Charcot-Marie-Tooth disease

Genetic and clinical spectrum in a Spanish clinical series

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ABSTRACT

Objectives: To determine the genetic distribution and the phenotypic correlation of an extensive series of patients with Charcot-Marie-Tooth disease 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 of 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. *Neurology*® 2013;81:1617–1625

GLOSSARY

AD = autosomal dominant; **AR** = autosomal recessive; **CMT** = Charcot-Marie-Tooth; **MMNCV** = median motor nerve conduction velocity.

Charcot-Marie-Tooth (CMT) disease refers to the genetically heterogeneous group of hereditary motor and sensory neuropathies. It is one of the most common inherited neurologic disorders, with a prevalence of 15.2 to 40 cases per 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/, http://neuromuscular.wustl.edu/, both accessed June 24, 2013). Most of the patients with CMT disease have autosomal dominant (AD) inheritance, but many have X-linked or autosomal recessive (AR) inheritance. CMT disease can be classified according to clinical, electrophysiologic, and nerve pathology findings into demyelinating forms (CMT1, CMT4), 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 pathologic 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 toward the second decade, with foot deformities and absent reflexes. However, other patients develop a much more severe form with onset in infancy or early

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childhood and great disability within a 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 on the populational genetic distribution and genotype-phenotype correlation in CMT disease.[6,7] Herein, we present the genetic distribution and phenotypic characterization of an extensive series of CMT disease after an exhaustive genetic screening in the Region of Valencia, a geographically well-defined Mediterranean area.

METHODS

Subjects. This is a longitudinal descriptive study, which includes all of the patients with the diagnosis of CMT disease and evaluated at the inherited neuropathy clinic of Hospital Universitari i Politècnic La Fe in Valencia from 2000 to 2012. Patients with sensory-motor neuropathy were considered to have CMT disease 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 disease, and other known causes of acquired neuropathy were reasonably discarded. Patients with inherited neuropathies with exclusive motor (distal hereditary motor neuropathies) or sensory and autonomic (hereditary sensory and autonomic neuropathies) signs were excluded from this study, as well as those with hereditary neuropathy with liability to pressure palsies, and those with complex disorders in which neuropathy was not the most predominant phenotypic feature. Patients were subclassified with demyelinating or axonal CMT disease according to MMNCVs of the proband, except when the amplitudes of median compound motor action potentials 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 such as the axillary nerve, or pathologic evidence were considered.

Standard protocol approvals, registrations, and patient consents. This study protocol was approved by the Institutional Review Board of the Hospital Univesitari i Politècnic La Fe. Written informed consents were obtained from all of 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 scale. CMT neuropathy score was recorded in all patients followed since 2006,[8] and the Functional Disability Scale score in those after 2000[9]; previous clinical data were extrapolated to CMT neuropathy score and Functional Disability Scale score when possible. Comprehensive electrophysiologic studies were performed in 401 of 438 patients (91.6%), and were not performed only when the genetic diagnosis of another family member was already available. Lower limb muscle MRI and sural nerve biopsy were performed only 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 of 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 Biosystems, Foster City, CA). Once the CMT1A duplication was discarded, a mutational screening of genes involved in CMT disease 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 of 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 version 3.05 software was used for designing primers (available on request). The PCR products were analyzed by using denaturing high-performance liquid chromatography (WAVE System; Transgenomic Inc., Omaha, NE), 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

Table 1 Genes analyzed in the mutational screening

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

Abbreviation: CMT = Charcot-Marie-Tooth.
[a] 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.
[b] More than one sequence reference was used because of the presence of isoforms.

MLPA using the SALSA kits P143 and P129 (MRC-Holland) in an ABI Prism 3130xl autoanalyzer. We did not screen *MT-ATP6*, *PDK3*, *DHTKD1*, *GNB4*, or *TRIM2* genes because 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 June 24, 2013) and PolyPhen (http://genetics.bwh.harvard.edu/pph, accessed June 24, 2013) programs. When the detected alteration modified a splicing sequence, we used the NNSPLICE (http://fruitfly.org:9005/seq_tools/splice.html, accessed June 24, 2013) and the Splice View (http://zeus2.itb.cnr.it/~webgene/wwwspliceview_ex.html, accessed June 24, 2013) software.

RESULTS A total of 1,009 patients were evaluated at our inherited neuropathy clinic during the timeframe 2000 to 2012; 438 of them were considered to have CMT disease 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 of 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 of 242 patients (88.4%) with AD inheritance, 45 of 51 (88.2%) with AR inheritance, 52 of 52 (100%) with X-linked inheritance, and in only 54 of 93 (58.1%) with a sporadic presentation. In table 2, the detailed genetic diagnosis can be analyzed and compared with the latest published data, and in the figure, the distribution according to CMT subtype is shown. All of the genetic and clinical information has also been recorded in a readily accessible mutation database (http://www.treat-cmt.es/db, accessed June 24, 2013).

Patients with demyelinating CMT disease. Of the 275 patients with demyelinating CMT disease, 241 were of Caucasian ethnicity and 34 were of Gypsy origin. Of the Caucasian patients with the demyelinating form, 184 (76.3%) carried the CMT1A duplication, which is the most frequent cause of CMT disease. 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 patients (4.9%). 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 cases, and consisted exclusively of founder mutations related to CMT disease in the Gypsy population.[11]

Table 2 Genetic distribution and comparison to other series

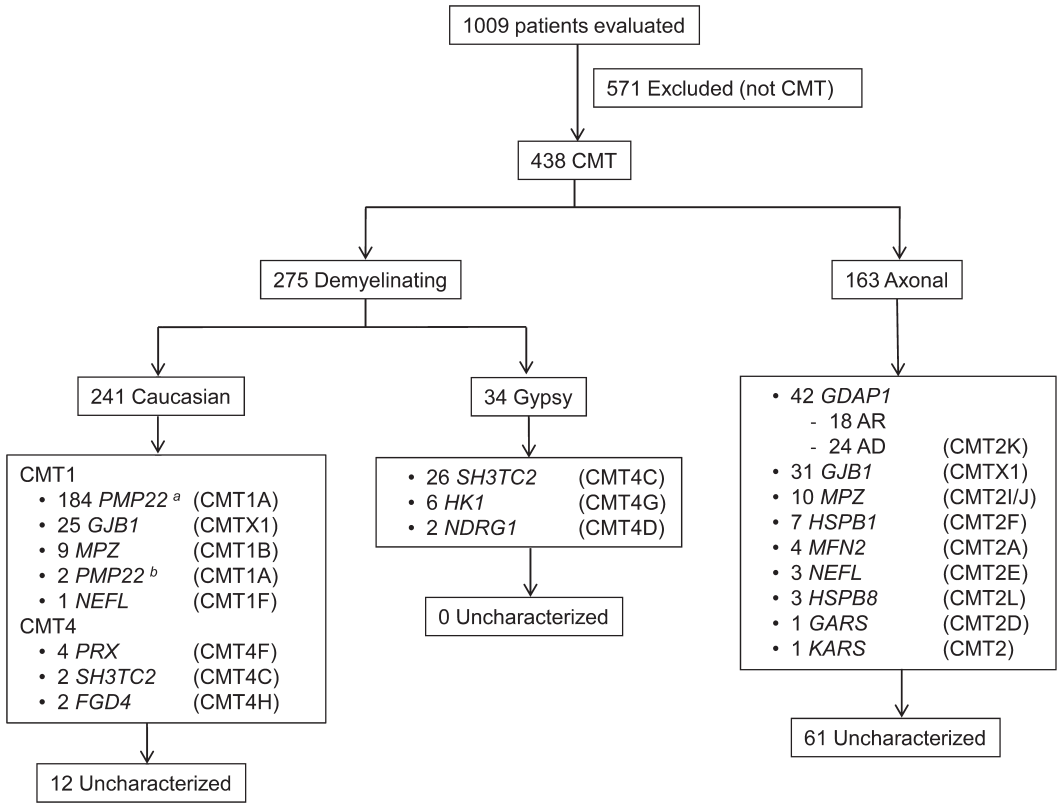
	No. of patients (frequency, %)		
Gene	Present study	Saporta et al.[6]	Murphy et al.[7]
PMP22[a]	184 (48.8)	290 (55)	168 (63.2)
GJB1	56 (14.9)	80 (15.2)	46 (17.3)
GDAP1	42 (11.1)	6 (1.2)	2 (0.8)
SH3TC2	27 (7.2)	3 (0.6)	5 (1.9)
MPZ	19 (5.0)	45 (8.5)	13 (4.9)
NDRG1	7 (1.9)		
HSPB1	7 (1.9)		2 (0.8)
MFN2	6 (1.6)	21 (4.0)	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 (1.0)	6 (2.3)
FGD4	2 (0.5)		
KARS	1 (0.3)		
YARS	1 (0.3)		
TRPV4	1 (0.3)		3 (1.1)
LITAF		5 (1.0)	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 4 shows the relevant clinical features associated with AR forms of demyelinating CMT disease (CMT4). These forms have certain common characteristics such as early onset, delayed motor development, and severe disability, but other features differ between the CMT4 subtypes.

Patients with axonal CMT disease. The mutational screening detailed in table 1 led to identification of the disease-causing mutation in 102 of 163 patients with axonal CMT disease (62.6%). In this set of patients, there is a marked genetic heterogeneity, with mutations in the *GDAP1* and *GJB1* genes being the 2 most frequent causes of axonal CMT disease. Mutations in the *GDAP1* gene correspond to 24 patients

Figure Genetic characterization of CMT disease subtypes



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. AD = autosomal dominant; AR = autosomal recessive; CMT = Charcot-Marie-Tooth.

(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 of our patients with *GDAP1* mutations were defined as CMT2 because 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 whereas patients with dominant *GDAP1* mutations presented with a mild to moderate phenotype with certain clinical and MRI particularities reported previously.[10]

Mutations in the *GJB1* gene were detected in 31 patients with axonal CMT disease (19.0%). It is interesting to note that although the patients were classified as having demyelinating or axonal CMT disease according to the MMNCVs of the proband, more than 80% of these families would be classified as having intermediate forms of CMT disease.

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 remained unknown in 61 patients (37.4%). No change was identified in the following genes: *RAB7*, *DNM2*, *YARS*, *AARS*, *LRSAM1*, and *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 disease 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 disease. In demyelinating patients, these rates are comparable to the other series in which a comprehensive genetic screening was performed (table 2),[6,7,17] suggesting that few genes involved in this form of CMT disease remain undiscovered. However, in CMT2, although the success rate is higher than in other series, 37.4% of patients remain without genetic diagnosis. The mutational distribution described confirms the

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

Gene	Mutation		Presentation	No. of patients	Onset, y	Age at exam, y	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 and 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. Hearing loss. Wheelchair bound in the 4th decade, death at 58 y.
	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.

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; CMT = Charcot-Marie-Tooth; CMTNS = CMT neuropathy score; FDS = Functional Disability Scale; hom = homozygous; 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 been widely described; we have included it because this patient is a compound heterozygote for a novel mutation.

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 with 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 >30 m/s. CMT1A accounts for 66.9% of the demyelinating forms, which is somewhat lower than other series that report slightly more than 70%.[18] Actually, these results are biased by the presence of 34 Gypsy patients affected by demyelinating CMT disease who harbored the previously described founder mutations associated with the Gypsy population as we have previously reported.[11,19] These 34 Gypsy patients and 8 others 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 in other series[6] and may reflect certain populational peculiarities,

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Table 4 Genotype-phenotype correlation of the series of patients with autosomal recessive demyelinating CMT disease

Gene	Mutations	Patients/ families	Onset, y	Age at exam, y	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%

Abbreviations: CMAP = compound muscle action potential of the median nerve (normal values >9.3 mV); CMT = Charcot-Marie-Tooth; CMTNS = CMT neuropathy score; FDS = Functional Disability Scale; hom = homozygous; LL = lower limbs; MMNCV = median motor nerve conduction velocity (normal values in our laboratory >51.6 m/s); NR = not recordable (expressed in % of the patients); SNAP = sensory nerve action potential in median nerve (normal values >16.5 μV); UL = upper limbs.

If more than one case, the numeric values are means and the percentages, are relative frequency of a characteristic.

[a] VIII nerve was considered affected when the patient reported relevant hypoacusia or the hearing loss was confirmed with audiometry.

[b] Nerve conduction studies of median nerve nearest to the moment of physical examination.

[c] The 2 patients with mutations in the *FGD4* gene had an early onset and moderate disability from infancy, but very slow progression thereafter.

as the Region of Valencia hosts a numerous Gypsy population (more than 50,000), and certain isolated areas have a high consanguinity rate.

Mutations in *GJB1* were the second 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 that was not so much influenced by conduction velocities, as by sex.[20,21] Only 5 patients (9%) had signs of CNS involvement (brisk reflexes and Babinski sign in 2 of them) with normal encephalic and spinal MRI. It is worth noting that in 2 of these patients after a long follow-up (>20 years), 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 (first 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*).

There is a great genetic diversity in axonal forms of CMT disease, 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 because of the ample genetic screening that has been performed, and the high relative frequency of *GDAP1*. The genetic distribution in CMT2 shows that the 2 most frequent causes of axonal CMT disease were mutations in the *GDAP1* and *GJB1* genes, which combined accounted for 44.8% of patients who had axonal CMT disease. However, 37.4% of the patients with CMT2 remained 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 neurophysiologic features of axonal CMT disease. 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 compound motor action potential was <0.5 mV, 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, and were wheelchair-bound in the second or third decade in all cases except 2 (associated with p.L344R/p.Q163X compound heterozygote, and p.R282C/p.R282C homozygote genotypes) who had a relatively milder phenotype.[27] Twenty-four of 25 patients with dominant *GDAP1* mutations carried the p.R120W substitution, which is to date the most frequent dominant mutation detected in the *GDAP1* gene. Although this mutation has been described in families with different geographic origins,[28–30] the *GDAP1* p.R120W probably has 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 disease in many series,[7,8] accounting consistently for 10% to 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.

AUTHOR CONTRIBUTIONS

Dr. Sivera: acquisition of data, analysis and interpretation, initial manuscript elaboration. Dr. Sevilla: study concept and design, initial manuscript 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 and 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.

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