Autosomal dominant axonal Charcot-Marie-Tooth disease type 2 (CMT2G) maps to chromosome 12q12–q13.3

E Nelis, J Berciano, N Verpoorten, K Coen, I Dierick, V Van Gerwen, O Combarros, P De Jonghe, V Timmerman

Charcot-Marie-Tooth (CMT) disease is a clinically and genetically heterogeneous group of disorders that involve the peripheral nervous system. It is characterised by progressive distal neurogenic muscular atrophy and weakness that initially affects the peroneal muscles and later the hands. Charcot-Marie-Tooth disease type 1 (CMT1), also called hereditary motor and sensory neuropathy type 1 (HMSN 1), is a dominantly inherited demyelinating neuropathy characterised by reduced nerve conduction velocities (NCV) (motor median NCV <38 m/s). Charcot-Marie-Tooth disease type 2 (CMT) 2, or HMSN II, is a dominantly inherited axonal neuropathy characterised by normal or slightly reduced NCV. Both autosomal dominant CMT1 and autosomal dominant CMT2 are genetically heterogeneous, with five and six loci, respectively. Most patients with CMT1 have a 1.4 Mb tandem duplication on chromosome 17p11.2 (CMT1A (MIM 118220)). Other patients with CMT1 may have point mutations in the peripheral myelin protein 22 gene (PMP22 in CMT1A (MIM 601977)), myelin protein zero gene (MPZ in CMT1B (MIM 159440)), lipopolysaccharide induced tumour necrosis factor gene (LITAF in CMT1C (MIM 603795)), early growth response 2 gene (EGR2 in CMT1D (MIM 129010)), or the gap junction protein beta 1 gene (GJB1 in CMT1X (MIM 304040)). Patients with CMT2 may have point mutations in the kinesin family member 1B gene (KIF1B in CMT2A (MIM 605995)), RAB7, member RAS oncogene family (RAB7 in CMT2B (MIM 602298)), glycyl tRNA synthetase (GARS in CMT2D (MIM 600287)), or neurofilament light polypeptide (NEFL in CMT2E (MIM 607684)). The genes for CMT2C and CMT2F have not been identified yet. Some patients with CMT2E have been reported to have specific mutations in the MPZ gene.

METHODS
We previously described a large Spanish family diagnosed with autosomal dominant CMT2. For this study, we enlarged the pedigree to 28 people, of whom 11 were affected (fig 1). The age at onset was 9–76 (mean 29) years. Most patients developed symptoms in the second decade of life. The disease, which presented with foot deformity and difficulty walking, showed a very slow progression. Patients were mildly disabled. Ankle reflexes were absent or hypoactive in all patients, whereas knee reflexes sometimes were preserved. Mild stocking hypaesthesia was seen. The upper limbs were involved in only two patients. Scoliosis, pupillary abnormalities, foot ulcers, deafness, diaphragm and vocal cord paralysis, nerve enlargement, optic atrophy, tremor, and ataxia were absent.

Electrophysiological findings were updated for this study. Briefly, we found slight slowing of motor NCV (77–93% of lower limit of normal) in 30% of nerves tested. Interestingly, five patients had entirely normal NCV values. Distal compound motor action potentials and sensory nerve action potentials in the lower limbs had low amplitudes or were unobtainable. Electromyography of tibialis anterior muscles showed chronic neurogenic alterations. The histological study of two sural nerve biopsies revealed a sciatic nerve and its branches that we dissected in an amputated leg of patient 117.3 showed loss of myelinated fibres with a proximal to distal gradient, clusters of regenerating fibres, and atrophic axons. Autopsy of patient 117.102 showed loss of anterior horn and dorsal root ganglion neurons in the lumbosacral segments and degeneration of the fasciculus gracilis. Morphometric evaluation of the L5 ventral and dorsal roots showed a normal number of myelinated fibres, while diameter histograms were shifted to the left because of a significant loss of large myelinated fibres and regeneration.

DNA from 28 individuals, of whom 11 were affected, was available for genetic analysis. Informed consent was obtained according to the Declaration of Helsinki, and protocols were approved by the Institutional Review Board of the Marqués de Valdecilla University Hospital. We previously had excluded the CMT1A tandem duplication at 17p11.2 and mutations in PMP22, MPZ, and NEFL (data not shown). Furthermore, we performed genetic linkage studies and excluded known CMT2 loci with short tandem repeat markers from these regions (data not shown). We performed a genomewide scan with 382 short tandem repeat markers of the ABI Prism Linkage Mapping Set (version 2.5; Applied Biosystems, Foster City, CA, USA), which had an average intermarker distance of 10 cM. Markers were amplified by polymerase chain reaction (PCR) on a PTC-220 DNA Engine DYAD (MJ Research, Waltham, MA, USA) and pooled with a Biomek FX...
Laboratory Workstation (Beckman Coulter, Fullerton, CA, USA). We performed and processed fragment analysis by capillary electrophoresis on an ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and with GeneScan Analysis software (version 3.5; Applied Biosystems, Foster City, CA, USA) and Genotyper software (version 3.7; Applied Biosystems, Foster City, CA, USA). We performed genetic fine mapping with additional short tandem repeat markers selected from the ABI Prism Linkage Mapping Set HD-5 (Applied Biosystems, Foster City, CA, USA) (D12S1682, D12S1640, and D12S1663) or UCSC Human Genome Browser (April 2003 Freeze) and the genome database (D12S1687, D12S1713, D12S1632, D12S1644, and D12S90).

We computed two point linkage between the disease gene and each marker with Linkage software (version 5.1) and Fastlink software (version 2.1, freely available).20–23 We scored CMT2 as a 90% penetrant autosomal dominant trait with a gene frequency of 0.0001. We assumed equal recombination rates between men and women. For each short tandem repeat marker, we set the number of alleles in the logarithm of odds (LOD) score calculations at the observed number of alleles in the pedigree (N) and the allele frequencies at 1/N.

We designed primers for PCR amplification of the coding regions of the candidate genes with Primer3 (RAB5B) or our in house software SNPBox (AVIL, CEN1G1) (S. Weckx et al., personal communication, 2003) (table 2, supplementary data) or used published primers (DHH).24 We sequenced the PCR products, which were prepared for sequencing with the PCR Product Pre-Sequencing Kit (USB Corporation, Cleveland, OH, USA), with the BigDye Terminator Cycle Sequencing kit and AmpliTaq DNA polymerase FS (Applied Biosystems, Foster City, CA, USA), with the BigDye Terminator Cycle Sequencing kit and AmpliTaq DNA polymerase FS (Applied Biosystems, Foster City, CA, USA), or the ABI Prism 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA) and Genotyper software (version 3.7; Applied Biosystems, Foster City, CA, USA). We removed unincorporated dye terminators with MultiScreen 96-well filtration plates (Millipore, Bedford, MA, USA). We performed electrophoresis on an ABI Prism 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA). We collected sequencing data with the ABI DNA Sequencing Analysis software (version 3.6) and analysed them with the SeqMan II software (version 5.06 DNASTAR, Madison, WA, USA).

RESULTS

In the initial genomewide search, significant linkage to marker D12S368 (LOD score z = 4.10 at χ2 = 0) and suggestive linkage to the neighbouring marker D12S85 (LOD score z = 2.92 at χ2 = 0) were obtained on chromosome 12q13.1 (table 1). We performed genetic fine mapping with additional short tandem repeat markers. Table 1 summarises the two point LOD scores. Segregation analysis in the family identified the disease haplotype in all patients (163, 171, 122, 210, 275, and 213) with markers D12S1687, D12S1713, D12S85, D12S368, D12S1604, and D12S1632 (fig 1). Two key meiotic recombinants were identified between D12S1663 and D12S1687 in patient 117.9 and between D12S1632 and D12S1644 in patient 117.16. These two recombinants assigned the CMT2 gene to a 13.2 Mb region (UCSC Human Genome Browser, April 2003 Freeze) between centromeric D12S1663 and telomeric D12S1644 at 12q12–q13.3. Simultaneous with the fine mapping, we screened four candidate genes in the linked region: avilin (AVIL), centaurin gamma 1 (CEN1G1), RAB5B member RAS oncogene family (RAB5B), and desert hedgehog homologue (Drosophila) gene (DHH). Sequence analysis of the complete coding regions of the genes showed a nucleotide change in intron 13 (c.1550–49C→T) of CEN1G1. Segregation analysis of this non-coding single nucleotide polymorphism in the family indicated that the single nucleotide polymorphism was transmitted with the CMT2 phenotype, except in one patient (117.16). This recombination event was confirmed by marker D12S1644 in the same patient (figs 1 and 2). The CEN1G1 single nucleotide polymorphism is not related, therefore, to the disease in this family affected by CMT2. In addition, analysis of 154 control chromosomes did not identify the c.1550–49C→T single nucleotide polymorphism, which suggests that this single nucleotide polymorphism is rare.

DISCUSSION

We report a novel genetic locus of a 12.8 cm linkage region at 12q12–q13.3 in a Spanish autosomal dominant family with CMT2. It is the seventh locus for autosomal dominant CMT2, so we designated it CMT2G. The CMT2G region is covered by one sequenced contig (NT_029419) and contains more than 300 genes (UCSC Human Genome Browser, April 2003 Freeze). We screened the coding region of four candidate genes in the linked region. AVIL and CEN1G1 were considered functional candidates, as they are expressed in the peripheral nervous system (Verpoorten et al., unpublished data, 2003). RAB5B was considered a good candidate, as it is highly homologous to RAB7—the gene in which mutations cause CMT2B.21 DHH may be involved in perineural development in peripheral nerves.22 No disease causing mutations were detected, which excluded these four genes as the CMT2 genes.

Table 1 Two point LOD scores between the CMT2 locus and short tandem repeat markers on chromosome 12q12–q13.3

<table>
<thead>
<tr>
<th>Marker</th>
<th>LOD score at recombination fraction (F)</th>
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<tr>
<td></td>
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<tr>
<td>D12S364</td>
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<tr>
<td>D12S310</td>
<td>0.56</td>
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<tr>
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<tr>
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<tr>
<td>D12S1713</td>
<td>0.56</td>
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<tr>
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<tr>
<td>D12S368</td>
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<tr>
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<td>D12S1644</td>
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<tr>
<td>D12S90</td>
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Chromosome 12q contains several loci for neuromuscular disorders: CMT2C at 12q23–q24,14 dominant congenital distal spinal muscular atrophy at 12q23–q24,23 distal hereditary motor neuropathy type II (distal HMN II) at 12q24,24 spinocerebellar ataxia type 2 (SCA2; gene SCA2) at 12q24,25 scapuloperoneal spinal muscular atrophy (SPSMA) at 12q24.1–q34.31,26 scapuloperoneal muscular dystrophy (SPMD) at 12q15–q23.1,27 gonadal dysgenesis with minifascicular neuropathy (gene DHH) at 12q13.1,21 spastic paraplegia type 10 (SPG10; gene KIF5A) at 12q13.3,28 centronuclear myopathy (gene MYF6) at 12q21,29 and restless leg syndrome at 12q12–q21.30 Only the gonadal dysgenesis with minifascicular neuropathy region overlaps with the CMT2G region. The gene associated with this disorder, DHH, was excluded by mutation analysis.

The electrophysiological and pathological findings in the Spanish pedigree affected by CMT showed that the neuropathy primarily is axonal and therefore can be classified as CMT2.31 32 The clinical phenotype is characterised by a classic, although mild, peroneal muscular atrophy syndrome. Additional features were absent, which indicates that this neuropathy presents as a pure variant of CMT.

**Figure 1** Haplotype analysis in the pedigree of the Spanish autosomal dominant family with CMT2 CMT-117. For confidentiality reasons, we did not reveal the genotypes of the asymptomatic people who were at risk in the youngest generation. Alleles were sized according to the Centre d'Etude du Polymorphisme Humain (CEPH) control DNA (1347.02) and are shown in base pairs. Short tandem repeat markers are shown from centromere (top) to telomere (bottom). The haplotype associated with the disease is boxed. Patient 117.9 defines the centromeric border of the disease at D12S1663, while patient 117.16 defines the telomeric border at D12S1644. ● affected woman; ■ affected man; ○ unaffected woman; □ unaffected man; ▲ man of unknown status; / deceased; 0 failed genotype.
Figure 2 Genetic map of chromosome 12q12–q13 region. Physical distances between the markers (Mb) were obtained from the UCSC Human Genome Browser (April 2003 Freeze). A bold line indicates distances between the markers (Mb) were obtained from the UCSC Human Genome Browser (April 2003 Freeze). The CMT2G region. Markers in bold are from the ABI Prism Linkage Mapping Set (version 2.5).

**Conclusion**
We report a novel genetic locus of a 12.8 cM linkage region at 12q12–q13.3 in a Spanish autosomal dominant CMT2 family. Clinical and genetic analysis of additional CMT2 families will help to fine map the locus and identify the genetic defect that causes CMT2G.

**ACKNOWLEDGEMENTS**
The authors gratefully acknowledge the cooperation and participation of all family members in this study and the contribution of the VIB Genetic Service Facility (http://www.vibgeneticserivcfacility.be/) in the genetic analyses. This research project was supported by Fund for Scientific Research—Flanders (Belgium) (FWO-Vlaanderen), Special Research Fund of the University of Antwerp, Medical Foundation Queen Elisabeth, Association Belgique contre les Maladies Neuro-Musculaires, Interuniversity Attraction Poles programme P5/19 of the Belgian Federal Science Policy Office, Muscular Dystrophy Association (USA), and Centro de Investigación de Enfermedades Neurologicas (ISCCIII, Madrid, Spain). EN is a postdoctoral fellow and KC is a research assistant of the FWO-Vlaanderen. NV and ID are supported by a fellowship from the Institute for Science and technology (IWT), Belgium.

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Received: 21 July 2003
Accepted: 5 September 2003

Conflict of interest: None declared.

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*J Med Genet* 2004 41: 193-197
doi: 10.1136/jmg.2003.012633

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