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


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 electrophysiologic 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-P).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 MPZ gene are expressed as dominant mutations, but there are some mutations in MPZ 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 MPZ, PMP22, 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, Déjerine-Sottas neuropathy; NCVs, nerve conduction velocities; SNAP, sensory nerve action potential.
Mutations in the \textit{GDAP1} gene have been observed particularly in patients from Mediterranean countries,\textsuperscript{15} 16 19 21 but also in those from other European regions.\textsuperscript{17 21 24} The most prevalent mutation in Spain is Q163X,\textsuperscript{16} which has also been found in three North American Hispanic families.\textsuperscript{22} Moreover, S194X, the most frequent mutation in North African countries—Morocco, Tunisia—\textsuperscript{15} 19 21—has also been found in Spain.\textsuperscript{16} Haplotype analysis of one Moroccan family and one Spanish family suggested a common origin of the S194X mutation found in both countries.\textsuperscript{18}

We report here the genetics of CMT4A investigated through genetic analysis of the \textit{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.

\section*{METHODS}

\subsection*{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 \textit{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 \textit{PMP22}, \textit{MPZ}, and \textit{GJB1} genes had previously been excluded. Mutation analysis\textsuperscript{20} and clinical descriptions\textsuperscript{16} 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.\textsuperscript{21} 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.

\subsection*{SSCP analysis and DNA sequencing}

The primers used for amplification of exons 1–6 have been reported elsewhere\textsuperscript{22} except a new direct primer for exon 1. The new designed primer is: 5'-CGGCCTCTTCTGCGGCCAGG 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.

\section*{RESULTS}

\subsection*{Mutation analysis}

In order to investigate the frequency and type of \textit{GDAP1} mutations in the Spanish population, we screened each coding exon and flanking intronic sequences of \textit{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 T288fsX290\textsuperscript{16}—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 GDAPILLI mapped on human chromosome 20, and its orthologues in mouse, rat, and zebrafish 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. By

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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.
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 electrophysiological 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 more affected in recessive than in 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.

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. 22 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 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 DBD551-c.507T/G-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 7-3-3-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.

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

<table>
<thead>
<tr>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Effect on coding sequence</th>
<th>Number of mutant chromosomes</th>
<th>Corresponding amino acid in mouse, rat, and zebrafish GDAP1, and in human, mouse, rat, and zebrafish GDAP1L1</th>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>c.469A→C</td>
<td>T157P</td>
<td>1</td>
<td>T, T, T, T, T, T</td>
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</table>

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.

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.
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 the existence of a founder effect for the Q163X mutation. 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 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. 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 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

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### Table: GDAP1 haplotypes associated with the Q163X mutation

<table>
<thead>
<tr>
<th>Family</th>
<th>Chromosome no.</th>
<th>rs1025928</th>
<th>D8S279</th>
<th>D8S286</th>
<th>D8S551</th>
<th>c.507G/T</th>
<th>D8S1474</th>
<th>D8S1829</th>
<th>D8S84</th>
<th>Ethnic origin</th>
<th>Ref.</th>
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<tr>
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<td>G</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td>Spain/Leon</td>
<td>PR, Cuesta et al.16</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>C</td>
<td>7</td>
<td>3</td>
<td>5</td>
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<td>3</td>
<td>6</td>
<td>6</td>
<td></td>
<td>PR, Cuesta et al.16</td>
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<tr>
<td>LF249</td>
<td>C</td>
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<td>2</td>
<td>4</td>
<td>G</td>
<td>3</td>
<td>5</td>
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<tr>
<td></td>
<td>F</td>
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<td></td>
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<td>C</td>
<td>5 mut</td>
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<td>5</td>
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<tr>
<td>HOU531</td>
<td>R</td>
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<td>6</td>
<td>6</td>
<td>Peru</td>
<td>PR, Boerkoel et al.25</td>
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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).
origin in Spain and Morocco, as previously reported by Nelis et al. 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 and one new mutation, in 12 families. Interestingly, we found mutations in seven isolated cases, three autosomal recessive families previously reported, 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, 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 phenotype in heterozygous patients is in contrast with the phenotype in heterozygous patients. Since chromosomes H and J are identical to chromosomes E, D8S279 found in the Spanish chromosomes (chromosomes E, H, and J) were also observed in the Spanish 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 Palaolithic expansion. 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 families and in Tunisian and Moroccan families from the countries referred to as the Maghreb (Morocco, Algeria, and Tunisia). As previously reported, we have confirmed the common origin of this mutation in two unrelated Spanish families and one Belgian family with Morrocan 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>
<thead>
<tr>
<th>Family proband</th>
<th>Mutations</th>
<th>Age (years)/gender</th>
<th>Age at onset</th>
<th>Presenting symptom</th>
<th>Age of walking (months)</th>
<th>Age wheelchair needed</th>
<th>Muscle weakness</th>
<th>Sensory disturbance</th>
<th>Haarne voice</th>
<th>Deep tendon reflexes</th>
<th>Motor NCV/CMAP (m.s⁻²/mV)</th>
<th>Sensory NCV/SNAP (m.s⁻²/mV)</th>
<th>Sural nerve biopsy</th>
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<tr>
<td>Autosomal recessive</td>
<td>LF249 Q163X/S194X</td>
<td>46/M</td>
<td>18 months</td>
<td>Gait clumsy, frequent falls</td>
<td>12</td>
<td>12</td>
<td>Dist. LL 0/5, dist. UL 0/5</td>
<td>Decreased P, V, T, Po</td>
<td>Yes</td>
<td>(vocal cord paresis)</td>
<td>Absent</td>
<td>Median nerve: NR/NR</td>
<td>Sural nerve: 37/1.3</td>
</tr>
<tr>
<td>LF20 Q163X/T288fs</td>
<td>42/M</td>
<td>Birth</td>
<td>Floppy infant, retarded milestones</td>
<td>24</td>
<td>9</td>
<td>Dist. LL 0/5, dist. UL 0/5</td>
<td>Decreased P, V, T, Po</td>
<td>Yes</td>
<td>(vocal cord paresis)</td>
<td>Absent</td>
<td>Median nerve: NR/NR</td>
<td>Sural nerve: 46.3/0.2</td>
<td>Reduced number of large myelinated fibres, occasional onion bulbs and regenerative clusters</td>
</tr>
<tr>
<td>LF38 Q163X/Q163X</td>
<td>56/M</td>
<td>2 years</td>
<td>NA</td>
<td>Delayed</td>
<td>30</td>
<td>Dist. LL 0/5, dist. UL 0/5</td>
<td>Decreased P, V, T, Po</td>
<td>Yes</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF127 S194X/T288fs</td>
<td>13/F</td>
<td>2 years</td>
<td>Walking problems</td>
<td>11</td>
<td>13</td>
<td>Prox. LL 4/5, dist. UL 0/5</td>
<td>Normal</td>
<td>Yes</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LF35 Q163X/Q163X</td>
<td>17/F</td>
<td>18 months</td>
<td>Walking problems</td>
<td>NA</td>
<td>NA</td>
<td>Dist. LL 0–1/5, dist. UL 2/5</td>
<td>Normal</td>
<td>Yes</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAL1 Q163X/Q163X</td>
<td>54/F</td>
<td>14 months</td>
<td>Muscle weakness, frequent falls</td>
<td>10</td>
<td>10</td>
<td>Prox. LL 2/5, dist. UL 0/5</td>
<td>Normal</td>
<td>Yes</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAL3 Q163X/Q163X</td>
<td>19/M</td>
<td>18 months</td>
<td>Muscle weakness and atrophy</td>
<td>17</td>
<td>7</td>
<td>Prox. LL 2/5, dist. UL 0/5</td>
<td>Normal</td>
<td>Yes</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAL4 Q163X/Q163X</td>
<td>53/M</td>
<td>17 months</td>
<td>Foot walking problems</td>
<td>14–15</td>
<td></td>
<td>Prox. LL 3–4/5, dist. UL 0/5</td>
<td>Normal</td>
<td>Yes</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAL5 Q163X/E114fs</td>
<td>4/M</td>
<td>10 months</td>
<td>Retarded milestones</td>
<td>15</td>
<td>Walking</td>
<td>11 years</td>
<td>Normal</td>
<td>No</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autosomal dominant</td>
<td>LF292 R120W/+</td>
<td>36/M</td>
<td>18 years</td>
<td>Feet pain, stepgait gait</td>
<td>NA</td>
<td>Walking</td>
<td>Dist. LL 3–4/5</td>
<td>Reduction of pain and profound senses</td>
<td>No</td>
<td>Normal except ankle reflexes</td>
<td>Normal NCVs and SNAPs in median, peroneal, and posterior tibial nerves</td>
<td>Sural nerves: reduction of NCVs and SNAPs</td>
<td></td>
</tr>
<tr>
<td>LF293 R120W/+</td>
<td>34/M</td>
<td>14 years</td>
<td>Muscle weakness and atrophy, clumsiness, gait instability, Delayed gait</td>
<td>NA</td>
<td>Walking</td>
<td>Dist. LL 4/5</td>
<td>Normal</td>
<td>No</td>
<td>Normal except ankle reflexes</td>
<td>Peroneal nerve: 45/15 reduction of SNAP</td>
<td>NA</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>VAL8 T157P/+</td>
<td>10/F</td>
<td>12 months</td>
<td>Walking</td>
<td>15 months</td>
<td>Prox. LL 5/5, dist. UL 2–3/5</td>
<td>Reduced</td>
<td>No</td>
<td>Absent</td>
<td>EMG: neurogenic pattern, normal NCVs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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/sep/tfracks.html; and BDMC21 v2.1 software available from http://www.rannala.org/labpages/software.html.

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