

Genetics of Charcot-Marie-Tooth disease type 4A: mutations, inheritance, phenotypic variability, and founder effect

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Charcot-Marie-Tooth (CMT) disease is a motor and sensory neuropathy with clinical and genetic heterogeneity. Patients usually present in the first or second decade of life with distal muscle atrophy in the legs, areflexia, foot deformity (mainly pes cavus), and steppage gait. In most cases, hands are also involved as the disease progresses. CMT is the most frequent inherited neuropathy, with a prevalence in Spain of 28 in 100 000.¹ Based on electrophysiological studies and histopathologic findings in nerve biopsies, CMT has been subcategorised into two main and distinct neuropathies: (i) demyelinating CMT (CMT1, MIM 118200) associated with reduction in a nerve conduction velocities (NCVs) in all nerves and segmental demyelination and remyelination (“onion bulbs”); and (ii) axonal CMT (CMT2, MIM 118220) associated with normal or almost normal NCVs and loss of myelinated axons. Other phenotypes are associated with motor and sensory nerve involvement: Déjérine-Sottas neuropathy (DSN, MIM 145900) is a severe demyelinating neuropathy with onset in infancy, delayed motor milestones, and NCVs less than 10 m/s; congenital hypomyelinating neuropathy (CHN, MIM 605253) is a dysmyelinating neuropathy characterised by infantile hypotonia, distal muscle weakness, and marked reduction of NCVs; hereditary neuropathy with liability to pressure palsies (HNPP, MIM 162500) is a milder sensory and motor neuropathy with periodic episodes of numbness, muscular weakness, and atrophy.²

Genetic heterogeneity is characteristic of the disease not just because of the large number of genes and loci associated with CMT (currently 21 genes),^{3–5} but also because the disease may segregate with different Mendelian patterns. The most frequent pattern of inheritance is autosomal dominant, but autosomal recessive and X linked segregation are also observed.

The relationship of the type of CMT, demyelinating or axonal, with specific genes is not perfect. For instance, *MPZ* mutations are usually manifested clinically as an autosomal dominant demyelinating neuropathy, CMT1B.^{6–7} However, some mutations in *MPZ* have also been found in patients with axonal neuropathy (CMT2-P₀).^{8–9} Moreover, some patients with mutation in *MPZ* expressed the disease as either DSN or CHN.^{10–11} On the other hand, mutations in the same gene may be expressed with a different Mendelian pattern. Mutations in the *PMP22* gene are expressed as dominant mutations, but there are some mutations in *PMP22* that convey an autosomal recessive trait.^{12–13}

CMT disease caused by mutations in the ganglioside-induced differentiation-associated protein 1 (*GDAP1*) gene is a severe autosomal recessive neuropathy originally reported in families with either demyelinating CMT4A neuropathy (MIM 214400)^{14–15} or axonal neuropathy with vocal cord paresis (MIM 607706),¹⁶ which maps to the CMT4A locus on

Key points

- We investigated the genetics and inheritance of the *GDAP1* gene and phenotype expression in a series of 106 isolated and 19 familial cases with Charcot-Marie-Tooth disease and Spanish ancestry, for whom mutations in the *PMP22*, *MPZ*, and *GJB1* genes had previously been excluded.
- We also investigated the existence of founder effects for some recurrent mutations and the origin of these mutations in patients from different countries.
- We found mutations in seven isolated patients, three autosomal recessive families, and two autosomal dominant families. Six out of seven isolated patients were homozygotes or compound heterozygotes with autosomal recessive inheritance and one patient carried a de novo dominant mutation. The mutation detection rate in the sporadic patients was 5.6%. Patients with homozygous or compound heterozygous genotypes showed a severe disease, whereas heterozygous patients from the two autosomal dominant families that segregated the R120W mutation showed a milder phenotype.
- We also report a de novo mutation, T157P, which has not been previously described. Haplotype analysis of the CMT4A locus confirmed a unique origin for the Q163X mutation in 13 Spanish chromosomes and six American Hispanic chromosomes. A common origin for two Spanish chromosomes and two Moroccan chromosomes carrying the S194X was also confirmed.
- We concluded that: (i) although it used to be considered that CMT4A was inherited as an autosomal recessive disorder, some mutations may be expressed in heterozygous patients and segregate dominantly in some families; (ii) *GDAP1* mutations are relatively frequent in our population, thus, the gene could be included in the routine genetic testing of CMT regardless of the inheritance pattern; and (iii) the most frequent mutation, Q163X, is the result of a founder effect as the consequence of a unique origin.

Abbreviations: CHN, congenital hypomyelinating neuropathy; CMAP, compound motor action potential; CMT disease, Charcot-Marie-Tooth disease; DSN, Déjérine-Sottas neuropathy; NCVs, nerve conduction velocities; SNAP, sensory nerve action potential

chromosome 8q21.1. GDAP1 is a 358 amino acid protein whose function is not well known. It is expressed in both the central and peripheral nervous system.^{16,17} A putative role as glutathione S-transferases has been postulated for GDAP1.^{16,17}

Mutations in the *GDAP1* gene have been observed particularly in patients from Mediterranean countries,^{15,16,18–22} but also in those from other European regions.^{15,23,24} The most prevalent mutation in Spain is Q163X,¹⁶ which has also been found in three North American Hispanic families.²⁵ Moreover, S194X, the most frequent mutation in North African countries—Morocco, Tunisia^{15,19,21}—has also been found in Spain.¹⁶ Haplotype analysis of one Moroccan family and one Spanish family suggested a common origin of the S194X mutation found in both countries.¹⁸

We report here the genetics of CMT4A investigated through genetic analysis of the *GDAP1* gene and locus in a series 125 isolated or familial CMT patients with Spanish ancestry. We show that the most frequent mutation, Q163X, has a common origin in families from both Spain and North America. We also present genetic data suggesting that CMT4A displays both autosomal recessive and autosomal dominant inheritance, and we document a possible correlation between the severity of the disease and genetic status.

METHODS

Patients

A cohort of 161 patients belonging to 125 families with Spanish ancestry and with a diagnosis of CMT disease, either axonal or demyelinating, was available for genetic analysis of *GDAP1*. The cohort was distributed as follows: 106 isolated cases, 13 families with autosomal recessive inheritance (categorised thus because more than one sib was affected and parents were normal by examination or history), and six families with autosomal dominant inheritance. Consanguinity was observed in 10 isolated cases and three families. In all cases mutations in the *PMP22*, *MPZ*, and *GJB1* genes had previously been excluded. Mutation analysis¹⁶ and clinical descriptions²⁶ for families LF38, LF249, and LF20 has previously been reported. Mutation studies and clinical data of the Hispanic families HOU531, HOU364, and HOU726, have also been reported elsewhere.²⁵ Informed consent was obtained from patients and relatives before genetic studies. The study was approved by both the Hospital Universitari La Fe and the CSIC Institutional Boards on Bioethics.

SSCP analysis and DNA sequencing

The primers used for amplification of exons 1–6 have been reported elsewhere¹⁶ except a new direct primer for exon 1. The new designed primer is: 5'-CGCCCTCCTCCGGCAGG TACC-3'. PCR conditions were set as follows: 4 min initial denaturation at 95°C, 35 cycles of 30 s denaturation at 94°C, 30 s annealing and elongation at 54–69°C (exon 1, 68°C; exon 2, 55°C; exon 3, 60°C; exon 4, 54°C; exon 5, 60°C; and exon 6, 69°C), and a 7 min final extension at 72°C.

For single-strand conformation polymorphism (SSCP) analysis, 5 µl of PCR products were diluted in 3 µl of 250 mM EDTA, 98% deionised formamide, 0.25% xylene cyanol, and 0.25% bromophenol blue. DNA was denatured for 10 min at 95°C and was kept on ice for more than 2 min. Then 6 µl of the mix was loaded onto 12% polyacrylamide with or without 5% glycerol. The non-denaturing gels were run at 800 V for 16 h at room temperature and visualised by silver staining.

Mutation screening was performed by direct sequencing of purified PCR products (Qiagen, Hilden, Germany) in an ABI PRISM 3100 sequencing analyser by using fluorescent

dideoxynucleotides and one of the PCR primers. All sequences on both strands were determined.

Microsatellite and SNP genotyping

Genotyping for the six microsatellite markers linked to the CMT4A locus was performed using a similar PCR protocol: 4 min initial denaturation at 95°C, 35 cycles of 30 s denaturation at 94°C, 30 s annealing and elongation at 55°C (*D8S279*, *D8S286*, and *D8S551*) or 58°C (*D8S1474*, *D8S1289*, and *D8S84*), and a 7 min final extension at 72°C. PCR products were run on a 12% non-denaturing polyacrylamide gel at 800 V for 10 h at room temperature. Allele fragments were visualised by silver staining. Allelic numbers for every marker except *D8S1474* are according to the Genome Database (<http://www.gdb.org/>). *D8S1474* alleles and frequencies were established in the general Spanish population. The determination of haplotype phase was based either on homozygosity or on analysis of parental samples, which allowed us to fully or partially define the phase.

Analysis of the c.507T/G single nucleotide polymorphism (SNP) was performed by PCR amplification of exon 4 and restriction digestion with *DdeI*. After digestion the presence of two bands of 168 and 120 bp indicates allele T, whereas a 288 bp undigested band indicates allele G. Both rs1025928 and rs959424 SNPs (UCSC Human Genome Browser, Human Genome Working Draft, <http://genome.ucsc.edu/goldenPath/septTracks.html>) were investigated by SSCP analysis.

Estimation of Q163X mutation age was performed by means of the maximum likelihood method implemented in the program BDMC21 v2.1 (available at <http://www.rannala.org/labpages/software.html>).²⁷ The program parameter setting we used were: growth rate = 0.005, sample fraction = 0.000325 (assuming a carrier frequency of 1/200 in the general population, a total population size of 40 million individuals, and a mutation rate of short tandem repeat (STR) linked markers of 0.0001). Program data settings were: 13 mutant copies, which are the mutant alleles in our sample, and two segregating sites. Program option settings were: conditional on copy number = yes, number of Monte Carlo replicates = 10 000, initial time = 10, final time = 500, and interval increments = 10.

Exclusion of false paternity was performed by using 10 microsatellite markers with high heterozygosity from the CHLC Human Screening Set/Weber version 6 (Research Genetics, Huntsville, AL, USA): *D1S518*, *D2S1326*, *D3S2459*, *D4S2368*, *D5S2494*, *D6S1956*, *D7S1808*, *D8S1477*, *D9S301*, and *D10S1432*.

RESULTS

Mutation analysis

In order to investigate the frequency and type of *GDAP1* mutations in the Spanish population, we screened each coding exon and flanking intronic sequences of *GDAP1* for mutations in a series of 125 CMT probands from unrelated families or isolated cases. All patients had Spanish ancestry. We observed mutations in 12 probands. The mutations described elsewhere—Q163X, S194X, and T288fsX290¹⁶—were found in one or more additional families. Three novel mutations were found (table 1). One patient with an S194X mutation in one chromosome presented a 4 bp AAAG deletion on the other chromosome. This deletion is a frameshift mutation that generates a stop codon at position 145 (E114fsX145) and predicts a major disruption of *GDAP1* synthesis. Two mutations were missense amino acid changes, R120W and T157P. The R120W mutation was found in the heterozygous state in two families segregating the CMT phenotype as an autosomal dominant trait and the T157P mutation was detected in one sporadic case again expressing the disease as a dominant trait (fig 1). We found no other

mutations in the dominant patients after sequencing all exons. Comparison of the mutated amino acids with the corresponding amino acids in the orthologous genes of *GDAP1* in mouse, rat, and zebrafish, and the paralogous gene *GDAP1L1*, mapped on human chromosome 20, and its orthologues in mouse, rat, and zebrafish¹⁷ revealed that the two missense mutations were non-conservative substitutions that altered highly conserved amino acids (table 1). Moreover, we did not find these nucleotide changes in 196 control chromosomes in individuals from the general Spanish population, suggesting again that neither R120W nor T157P are neutral polymorphisms. In short, we found 21 chromosomes bearing a *GDAP1* mutation in five families and seven sporadic cases.

Mendelian inheritance

Both types of CMT4A, demyelinating and axonal, were originally described in families segregating the disease as an autosomal recessive trait. Further studies have confirmed that patients carry two mutations and healthy parents are heterozygotes. By analysing the present series we found two families and one sporadic case with just one mutation in the coding sequence of the gene. In family LF292, the R120W

mutation segregates with the disease phenotype in three generations (fig 1A). The mutation was associated with an extended haplotype constructed with microsatellite markers *D8S279* to *D8S84* (figs 1A and 2). In family LF293 we detected the R120W mutation in two affected brothers (fig 1B). In this family the parents were not available for genetic and clinical studies but the father was affected by history. The proband from family VAL8 was heterozygous for the T157P mutation (fig 1C); the mutation was not found in the parents. By genotyping 10 non-linked STRs we excluded false paternity and we confirmed that the mutation is a de novo event. Taken as a whole, the three families represent expression of the CMT phenotype as a dominant disorder.

Phenotypic variability

In order to correlate the mutations and inheritance patterns with disease severity, electrophysiological data and information on the clinical features of probands were compiled (table 2). Patients carrying two mutations and recessive inheritance showed a severe phenotype with very early onset in infancy and were wheelchair bound at the end of their first or the beginning of their second decade. In family LF38 affected members needed a wheelchair by the age of 30.²⁶ By

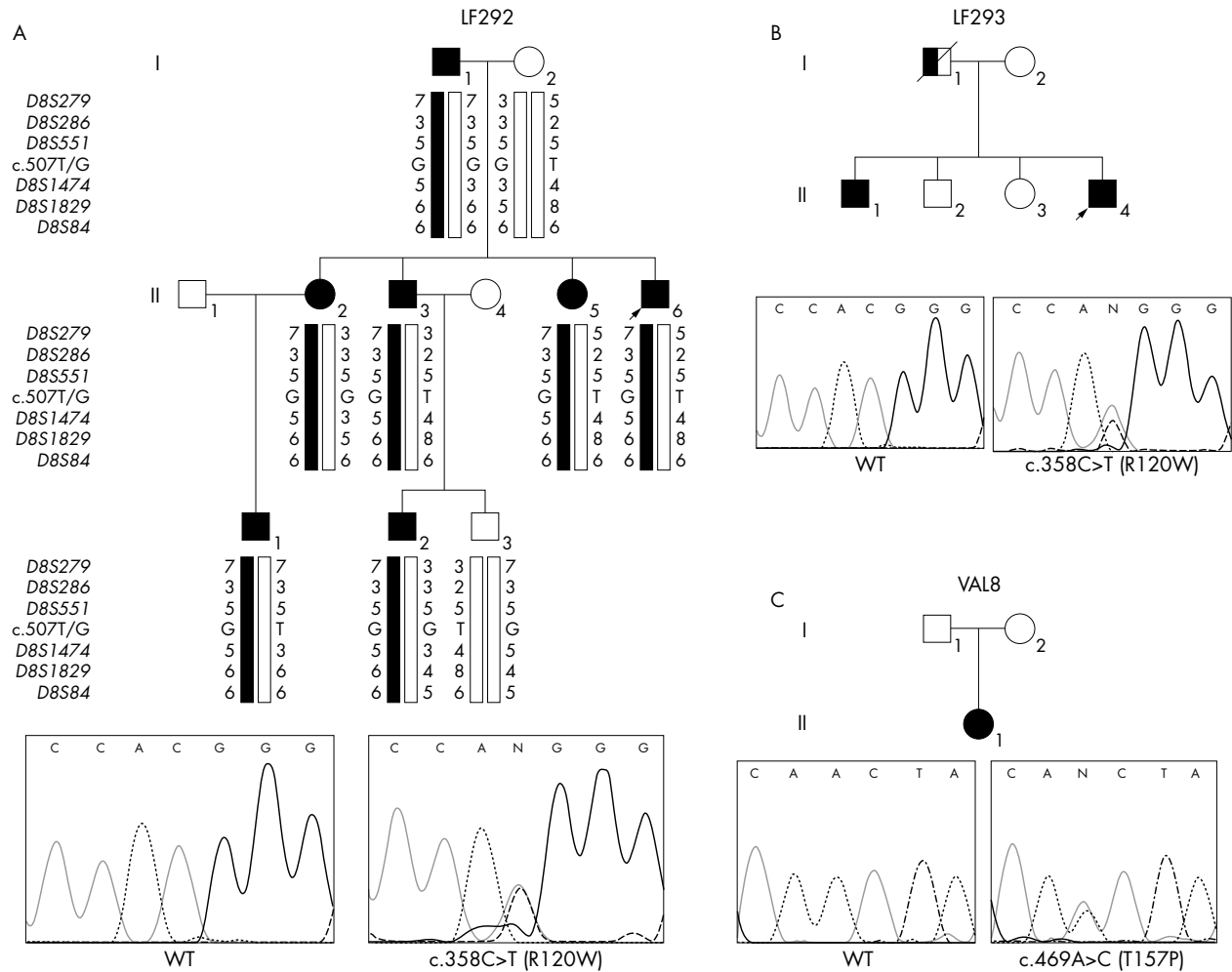


Figure 1 Pedigrees, mutations, and haplotype segregation of autosomal dominant CMT4A families. Affected individuals are indicated by filled symbols, unaffected individuals by unfilled symbols. Direct sequences of *GDAP1* mutations are identified in the three probands. Wild type sequence is on the left and mutation is on the right for each family. (A) Family LF292 with three affected generations; below the symbol for each individual are the genotypes for seven markers; the shared disease associated haplotype is in black. (B) Family LF293 for which dominant segregation is postulated because of involvement of the dead father by history (indicated as a half filled symbol). (C) Sporadic patient as the consequence of a de novo T157P mutation.

Table 1 Mutations in the *GDAP1* gene of CMT4A patients

Exon	Nucleotide change	Effect on coding sequence	Number of mutant chromosomes	Corresponding amino acid in mouse, rat, and zebrafish <i>GDAP1</i> , and in human, mouse, rat, and zebrafish <i>GDAP1L1</i>
3	c.342-345delAAAG	E114fsX145, protein truncation	1	–
3	c.358C→T	R120W	2	R, R, R, R, R, R, R
4	c.469A→C	T157P	1	T, T, T, T, T, T, T
4	c.487C→T	Q163X, protein truncation	13	–
5	c.581C→G	S194X, protein truncation	2	–
6	c.863-864insA	T288fsX290, protein truncation	2	–

contrast, patients from the dominant families LF292 and LF293, which carry the identical mutation R120W, show a mild phenotype with onset at the end of second decade and very slow evolution. They are currently able to walk and need no orthopaedic devices. Muscle strength and deep tendon reflexes were more affected in recessive than in dominant patients. A hoarse voice was evident in patients from seven of eight recessive families but not in patients from dominant families. The sporadic patient from family VAL8 is a 9 year old girl who shows a severe phenotype with early onset of symptoms in the first year of life, moderately reduced distal strength in lower limbs, absent tendon reflexes, and optic atrophy.

It is noteworthy that electrophysiologic studies indicated that all patient except for probands from families LF127 and LF135 showed normal or mildly reduced NCVs. In these two patients, however, both compound motor action potential (CMAP) and sensory nerve action potential (SNAP) were strongly reduced. These findings suggest that the main lesion in all patients is localised to the axon.

Haplotype analysis and founder effect

We found four mutations in more than one chromosome (table 1). To determine whether each mutation had a common ancestral origin, we proceeded to investigate the presence of a shared common haplotype. To construct haplotypes we searched for SNPs in the coding sequence and flanking intronic sequences of *GDAP1*. After screening 224 chromosomes by SSCP analysis, we only found the c.507T/G SNP in the coding sequence (exon 4) which represents a synonymous change, S169S. This SNP has also been reported by others.²⁵ We then proceeded to construct extended haplotypes by analysis of six flanking STR markers, *D8S279*, *D8S286*, *D8S551*, *D8S1474*, *D8S1829*, and *D8S84*, which span ~2.83 Mb around the *CMT4A* locus (fig 2).

The most frequent mutation in our series was Q163X. Five patients were homoallelic for Q163X and three patients were

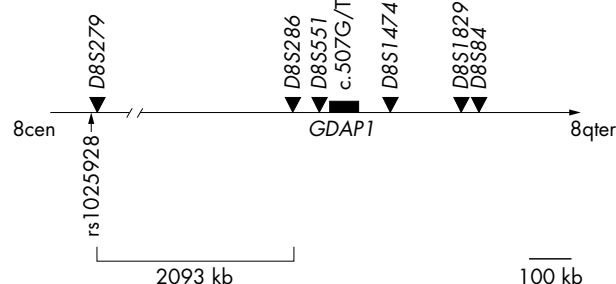


Figure 2 Physical map of the *CMT4A* locus. The *GDAP1* gene is indicated as a box. Linked microsatellite markers are indicated at the top. The rs1025928 SNP at the *D8S279* locus is shown at the bottom.

compound heterozygous for Q163X and a different mutation. Four patients, including three homozygotes, came from valleys in the Basque Country; thus, seven Q163X bearing chromosomes had a Basque origin. The other six mutant chromosomes came from Castile and Leon and the Valencia region of Spain (fig 3).

We constructed haplotypes at the *CMT4A* locus in our families. When considering only the *D8D551*-c.507G/T-*D8S1474* core haplotype in the Spanish patients, three different haplotypes were associated with the Q163X mutation: the main haplotype 5-G-3 was observed in 11 chromosomes, whereas haplotypes 4-G-3 and 5-G-2 were associated with chromosomes C and F, respectively (fig 4). The observed allele variations represent a change of just one repeat that could be produced by mutation. Thus, we suggested that the three haplotypes represent a common ancestral haplotype (the C and F chromosomes) caused by mutations of repeat elements. We postulated that the Q163X mutation originated once in the Iberian peninsula and that the present patients and families segregating the *CMT4A* phenotype are the consequence of a founder effect.

To confirm this hypothesis we extended haplotype analysis with more STRs. Analysis of all markers again suggested the presence of a common ancestral haplotype 7-3-5-G-3-6-6. Seven out of 13 Spanish chromosomes shared the complete haplotype, while we found variations in some STRs in the other six chromosomes (fig 4). We observed different alleles at markers *D8S286* and *D8S1829* on chromosome C. This finding suggests chromosome C derived from the ancestral chromosome after several mutation or recombination events.

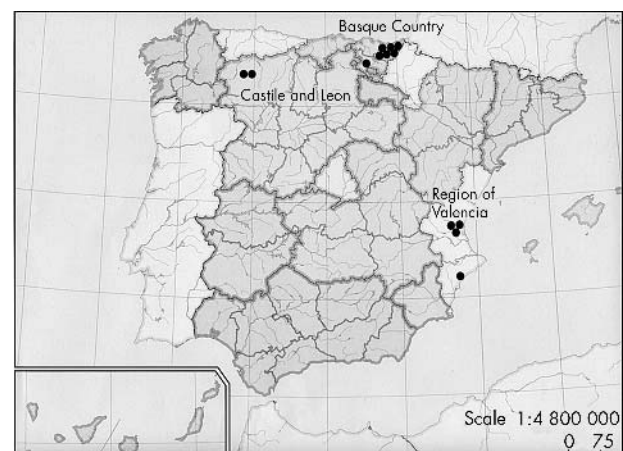


Figure 3 Map of Spain showing the 17 autonomous regions. Chromosomes bearing Q163X mutations are represented as filled circles: there are seven in the Basque Country, four in the Valencia region, and two in Castile and Leon.

Family	Chromosome no.	5' flanking markers				GDAP1 SNP	3' flanking STRs			Ethnic origin	Ref.
		rs1025928	D8S279	D8S286	D8S551	c.507G/T	D8S1474	D8S1829	D8S84		
LF38	A	C	7	3	5	G	3	6	6	Spain/Leon	PR, Cuesta et al ¹⁶
	B	C	7	3	5	G	3	6	6		
LF249	C	C	7	2	4	G	3	5	6	Spain/Valencia	PR, Cuesta et al ¹⁶
LF20	D	C	7	3	5	G	3	6	6	Spain/Valencia	PR, Cuesta et al ¹⁶
LF135	E	C	5 mut	3	5	G	3	6	6	Spain/Valencia	PR
	F	T	5 rec	3	5	G	2	6	6		
VAL1	G	C	7	3	5	G	3	6	6	Spain/Basque	PR
	H	C	5 mut	3	5	G	3	6	6		
VAL3	I	C	6 mut	3	5	G	3	6	6	Spain/Basque	PR
	J	T	3 rec	3	5	G	3	6	6		
VAL4	K	C	7	3	5	G	3	6	6	Spain/Basque	PR
	L	C	7	3	5	G	3	6	6		
VAL5	M	C	7	3	5	G	3	6	6	Spain/Basque	PR
HOU364	N	C	7	3	5	G	3	6	6	Costa Rica	PR, Boerkoel et al ²⁵
	O	C	7	3	5	G	3	6	6		
HOU726	P	C	7	3	5	G	3	6	6	Costa Rica	PR, Boerkoel et al ²⁵
	Q	C	7	3	5	G	3	6	6		
HOU531	R	T	3 rec	3	5	G	3	6	6	Peru	PR, Boerkoel et al ²⁵
	S	C	5 mut	3	5	G	3	6	6		

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Figure 4 GDAP1 haplotypes associated with the Q163X mutation. The figure shows haplotypes associated with the 13 Spanish chromosomes (A to M) and the six (N to S) Hispanic North American chromosomes bearing the Q163X mutation. Families are indicated in the first column. Ethnic origin is indicated. A grey colour code is used to identify the marker alleles shared by all chromosomes. Alleles for some markers diverge from the common ancestral haplotype. Light grey labelling indicates that it may have been generated by mutation (mut); by contrast, white labelling suggests that it was the result of a recombination event (rec).

However, since flanking markers *D8S279* (allele 7 represents 0.08% of normal chromosomes) and *D8S84* (allele 6 represents 0.54% of normal chromosomes) did not change with regard to the common haplotype, we suggest a unique origin for the Q163X mutation is most likely. At locus *D8S279* the ancestral chromosome is split into three different haplotypes: three chromosomes carry allele 5 (chromosomes E, F, and H), one chromosome allele 3 (chromosome J), and another chromosome allele 6 (chromosome I). To determine if variation was generated by recombination (*D8S279* maps 2.1 Mb away from *D8S286*) or by mutation of repeats, we extended the genotype of the *D8S279* locus with closely linked SNPs. We reasoned that analysis of closely linked SNPs would reduce the risk of marker mutation whereas the chance of recombination is minimal. We typed rs1025928 (allele T 70%, allele C 30% in the normal population) in patients. We observed five allele segregations of rs1025928-*D8S279* markers in Spanish chromosomes: C-7, T-5, C-5, T-3, and C-6. We postulated that the short haplotype C-7 represents the ancestral chromosome carrying the C-7-3-5-G-3-6-6 extended haplotype. Thus, when allelic variation at *D8S279* was associated with allele C we interpreted it as the consequence of mutation (chromosomes E, H, and I), whereas the presence of allele T suggested that variation at *D8S279* was the consequence of a recombination event (chromosomes F and J). By using intra-allelic variability of the closest markers *D8S551* and *D8S1474*, we estimated the age of the Q163X mutation to be approximately 33 000 years old (1650 generations, 20 year generations).

Recently, Boerkoel *et al*²⁵ reported three North American Hispanic families from Texas with patients homozygous for the Q163X mutation. Two families came from Costa Rica

(families HOU364 and HOU726) and one from Peru (family HOU531). Since patients of the three families shared a common haplotype and they are Hispanic in origin, a founder mutation originating in the Iberian peninsula was postulated. To confirm this hypothesis we compared haplotypes at the CMT4A locus with those of the Spanish patients. We observed that four of the Hispanic chromosomes shared the common haplotype C-7-3-5-G-3-6-6 with Spanish chromosomes. The other two chromosomes showed allelic differences for the proximal markers rs1025928 and *D8S279* (fig 4). These data confirm that the Q163X mutation in patients from both sides of the Atlantic has a common ancestral origin and suggest the existence of a founder effect for the Q163X mutation in the Iberian peninsula. Interestingly, we observed identical variation at locus *D8S279* in both Spanish and Hispanic chromosomes. Chromosomes R and J share the same haplotype, whereas chromosome S has the identical haplotype to chromosomes E and H. These findings suggest two possible hypotheses about migration: first, the Q163X mutation moved from Spain to America once, probably associated with allele 7, and later the other haplotypes appeared either by recombination or by mutation; second, the Q163X mutation arrived into America in more than one migration movement.

We also applied haplotype analysis to the other mutations each found in two chromosomes: S194X and T288fsX290 (fig 5). We observed that every mutation could be associated with a unique haplotype confirming a unique origin for each one. The S194X mutation was associated with a common *D8S551* to *D8S84* haplotype in the two Spanish chromosomes. By studying the extended haplotype in the Moroccan family PN860 we confirmed that S194X mutations have a common

Mutation	Family	5' flanking STRs			GDAP1 SNP	3' flanking STRs			Origin	Ref.
		D8S279	D8S286	D8S551	c.507G/T	D8S1474	D8S1829	D8S84		
S194X	LF249	3	4	2	T	1	5	5	Spain	PR, Cuesta et al ¹⁶
	LF127	7	3	2	T	1	5	5	Spain	PR
	PN860	6	4	2	T	1	5	5	Morocco	PR, Nelis et al ¹⁸
		6	4	2	T	1	5	5		
T288fsX290	LF20	5	3	5	T	1	6	7	Spain	PR, Cuesta et al ¹⁶
	LF127	5	3	5	T	2	5	6	Spain	PR

PR, present report

Figure 5 GDAP1 haplotypes associated with S194X and T288fsX290 mutations in Spanish and Moroccan chromosomes. Shared alleles for each marker are indicated by a grey shading. When a recombination event is suspected alleles are labelled in white.

origin in Spain and Morocco, as previously reported by Nelis *et al.*¹⁸ The two chromosomes bearing the T288fsX290 mutation shared a common haplotype between markers D8S279 and c.507G/T, but differed in the three distal markers. This difference suggests that a recombination event occurred between GDAP1 and D8S1474.

DISCUSSION

The genetics of motor and sensory hereditary neuropathies is complex. These disorders show a wide phenotypic and genetic heterogeneity. We addressed the genetics of CMT4A in a series of 125 unrelated families with Spanish ancestry. We found six different mutations in GDAP1, five previously reported^{15, 16, 23} and one new mutation, in 12 families. Interestingly, we found mutations in seven isolated cases, three autosomal recessive families previously reported,¹⁶ and two autosomal dominant families. Six out of seven sporadic patients were homozygotes or compound heterozygotes, whereas one patient was heterozygous for the T157P mutation that originated as a de novo mutation and, hence, was expressed as a dominant mutation. In any case there was a history of consanguinity. The seven sporadic patients represent a detection rate of 5.6% in the 106 isolated cases of the series, a screening yield suggesting that GDAP1 testing may be indicated in the mutation analysis of isolated CMT patients. Nevertheless, as no mutation has been reported in isolated cases from other series,²³ inclusion of GDAP1 in the molecular diagnosis routine of CMT may depend on the prevalence of CMT4A in each country.

Autosomal dominant inheritance has not previously reported in CMT patients and families with GDAP1 gene mutations. Patients from families LF292 and LF293 carried the R120W mutation and segregated the disease as an autosomal dominant trait. They showed a mild phenotype with age of onset in the second decade or even later and are still walking (I-1 in family LF292 is 70 years old). This mild phenotype in heterozygous patients is in contrast with the apparently healthy parents of patient CMT-82.3 reported by Ammar *et al.*²³ who is compound heterozygous for the R120W and G271R mutations. However, while the patient inherited the G271R mutation from her mother, the R120W mutation had to be inherited from her deceased father; thus, it is impossible to know whether or not the father would have developed the symptoms of CMT disease. It is possible that R120W substitution may produce a mild clinical picture in heterozygotes but a more severe phenotype when it is associated with another mutation.

The Q163X mutation is clearly the most frequent mutation in Spain. It has not been found in patients from other countries except for three Hispanic North American families.

These three families shared a common associated haplotype indicating a founder mutation with a possible origin in the Iberian peninsula.²⁵ Haplotype analysis suggests that the Q163X mutation in several families from different regions in Spain is the consequence of a founder effect that we have dated to many centuries ago, and confirms the Spanish origin of the mutant chromosomes in the Hispanic families. Interestingly, identical variations at markers rs1025928 and D8S279 found in the Spanish chromosomes (chromosomes E, H, and J) were also observed in the Hispanic chromosomes (chromosomes R and S). This coincidence suggests that D8S279 divergence might have occurred before carrier individuals went to America. Thus, the most likely series of events is that Q163X migrated from the Iberian peninsula to America in three separate migrations. Seven out of 13 Spanish chromosomes are from the Basque Country, six of them from two valleys close together in the province of Guipuzkoa. Chromosomes H and J have been found in two Basque patients while the other Basque patients carry the common ancestral chromosomes also found in other Spanish patients. Since chromosomes H and J are identical to chromosomes S and R, respectively, we propose that the latter arrived in Peru from the Basque Country. By contrast, determination of the origin of the four Costa Rican chromosomes is more difficult because we have no data on the origin of the mutation or the distribution of the ancestral chromosome in the Iberian peninsula.

We estimated the age of the Q163X mutation to be 33 000 years, which makes it a relatively ancient mutation. It predates the most recent Neolithic expansion of modern humans into Europe, roughly 10 000–15 000 years ago, and corresponds more closely with the early Palaeolithic expansion.²⁸ It is interesting to speculate that the mutation may have originated in or arrived into the Basque Country and then extended to the rest of the Iberian peninsula.

A second mutation, S194X, has been described both in Spanish families¹⁶ and in Tunisian¹⁵ and Moroccan families^{18, 19} from the countries referred to as the Maghreb (Morocco, Algeria, and Tunisia). As previously reported,¹⁸ we have confirmed the common origin of this mutation in two unrelated Spanish families and one Belgian family with Moroccan ancestry. Moreover, we also observed that the T288fsX290 mutation, found in two chromosomes from unrelated families, has a common origin. In total, we have demonstrated that three mutations in the Spanish population are the consequence of founder effects.

In summary, both the genetics of the GDAP1 gene and clinical expression of CMT4A neuropathy is complex. Most families segregate the neuropathy as a recessive trait although some families show dominant segregation of mutations that may be associated with a milder phenotype.

Table 2 Clinical summary of CMT4A proband patients

Family proband	Mutations	Age (years)/gender	Age at onset	Presenting symptom	Age of walking (months)	Age wheelchair needed	Muscle weakness	Sensory disturbance	Hoarse voice	Deep tendon reflexes	Motor NCV/CMAP (m.s ⁻¹ /mV)	Sensory NCV/SNAP (m.s ⁻¹ /μV)	Sural nerve biopsy
Autosomal recessive													
IF249	Q163X/S194X	46/M	18 months	Gait clumsy, frequent falls	12	12	Dist. LL 0/5, dist. UL 0/5	Decreased P, V, T, Po	Yes (vocal cord paresis)	Absent	Median nerve: NR/NR	Sural nerve: 37/1.3	Reduced number of large myelinated fibres, occasional onion bulbs and regenerative clusters
IF20	Q163X/T288fs	42/M	Birth	Floppy infant, retarded milestones	24	9	Dist. LL 0/5, dist. UL 0/5	Decreased P, V, T, Po	Yes (vocal cord paresis)	Absent	Median nerve: NR/NR	Sural nerve: 46.3/0.2	Reduced number of large myelinated fibres, occasional onion bulbs and regenerative clusters
IF38	Q163X/Q163X	56/M	2 years	NA	Delayed	30	Dist. LL 0/5, dist. UL 0/5	Decreased P, V, T, Po	Yes	Absent	Median nerve: 41/1.4	NA	ND
IF127	S194X/T288fs	13/F	2 years	Walking problems	11	13	Prox. LL 4/5, dist. LL 0/5	NA	Yes	Absent	Median nerve: NR/NR	Sural nerve: NR/NR	ND
IF135	Q163X/Q163X	17/F	18 months	Walking problems	NA	NA	Dist. LL 0-1/5, dist. UL 2/5	Normal	Yes	Absent	Median nerve: 26/0.7	Median nerve: 53/3	NA
VAL1	Q163X/Q163X	54/F	14 months	Muscle weakness, frequent falls	10	10	Prox. LL 2/5, dist. LL 0/5	Normal	Yes (vocal cord paresis)	Absent	Normal NCV with reduced CMAP	Normal NCV with reduced SNAP	Reduced number of large myelinated fibres, no onion bulbs
VAL3	Q163X/Q163X	19/M	18 months	Muscle weakness and atrophy	17	7	Prox. LL 2/5, dist. LL 0/5	Normal	Yes	Absent	EMG: neurogenic pattern, normal NCVs	EMG: neurogenic pattern, unexcitable nerves	ND
VAL4	Q163X/Q163X	53/M	17 months	Foot walking problems	14-15	NA	Prox. LL 3-4/5, dist. LL 0/5	Normal	Yes (vocal cord paresis)	Absent	EMG: neurogenic pattern, unexcitable nerves	EMG: neurogenic pattern, unexcitable nerves	ND
VAL5	Q163X/E114fs	4/M	10 months	Retarded milestones	15	Walking	11 years	Normal	No	Absent	Median nerves: right UL 31.9/9.4; left UL 24/1.2	NA	ND
Autosomal dominant													
IF292	R120W/+	36/M	18 years	Feet pain, stepage gait	NA	Walking	Dist. LL 3-4/5	Reduction of pain and profound senses	No	Normal except ankle reflexes	Normal NCVs and CMAPs in median, peroneal, and posterior tibial nerves	Sural nerves: reduction of NCVs and SNAPs	ND
IF293	R120W/+	34/M	14 years	Muscle weakness and atrophy, clumsiness, gait instability, Delayed gait	NA	Walking	Dist. LL 4/5	Normal	No	Normal except ankle reflexes	Peroneal nerve: 45/mild reduction of CMAP	NA	ND
VAL8	T157P/+	10/F	12 months	Delayed gait	15 months	Walking	Prox. LL 5/5, dist. LL 2-3/5	Reduced	No	Absent	45/15	NA	ND

CMAP, compound motor action potential; LL, lower limbs; SNAP, sensory nerve action potential; UL, upper limbs (the Medical Research Council (MRC) scale is used), sensory disturbance: P, pinprick; Po, positional; T, touch; V, vibratory. NA, not available; ND, not done; NR, not recorded.

Finally, most of the mutations causing the rare disease of CMT4A originated once in human history.

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ELECTRONIC-DATABASE INFORMATION



The URLs mentioned in this study are as follows: Genome Database, <http://www.gdb.org/>; UCSC Human Genome Browser, Human Genome Working Draft, <http://genome.ucsc.edu/goldenPath/septTracks.html>; and BDMC21 v2.1 software available from <http://www.rannala.org/labpages/software.html>.

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