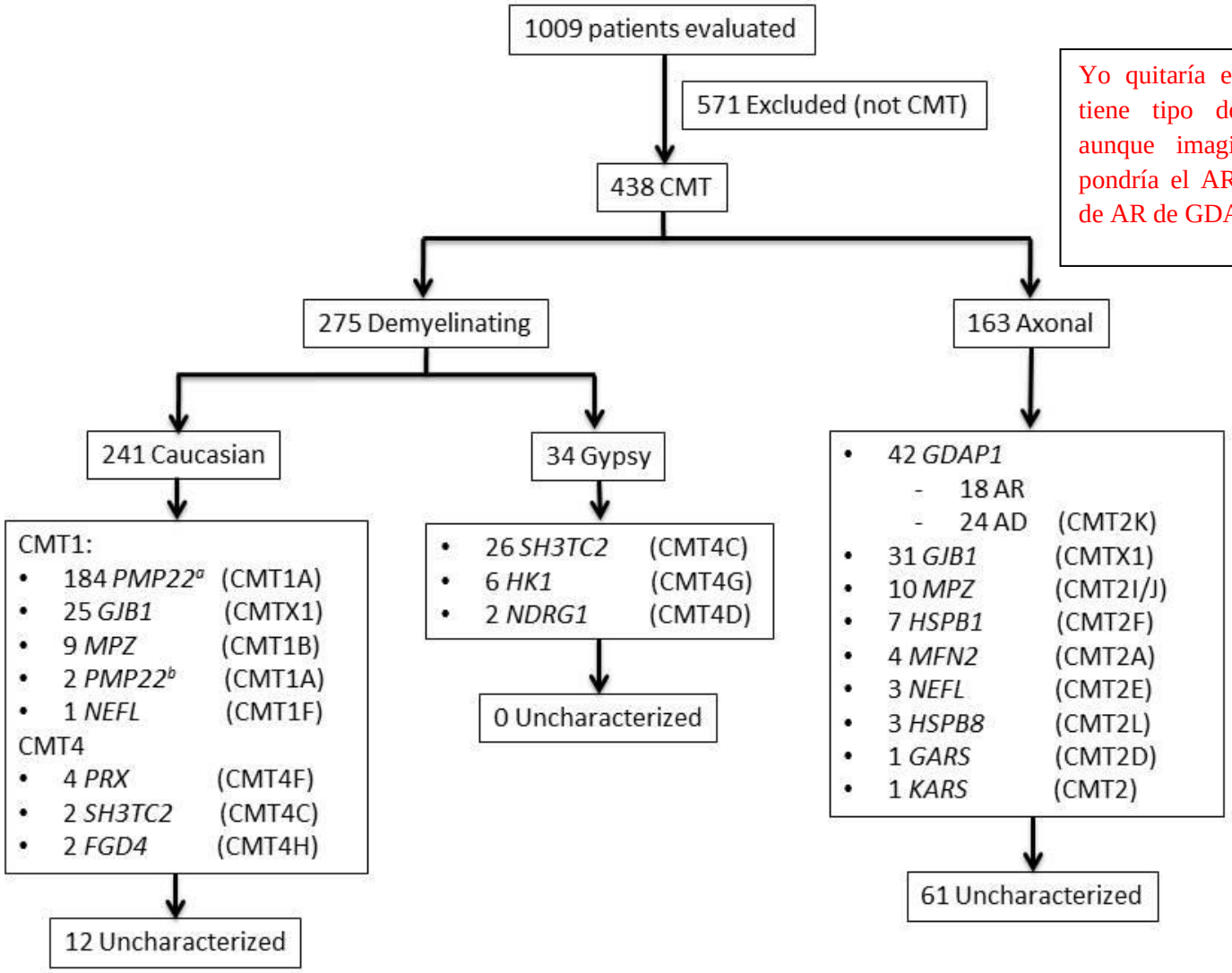
32. Calvo J, Funalot B, Ouvrier RA, et al. Genotype-phenotype correlations in Charcot-Marie-Tooth disease type 2 caused by mitofusin 2 mutations. Arch Neurol 2009;66:1511-1516.

33. Auranen M, Ylikallio E, Toppila J, Somer M, Kiuru-Enari S, Tyynismaa H. Dominant GDAP1 founder mutations is a common cause of axonal Charcot-Marie-Tooth disease in Finland. Neurogenetics 2013;14:123-132.

34. Casasnovas C, Banchs I, Cassereau J, et al. Phenotypic spectrum of MFN2 mutations in the Spanish population. J Med Genet. 2010;47:249-256.

35. Braathen GJ. Genetic epidemiology of Charcot-Marie-Tooth disease. Acta Neurol Scand Suppl 2012;126:1-22.

36. Lupo V, Galindo MI, Martínez-Rubio D, et al. Missense mutations in the SH3TC2 protein causing Charcot-Marie-Tooth disease type 4C affect its localization in the plasma membrane and endocytic pathway. Hum Mol Genet 2009;18:4603-4614.



Yo quitaría el CMT2 de KARS; no tiene tipo de CMT2 asignado. Y aunque imagino que no os gusta, pondría el ARCMT2K a los 18 casos de AR de GDAP1.

LEGEND

Figure 1: Genetic characterization of CMT subtypes.
Legend: Patients evaluated at the inherited neuropathy clinic during the timeframe 2000-2012. [a] Carriers of the CMT1A duplication. [b] Carriers of point mutations in the *PMP22* gene.

Table 1: Genes analyzed in the mutational screening

CMT1		CMT2
Caucasian	**Gypsy**	
PMP22	*SH3TC2*[b]	*MFN2*
GJB1	*NDRG1*[b]	*GJB1*
MPZ	*HK1*[b]	*MPZ*
GDAP1		*GDAP1*
SH3TC2		*HSPB1*
FGD4		*HSPB8*
NEFL		*LITAF*
LITAF		*NEFL*
GAN1		*DNM2*[a]
BSCL2		*GARS*
FIG4		*AARS*
ERG2		*KARS*
PRX[a]		*YARS*
MTMR2		*TRPV4*
MTMR13		*RAB7*
PRPS1		*MED25*[b]
DNM2[a]		*LMNA*[b]
YARS		*LRSAM1*
SOX10		

[a]More than one sequence references were used due to the presence of isoforms. [b]Only founder mutations were analyzed: *SH3TC2* p.C737_P738delinsX, *SH3TC2* p.R1109X, *NDRG1* p.R148X, *HK1* g.9712G>C, *MED25* p.A335V, and *LMNA* p.R298C.

Table 2: Genetic distribution and comparison to other series

Gene	No. of patients (Frequency)		
	Present study	**Saporta et al.[6]**	**Murphy et al.[7]**
PMP22 [a]	184 (48.8%)	290 (55%)	168 (63.2%)
GJB1	56 (14.9%)	80 (15.2%)	46 (17.3%)
GDAP1	42 (11.1%)	6 (1.2%)	2 (0.8%)
SH3TC2	27 (7.2%)	3 (0.6%)	5 (1.9%)
MPZ	19 (5.0%)	45 (8.5%)	13 (4.9%)
NDRG1	7 (1.9%)		
HSPB1	7 (1.9%)		2 (0.8%)
MFN2	6 (1.6%)	21 (4%)	12 (4.5%)
HK1	5 (1.3%)		
NEFL	4 (1.1%)	4 (0.8%)	2 (0.8%)
GARS	4 (1.1%)	3(0.6%)	
PRX	4 (1.1%)	1 (0.2%)	
HSPB8	3 (0.8%)		
PMP22 [b]	2 (0.5%)	5 (1%)	6 (2.3%)
FGD4	2 (0.5%)		
KARS	1 (0.3%)		
YARS	1 (0.3%)		
TRPV4	1 (0.3%)		3 (1.1%)
LITAF		5 (1%)	4 (1.5%)
MTMR2			1 (0.4%)
GAN			1 (0.4%)
BSCL2			1 (0.4%)
FIG4		2 (0.4%)	

[a] Carriers of the CMT1A duplication. [b] Carriers of point mutations in the *PMP22* gene.

Table 3: Novel mutations with detailed assessments and conduction velocities of the probands, and phenotypic peculiarities

Gene	Mutation		Presentation	No. patients	Onset (ys)	Age at exam (ys)	CMTNS	FDS	MMNCV (m/s)	Phenotypic characteristics
	Nucleotide	Amino acid (aa)								
GJB1	c.44_45delinsTT	p.R15L	X-linked	3	20	59	14	3	36.1	Early distal upper limb atrophy and weakness. Intrafamily variability regarding severity.
	c.529G>A	p.V177M	Sporadic	1	18	34	14	1	30	Early distal upper limb atrophy and weakness.
	c.-540C>G	*(No aa change)*	X-linked	10	26	36	15	2	31	Lower limb distal weakness earlier & more prominent than upper limb. Includes 2 asymptomatic women.
	c.484dupA	p.M162NfsX81	X-linked	2	15	34	13	2	40	Early distal upper limb atrophy and weakness.
	c.141_143dupGAA	p.K48_S49insK	X-linked	4	24	44	12	2	41	Early distal upper limb atrophy and weakness. Brisk reflexes only in proband.
SH3TC2	c.3305delA (hom)[a]	p.H1102LfsX14[a]	Sporadic	1	9	43	15	3	27	Early sensory ataxia, scoliosis. Lower > upper limb distal weakness and atrophy. No hearing loss.
PRX	c.589G>T c.642insC	p.E197X p.R215QfsX8	AR	2	2	42	27	8	4	Early onset, sensory ataxia, scoliosis. Refractory trigeminal neuralgia in 1/2. Few motor signs.
FGD4	c.1886delGAAA (hom)	p.K630NfsX5	AR	2	3	34	14	4	11	Early onset but slow progression. Sensory ataxia. Lower > upper limb distal weakness and atrophy. Spinal syringomyelia in 1/2
GDAP1	c.1031T>G c.487C>T[b]	p.L344R p.Q163X[b]	Sporadic	1	12	49	12	2	57	Mild phenotype for a recessive mutation. Distal lower limb weakness, no vocal cord or diaphragmatic palsy.
MFN2	c.306dupT	p.G103WfsX41	AD	2	22	40	11	2	52	Classic CMT2 phenotype, moderate instability.
	c.752C>T	p.P251L	Sporadic	1	25	47	13	2	51	Classic CMT2 phenotype.
MPZ	c.21_26dupTGGGGG	p.P9_A10dup	AD	2	30	39	9	1	54	Proband with mild phenotype and his father is mostly asymptomatic. Upper limb reflexes are present.
NEFL	c.293A>C	p.N98T	Sporadic	1	3	54	26	8	44	Early onset, severe phenotype. Wheelchair bound in the 4th decade, death with 58 ys. Hearing loss.
	c.1315T>A	p.F439I	Sporadic	1	23	41	8	2	45	Early distal upper limb atrophy and weakness. Brisk reflexes.
GARS	c.1171C>T	p.R391C	Sporadic	1	18	39	10	1	53	Early distal upper limb atrophy and weakness. Brisk reflexes. Motor > sensory involvement.

Hom: homozygous; AD: autosomal dominant; AR: autosomal recessive; CMTNS: CMT neuropathy score; FDS: Functional disability scale; MMNCV: Median motor nerve conduction velocity (Normal values in our laboratory > 51.6 m/s) [a]We cited this mutation in Lupo et al[36], but clinical features were not included. [b] This mutation has widely been described; we have included it because this patient is a compound heterozygote for a novel mutation.

Table 4: Genotype-phenotype correlation of the series of autosomal recessive demyelinating patients

Gene	Mutations	Patients/ families	Onset	Age exam	Weakness	Sensory loss	Foot deformity	Scoliosis	Cranial nerves[a]	CMTNS FDS	MMNCV (m/s) CMAP (mV)[b]	SNAP (μV)
SH3TC2	p.R1109X (hom)	21/11	3.2	23.4	LL>UL Proximal 38%	Prominent Vibratory = pinprick Ataxia 100%	100%	91%	V-trigeminal neuralgia (5%) VIII (48%)	16.8 3.7	24.6 4.2	0.7 NR 52%
	p.R1109X / p.C737_P738delinsX	5/3	4.1	20.3	LL>UL Proximal 40%	Same	100%	100%	VIII (40%)	15.6 4.1	22.7 4.8	0.3 NR 80%
	p.H1102LfsX14 (hom)	1/1	9	30	LL>UL Distal>Proximal	Same	Yes	Yes	No	15 2	18 8.7	NR
	p.R529Q (hom)	1/1	8	43	LL>UL Only distal	Same	Yes	Yes, mild	VIII	10 2	28 9.6	NR
HK1	g.9712G>C (hom)	6/3	4.8	24.2	LL>UL Proximal 33	Prominent Vibratory > pinprick Ataxia 100%	100%	50%	VIII (33%)	14.1 3	26.3 5.1	1.9 NR 17%
NDRG1	p.R148X (hom)	2/2	3.8	18.1	LL>UL Proximal 50	Prominent Vibratory > pinprick Ataxia 100%	100%	100%	VIII (50%)	16,3 3,1	16,7 6,2	0.9 NR 50%
PRX	p.E197X / p.R215QfsX8	3/1	2.7	25.7	LL>UL Only distal	Prominent Vibratory > pinprick Ataxia 100%	100%	100%	V- trigeminal neuralgia (33%)	22.7 5.3	4.9 1.2	NR 100%
	p.E113fsX3 (hom)	1/1	1	12	LL>UL Only distal	Prominent Vibratory > pinprick Ataxia	Yes	Yes	No	18 3	5.8 0.5	NR
FGD4[c]	p.K630NfsX5 (hom)	2/1	2.5	32	LL>UL Only distal	Prominent Vibratory > pinprick Ataxia 100%	Yes	Yes	No	12 3	11.5 5.2	NR 100%

If more than one case, the numeric values are means, and the percentages, relative frequency of a characteristic. CMTNS: CMT neuropathy score; FDS: Functional disability scale; MMNCV: Median motor nerve conduction velocity (normal values in our laboratory > 51.6 m/s); CMAP: Compound muscle action potential of the median nerve (normal values > 9.3 mV); SNAP Sensory nerve action potential in median nerve (normal values > 16.5 μV); NR: not recordable (expressed in % of the patients); UL: upper limbs, LL: lower limbs, hom: homzygous. [a]VIII nerve was considered affected when the patient referred relevant hypoacusia or the hearing loss was confirmed with an audiometry. [b]Nerve conduction studies of median nerve nearest to the moment of physical examination. [c]The two patients with mutations in the *FGD4* gene had an early onset, and moderate disability from infancy, but very slow progression thereafter.