

# Mutilating neuropathic ulcerations in a chromosome 3q13-q22 linked Charcot-Marie-Tooth disease type 2B family

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## Abstract

**Background**—Charcot-Marie-Tooth disease type 2 (CMT2) or hereditary motor and sensory neuropathy type II (HMSN II) is an inherited axonal neuropathy of the peripheral nervous system. Three autosomal dominant CMT2 loci have been located on chromosomes 1p35-p36 (CMT2A), 3q13-q22 (CMT2B), and 7p14 (CMT2D) indicating that CMT2 is a genetically heterogeneous disorder.

**Methods**—A CMT2 family was examined for linkage to the CMT2A, CMT2B, and CMT2D loci using short tandem repeat polymorphisms.

**Results**—Suggestive evidence for linkage to 3q13-q22 was found. Recombinations occurred with markers D3S1769 and D3S1267 indicating that the CMT2B locus is located distal to D3S1267 and resides in an interval of 25 cM. Some patients in this family have pronounced sensory disturbances leading to poorly healing ulcerations.

**Conclusions**—These unusual sensory signs for CMT were also noted in the only other CMT2B family reported so far, suggesting a distinct clinical phenotype for CMT2B. Exclusion of the locus for hereditary sensory neuropathy type I (HSN I) on chromosome 9q22 indicates that HSN I with mild motor symptoms and CMT2 with prominent sensory abnormalities are not allelic.

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**Keywords:** Charcot-Marie-Tooth type 2; hereditary motor and sensory neuropathy type II; hereditary sensory neuropathy type I; linkage analysis; neuropathic ulcerations

Charcot-Marie-Tooth disease (CMT) is clinically characterised by progressive weakness and atrophy initially of the distal muscles of the lower limbs and later on also of the upper limbs. Sensory symptoms are usually lacking but distal sensory loss can often be detected on routine neurological examination. Most patients with CMT belong to families in which the disease segregates according to an autosomal dominant inheritance pattern. An X linked and recessive mode of inheritance as well as sporadic patients have been described. Histopathological criteria of peripheral nerve biopsies differentiate CMT into three different subtypes. CMT1 or the hypertrophic form of

CMT is characterised by extensive demyelination and remyelination leading to the formation of onion bulbs. In CMT2 or the neuronal form of CMT axonal degeneration without extensive alterations of the myelin sheaths is the most striking feature. In the spinal form of CMT the axons of the motor anterior horn neurons seem to be primarily affected whereas the sensory neurons are spared.<sup>1</sup> In the classification of Dyck *et al*<sup>1</sup> CMT1 is synonymous with hereditary motor and sensory neuropathy type I (HMSN I), CMT2 is HMSN II, and the spinal form of CMT is described as distal hereditary motor neuropathy (distal HMN).

Fortunately, there is a good correlation between the results of routine neurophysiological tests—for example, nerve conduction velocity (NCV) measurements, EMG, and the histological findings obviating the need for nerve biopsies in most cases. In the demyelinating CMT1 motor and sensory NCVs are severely reduced with an upper limit of 38 m/s for the motor median nerve. Limits of NCV in CMT2 are less well defined. Harding and Thomas<sup>2</sup> considered motor NCVs higher than 38 m/s the hallmark of CMT2. Dyck *et al*<sup>1</sup> used a more restrictive definition for CMT2—that is, normal or slightly reduced NCVs—but they did not specify the lower limit. Also, female patients or carriers in X linked CMT1 families often meet the CMT2 criteria.<sup>3,4</sup> As an axonal neuropathy, CMT2 is characterised by low amplitudes of the compound muscle action potentials, whereas sensory nerve action potentials (SNAPs) are often absent. An essential distinguishing feature between CMT2 and distal HMN is the presence of normal SNAPs in distal HMN.<sup>1</sup>

CMT2 is not genetically homogeneous as both an autosomal dominant and a recessive mode of inheritance are found.<sup>2</sup> A rare X linked CMT2 subtype with deafness and mental retardation has been reported.<sup>5</sup> Ben Othmane *et al*<sup>6</sup> reported evidence for genetic linkage and genetic heterogeneity of autosomal dominant CMT2, with the localisation of a first CMT2 (CMT2A) locus to chromosome 1p35-p36 in three unrelated families. In an analysis of 11 CMT2 families we found suggestive evidence for linkage to the CMT2A locus in only one pedigree which indicates that only a minority of CMT2 families belong to the CMT2A subtype.<sup>4</sup> A second locus for autosomal dominant CMT2 has been assigned to chromosome 3q13-q22 (CMT2B) in a single large pedigree by Kwon *et al*.<sup>7</sup> A third locus for CMT2, CMT2D, has recently been mapped to chromosome 7p14.<sup>8</sup> Yoshioka *et*

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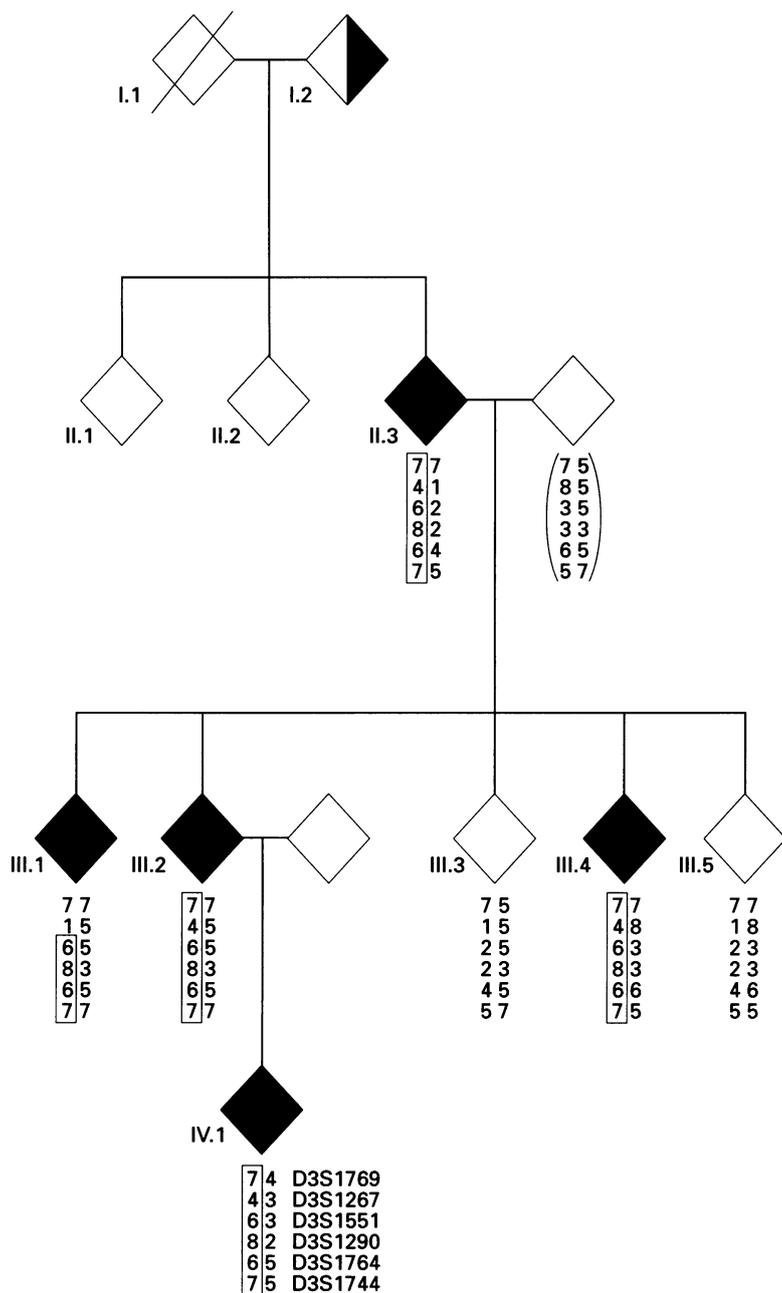


Figure 1 Segregation analysis of 3q13-q22 STRs in family CMT-90. The haplotypes segregating with the disease are boxed. Males and females are represented by diamonds to ensure anonymity; slashed = deceased; filled = affected members; blank = non-affected members; half filled = disease status unknown.

*al*<sup>9</sup> showed that autosomal dominant CMT2 with vocal cord paralysis is not allelic with CMT2A. This clinical entity has been designated as CMT2C. When the diagnosis of CMT2 is based on the electrophysiological data obtained in female patients of pedigrees without male to male transmission, inclusion of X linked CMT1 families is common. In our families we identified one connexin 32 (Cx32) mutation and another family showed suggestive evidence for Xq13 linkage but no mutation was found in the coding region of the Cx32 gene.<sup>4</sup>

**Materials and methods**

**FAMILY DATA**

We studied a multigenerational family (CMT-

90) in which an unusual CMT phenotype segregates as an autosomal dominant trait (fig 1). Five patients were identified. Patient II.3 was well until the age of 28 when he began to develop ulcers at the toes which, after several operations, eventually led to an amputation of all five toes of the right foot. He has gross wasting of the legs distal to the knee with associated profound weakness which necessitates the use of calipers. There is superficial sensory loss to midshin. His mother (I.2) had drop feet and wore a toe raising spring. She probably did not have any ulcers. His eldest son, III.1, had an amputation of the left leg below the knee and necrotic ulcers on the right foot at the age of 18 years (fig 2). He has grossly wasted muscles below the knees with some residual strength in the calves but complete paralysis of the peroneal muscles. The proximal muscle groups of the lower limbs remained normal. There was a considerable loss of touch and pain sensation to half way up the legs. Tendon reflexes were generally diminished and ankle jerks were absent. Patient III.4 has distal wasting in the lower limbs with weakness of dorsiflexion of the ankle and small foot muscles. Sensation for temperature was decreased to the lower shin. Nerve conduction studies in patients III.1, III 2, and III.4 showed normal motor NCVs in the median nerve. Sensory nerve action potentials had low amplitudes or could not be elicited. Examination with EMG showed chronic neurogenic alterations.

**DNA ANALYSIS**

Short tandem repeat genotype analysis was performed with the chromosome 1p35-p36 markers D1S160 (MIT-MS48), D1S170 (MIT-COS37), D1S244 (AFM220yf4), and D1S228 (AFM196xb4); the chromosome 3q13-q22 markers D3S1769 (GATA8D02), D3S1267 (AFM116xh2), D3S1551 (AFM198yc1), D3S1290 (AFM198yh6), D3S1764 (GATA4A10), and D3S1744 (GATA3C02); the chromosome 7p14 markers D7S435 (Mfd20) and D7S1806 (GGAA11C11); and the chromosome 9q22 markers, D9S196 (AFM212yb4), D9S197 (AFM238va7), and D9S280 (AFM304td9).<sup>10-12</sup> Genomic DNA (0,15 µg) was amplified using oligonucleotide primers labelled with fluorophores (Applied Biosystems Incorporation, ABI, Foster City, CA, USA). A polymerase chain reaction (PCR) was performed in a 25 µl reaction volume containing 10 pmol of each primer and 0.1 U Goldstar Taq DNA polymerase (Eurogentec, Belgium). The PCR amplifications were performed in a thermal cycler (Techne PHC-3; New Brunswick Scientific, The Netherlands). An aliquot of 1 µl of each amplified product was mixed with 2 µl formamide and 0.5 µl fluorescent labelled size standard GS350-ROX (ABI) and denatured for three minutes at 95°C. The samples were loaded on 4% polyacrylamide sequencing gels and electrophoresed in an ABI automated DNA sequencer 377. Finally, the data were collected and analysed using ABI GENESCAN software. The haplotypes were constructed according to the genetic map of



Figure 2 Necrotic ulcer at the left foot of patient III.1 at the age of 18 years.

Dib *et al*<sup>12</sup> and the Cooperative Human Linkage Center (<http://www.chlc.org>). The two point and multipoint linkage studies were performed with the FASTLINK computer package.<sup>13</sup> CMT2 was assessed in the linkage according to the methods previously described.<sup>4</sup> Allele frequencies of the 1p35–36, 3q13–22, and 9q22 short tandem repeat markers were obtained from the Genome Data Base (<http://gdbwww.gdb.org>).

### Results

To analyse linkage in family CMT-90 with the CMT2A locus on chromosome 1p35–p36,<sup>6</sup> we performed a genotype analysis with the short tandem repeat markers D1S160, D1S170, D1S244, and D1S228. Recombinants were noted and negative lod scores were obtained with D1S244, D1S228, and D1S170. Marker D1S160 was not informative (table). These results indicate that this CMT2 family is not linked to chromosome 1p35–p36. The segregation analysis and the linkage results obtained in family CMT-90 with D7S435 and D7S1806 indicated that there was also no evidence of linkage for the CMT2D locus on chromosome 7p14 (table).<sup>8</sup> As patients in this

family had prominent sensory abnormalities we also performed a linkage analysis with markers of chromosome 9q22 near the HSN I locus.<sup>14</sup> Negative lod scores were obtained with D9S196 and D9S280, and marker D9S197 was not informative. Next we examined chromosome 3q13–q22 short tandem repeat polymorphisms. A disease associated haplotype 7–4–6–8–6–7 for the loci D3S1769, D3S1267, D3S1551, D3S1290, D3S1764, and D3S1744 is segregated through the whole pedigree, except in patient III.1 (fig 1). In this patient a recombination at the loci D3S1769 and D3S1267 resulted in a disease associated haplotype 6–8–6–7. A two point linkage study was performed with all short tandem repeats and a maximum lod score of 1.27 in the absence of recombinants is obtained with D3S1551 and D3S1290. A lod score of  $-3.72$  at  $\theta = 0.00$  is obtained with D3S1267 and is due to the recombination event in patient III.1 (table). Similar results were obtained by multipoint linkage analysis (data not shown). Our results support the localisation of a CMT2B locus on chromosome 3q13–q22 reported by Kwon *et al*.<sup>7</sup> To map the CMT2B gene more precisely additional STR markers need to be analysed in more CMT2 families.

### Discussion

Linkage and segregation analysis with chromosome 1p35–p36 and 7p14 STR markers flanking the CMT2A and CMT2D candidate regions respectively yields negative lod scores indicating that these loci are most likely not present in our family CMT-90. STR analysis with chromosome 3q markers, however, showed cosegregation of a disease haplotype. A maximum two point lod score of 1.27 in the absence of recombinations was obtained with D3S1551 and D3S1290. This positive but non-conclusive maximum lod score is the highest that can be obtained in the pedigree. A recombination event in patient III.1 makes D3S1267 the centromeric flanking marker and reduces the 30 cM CMT2B candidate region between the flanking markers D3S1769 and D3S1744 of Kwon *et al* to 25 cM.<sup>7</sup>

The patients in the CMT2B family reported

Two point lod scores between CMT2 and chromosomes 1p, 3q, 7p, and 9q short tandem repeats in family CMT-90

Chromosome/locus	Lod score at $\theta$						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
1p35–p36 (CMT2A):							
D1S244	-1.97	-1.49	-0.89	-0.58	-0.27	-0.11	-0.03
D1S160	ni						
D1S228	-4.64	-2.74	-1.40	-0.87	-0.42	-0.21	-0.09
D1S170	-0.09	-0.10	-0.15	-0.19	-0.22	-0.16	-0.07
3q13–q22 (CMT2B):							
D3S1769	0.30	0.30	0.28	0.26	0.20	0.15	0.08
D3S1267	-3.72	-0.75	-0.12	0.08	0.18	0.16	0.08
D3S1551	1.27	1.25	1.15	1.03	0.76	0.47	0.47
D3S1290	1.27	1.25	1.15	1.03	0.76	0.47	0.18
D3S1764	0.97	0.95	0.87	0.77	0.55	0.32	0.10
D3S1744	0.46	0.45	0.40	0.34	0.22	0.11	0.03
7p14 (CMT2D):							
D7S435	-1.44	-1.25	-0.88	-0.64	-0.37	-0.21	-0.09
D7S1806	0.16	0.15	0.13	0.11	0.06	0.03	0.01
9q22 (HSN-I):							
D9S196	-0.31	-0.30	-0.24	-0.18	-0.09	-0.04	-0.01
D9S197	ni						
D9S280	-3.79	-1.18	-0.51	-0.25	-0.03	0.04	0.05

ni = Not informative.

by Kwon *et al*<sup>7</sup> have some unusual clinical features. Sensory symptoms are often prominent and poorly healing ulcerations eventually lead to extensive necrosis and amputation of the distal parts of the lower limbs. The diagnosis of HMSN II in this CMT2B family has been challenged and it has been argued that the diagnosis of HSN is more appropriate.<sup>15,16</sup> Poorly healing ulcerations leading to mutilations and amputations are the hallmarks of HSN. The descriptive terminology suggests that this peripheral neuropathy is an exclusively sensory neuropathy but patients in these pedigrees can have a varying degree of motor deficit making the clinical distinction between HSN and CMT2 less clear.<sup>1</sup> However, Nicholson *et al*<sup>14</sup> recently mapped the HSN I gene on chromosome 9q22 in autosomal dominant HSN I families in which patients also had some distal weakness. These genetic linkage data prove that despite some overlap in the clinical phenotype, CMT2B and HSN I are not allelic and that mutations in different genes must be responsible for these two inherited neuropathies. The clinical phenotype in our CMT-90 family is characterised by distal weakness and atrophy of the lower limbs. However, sensory disturbances are also prominent and poorly healing ulcerations occur in some patients. These neuropathic ulcerations eventually necessitate amputations. The electrophysiological data are typical for an axonal sensorimotor polyneuropathy. Therefore this family can be classified as HMSN II. Our data exclude linkage to the HSN I locus on chromosome 9q22, confirming that HSN I with mild motor symptoms and HMSN II with prominent sensory abnormalities are not allelic.

Our results confirm the presence of a HMSN II gene on chromosome 3q13-q22 and refine the CMT2B candidate region. There is a striking clinical similarity between our CMT-90 family and the one reported by Kwon *et al*.<sup>7</sup> The clinical similarity between these two families suggests that the prominent sensory features form an integral part of the CMT 2B phenotype.

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- 1 Dyck PJ, Chance P, Lebo R, Carney JA. Hereditary motor and sensory neuropathies. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, eds. *Peripheral neuropathy*. Philadelphia: Saunders, 1993:1094-136.
- 2 Harding AE, Thomas PK. The clinical features of hereditary motor and sensory neuropathy types I and II. *Brain* 1980;103:259-80.
- 3 Nicholson G, Nash J. Intermediate nerve conduction velocities define X-linked Charcot-Marie-Tooth neuropathy families. *Neurology* 1993;43:2558-64.
- 4 Timmerman V, De Jonghe P, Spoelders P, *et al*. Linkage and mutation analysis of Charcot-Marie-Tooth neuropathy type 2 families with chromosomes 1p35-p36 and Xq13. *Neurology* 1996;46:1311-8.
- 5 Priest JM, Fischbeck KH, Nouri N, Keats BJB. A locus for axonal motor-sensory neuropathy with deafness and mental retardation maps to Xq24-q26. *Genomics* 1995; 29:409-12.
- 6 Ben Othmane K, Middleton LT, Loprest LJ, *et al*. Localization of a gene (CMT2A) for autosomal dominant Charcot-Marie-Tooth disease type 2 to chromosome 1p and evidence of genetic heterogeneity. *Genomics* 1993;17:370-5.
- 7 Kwon JM, Elliott JL, Yee WC, *et al*. Assignment of a second Charcot-Marie-Tooth type II locus to chromosome 3q. *Am J Hum Genet* 1995;57:853-8.
- 8 Ionasescu V, Searby C, Sheffield VC, Roklina T, Nishimura D, Ionasescu R. Autosomal dominant Charcot-Marie-Tooth axonal neuropathy mapped on chromosome 7p (CMT2D). *Hum Mol Genet* 1996;5:1373-5.
- 9 Yoshioka R, Dyck PJ, Chance PF. Genetic heterogeneity in Charcot-Marie-Tooth neuropathy type 2. *Neurology* 1996;46:569-71.
- 10 Hudson TJ, Engelstein M, Lee MK, *et al*. Isolation and chromosomal assignment of 100 highly informative human simple sequence repeat polymorphisms. *Genomics* 1992;13:622-9.
- 11 Sheffield VC, Weber JL, Buetow KH, *et al*. A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution human genome-wide linkage maps. *Hum Mol Genet* 1995;4:1837-44.
- 12 Dib C, Fauré S, Fizames C, *et al*. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996;380:152-4.
- 13 Cottingham RW, Idury RM, Schäffer AA. Faster sequential genetic linkage computations. *Am J Hum Genet* 1993;53:252-63.
- 14 Nicholson GA, Dawkins JL, Blair IP, *et al*. The gene for hereditary sensory neuropathy type I (HSN-I) maps to chromosome 9q22.1-q22.3. *Nat Genet* 1996;13:101-4.
- 15 Vance JM, Speer MC, Stajich JM, *et al*. Misclassification and linkage of hereditary sensory and autonomic neuropathy type 1 as Charcot-Marie-Tooth disease, type 2B. *Am J Hum Genet* 1996;59:258-60.
- 16 Yee WC, Elliott JL, Kwon JM, Goodfellow P. Misclassification and linkage of hereditary sensory and autonomic neuropathy type 1 as Charcot-Marie-Tooth disease, type 2B (reply). *Am J Hum Genet* 1996;59: 260-2.