Localization of a gene responsible for autosomal recessive demyelinating neuropathy with focally folded myelin sheaths to chromosome 11q23 by homozygosity mapping and haplotype sharing

Alessandra Bolino1, Valeria Brancolini2, Francesco Bono3, Amalia Bruni4, Antonio Gambardella3, Giovanni Romeo1, Aldo Quattrone3 and Marcella Devoto1,2,*

1 Laboratorio di Genetica Molecolare, Istituto Gaslini, Genova, Italy, 2Department of Psychiatry, Columbia University, New York, NY, USA, 3Clinica Neurologica Facoltà di Medicina, Catanzaro, Italy and 4Unità Operativa CNR USL6, Lamezia Terme, Italy

Received February 28, 1996; Revised and Accepted April 22, 1996

Hereditary motor and sensory neuropathy (HMSN) with focally folded myelin sheaths, or Charcot–Marie–Tooth type 4B (CMT4B), is a distinct clinical entity belonging to the heterogeneous group of autosomal recessive demyelinating neuropathies. We first described a large pedigree with CMT4B, which showed a high consanguinity level and an autosomal recessive pattern of inheritance. Through conventional linkage analysis, we excluded linkage of the locus segregating in this pedigree to any of the known genes responsible for other HSMNs. Using homozygosity mapping and haplotype sharing analysis, we were able to localize the disease gene in a 4 cM interval on chromosome 11q23, between the D11S1332 and D11S917 loci. On the basis of the clinical characteristics of the disease, we propose that this locus corresponds to the CMT4B gene.

INTRODUCTION

The classification of autosomal recessive hereditary demyelinating motor and sensory neuropathies is still a matter of debate (1,2). They include different disorders with a broad spectrum of clinical severity (1,2). Three pathological forms are now recognized (1–3), one with classical onion bulbs, one with basal laminal onion bulbs or Charcot–Marie–Tooth (CMT) type 4A (CMT4A) and a third characterized by focally folded myelin sheaths or CMT type 4B (CMT4B). It is still unknown whether these pathological entities represent distinct genetic disorders or whether they are caused by different mutations occurring at the same gene. Up to now, only the gene responsible for the form characterized morphologically by basal laminal onion bulbs, namely CMT4A, has been mapped in the 8q13–q21.1 region (3).

We recently identified a large family with CMT4B (4). The full clinical, electrophysiological and pathological findings, and the natural clinical history of this disease have been reported elsewhere (4).

In this particular pedigree, we excluded linkage of the disease to the following loci already known to be associated with other forms of hereditary motor and sensory neuropathy (HMSN) (5,6): CMT1A, corresponding to the peripheral myelin protein 22 (PMP-22) gene, on chromosome 17p11.2–p12; CMT1B, corresponding to the myelin protein zero (MP0) gene, on chromosome 1q22–q23; CMT2A, on chromosome 1p35–p36 and, finally, CMT4A, on chromosome 8q13–q21.1. Then, taking advantage of the particular inbred structure of the family, we used homozygosity mapping and haplotype sharing to localize, through a genome-wide search, the CMT4B locus on chromosome 11 in a 4 cM interval in the q23.1 region.

RESULTS

In consideration of the high degree of heterogeneity with multiple allelism among the HSMNs, we first performed a two-point linkage study in order to assess whether the disease gene segregating in our pedigree was allelic to any of the other CMT loci already identified. Negative lod scores were obtained for all these regions (data not shown). In addition, no mutations occurring in PMP-22 and MP0 were detected in our patients (4).

In order to map this new locus, we took advantage of the particular structure of this pedigree, and initially genotyped only the five available affected members to identify regions of shared homozygosity (individuals 1945, 279, 273, 287 and 1943 in Fig. 1). Using 478 polymorphic markers from Généthon’s genetic map, we identified 30 markers on 14 chromosomes for which the same allele was present on at least seven of the 10 disease chromosomes. After genotyping all these markers in the other members of the family, only one marker located on chromosome 11q, namely D11S898, yielded a significant positive lod score, with a maximum Z of 5.54 at θ = 0 (upper limit of one-lod support interval = 0.06 cM). When the analysis was extended to eight more markers from the same region covering a total of 24 cM, three more markers gave significant lod scores.*}

*To whom correspondence should be addressed
Figure 1. Haplotypes defined by nine markers on 11q23 in a Southern Italian pedigree with autosomal recessive demyelinating neuropathy with focally folded myelin sheaths (CMT4B). Markers are reported from centromere (top) to telomere (bottom) following the order reported in the Généthon’s genetic map. The relative order of markers D11S917 and D11S919, which are reported to be at 0 cM, has been taken from a radiation hybrid map (18).

(Table 1) (D11S919, one lod support interval 0–0.08; D11S900, one-lod support interval 0–0.15; D11S908, one lod-support interval 0.001–0.14). One patient was homozygous for all nine markers included in the analysis, whereas the other four patients were homozygous for two to eight markers. Only marker D11S919 was homozygous in all five patients (Fig. 1). Haplotype analysis showed that, of the 10 disease-associated haplotypes, four were identical at all nine markers, and the others had from three to eight alleles in common (Fig. 1). Individual 164, who is an obligatory carrier but whose affected daughter was not available for analysis, also carried a copy of the disease-associated haplotype. Examination of the haplotypes showed that a recombination must have occurred between the disease locus and D11S1332 on at least two disease chromosomes (see individuals 1945 and 279 in Fig. 1), thus placing the disease locus proximal to D11S1332. On the other hand, another recombination which positions the disease locus proximal to D11S917 must have occurred on the maternal chromosome of individual 287. These two markers, which are located 4 cM apart, thus define the proximal and distal boundaries of the critical region of this new locus.

DISCUSSION

We recently identified a large inbred pedigree with 10 patients affected by CMT4B (4). Our findings enabled us to confirm definitely that CMT4B represents a distinct clinical and genetic entity. Because of the high level of consanguinity among the different patients, we hypothesized the segregation of a unique disease allele originating from a common founder. Therefore, we expected affected members of the family to be homozygous for the disease allele and for alleles at the marker loci tightly linked to it.
After exclusion of loci known to be responsible for other forms of HMSN, we optimized our genome-wide search by using homozygosity mapping and haplotype sharing analysis. We were thus able to identify a number of markers located on chromosome 11 which gave positive significant lod scores. Analysis of haplotypes revealed that several recombinations occurred between the disease allele and different microsatellite markers, placing the disease locus in the 4 cM interval delimited by markers D11S1332 and D11S917.

It is interesting that the paternal haplotype of patient 1945, whose father is apparently not related to the rest of the family, has four out of nine alleles present in the extended disease-associated haplotype. In spite of extensive investigation, it was impossible to detect any relationship between his father’s family and the rest of the pedigree. Nonetheless, this observation suggests that patient 1945 may be related to the rest of the pedigree also on his paternal side, and therefore may be homozygous for alleles identical by descent at the disease locus, as is expected in the case of a rare autosomal recessive disease in a small and genetically homogeneous population. Haplotype sharing of a small genomic region in two apparently unrelated families from the same homogeneous population. Haplotype sharing of a small genomic region in two apparently unrelated families from the same homogeneous population. Haplotype sharing of a small genomic region in two apparently unrelated families from the same homogeneous population.

To the best of our knowledge, only two genes, TYR (tyrosinase), associated with oculocutaneous albinism type I (OCA), and PGR (progesteron receptor) have been assigned to the interval between D11S1332 and D11S917 (8,9). Among the genes located on chromosome 11 whose function might be relevant to the disease phenotype, a cluster of genes encoding collagenase-related connective tissue degrading metalloproteinase (MMP0, MMP1, MMP3) has been mapped outside the candidate region between D11S900 and D11S927 (8,10), and the NCAM gene (neural cell adhesion molecule) is between markers D11S927 and D11S908 (8) and therefore excluded by the haplotype analysis. Based on the genes already identified and associated with other forms of CMT, it is possible to hypothesize that a mutation in a gene coding for a protein of the peripheral myelin could be responsible for the disease in this family.

Finally, the reconstruction of the at-risk haplotype segregating in this pedigree led us to the identification of several carriers of the disease gene among the individuals belonging to the last generation. In consideration of the high frequency of consanguineous marriages among its members, this information can be useful for genetic counselling in order to prevent recurrence of CMT4B in the subsequent generations of this family.

**MATERIALS AND METHODS**

**Family**

A genealogical study carried out on this pedigree led to the reconstruction of seven consecutive generations with >1900 members (4). The affected members of this family show early onset motor and sensory neuropathy with rather severe clinical course. Progressive distal and proximal weakness, foot and limb deformities and scoliosis are observed. Complex redundant loops and folds of myelin sheaths are visible on histological examination. A detailed clinical description is contained in ref.4.

All patients except one were born from consanguineous matings, and all of them are related to each other through multiple consanguineous marriages starting from an original couple of founders (Fig. 1). This observation strongly supports an autosomal recessive mode of inheritance for the trait segregating in this pedigree.

**Microsatellite analysis**

Blood samples were collected from individuals marked with numbers in Figure 1. Since patient no. 287 identifies a crucial recombination between the disease and one of the flanking markers, which is not seen in any other chromosome in the family, a second sample of this patient was drawn to confirm the results obtained and reported in Figure 1. Moreover, six additional microsatellite markers were tested independently in this patient and her father in order to exclude non-paternity.

Microsatellite markers used for exclusion of the known HMSN loci were: (i) RM11-GT at the D17S122 locus for CMT1A (11); (ii) SPTA1, CRP, APOA2 and D1S318, for CMT1B (12–15); (iii) D1S244 and D1S228, for CMT2A (15,16); and (iv) D8S164, D8S541, D8S543 and D8S167 for CMT4A (3,16).

The analysis of the microsatellite markers used for the exclusion of linkage was performed following conditions previously described (17). Microsatellite markers used for the genome-wide search of the disease gene are part of Généthon’s human genetic linkage map and they have been chosen with a heterozygosity of at least 70%, and an average spacing of 10–20 cM (16). PCR analysis and genotyping were performed as reported elsewhere (16).

**Linkage analysis**

Two-point lod scores were calculated using the MLINK program of the LINKAGE package version 5.1 on a SUN SparcStation. Because of the large number of consanguinity loops, a simultaneous analysis of the whole pedigree was computationally impossible. As an alternative, the pedigree was subdivided into its nuclear components corresponding to the families of patients no. 279, 273, 287 and 1943. The family of patient no. 1945, whose parents are apparently not related, was analysed together with the family of patient no. 279. For each sub-pedigree, all available members and all individuals necessary to connect them to the founder couple were included in the analysis. Lod scores reported in Table 1 are the sum of the lod scores obtained in these four sub-pedigrees. The disease was assumed to be caused by a fully penetrant recessive gene with a frequency of 0.001. Marker allele
frequencies used in the linkage analysis were obtained from GDB; lod scores calculated using equal marker allele frequencies are of the same order of magnitude of those reported in Table 1.

ACKNOWLEDGEMENTS

We wish to thank Dr Francesco De Stefano and Dr Lucia Casarino from the Laboratorio di Ematologia Forense, Istituto di Medicina Legale e delle Assicurazioni for the paternity testing, as well as Ms Monica Scaranari and Ms Tatiana Tocco for technical help. Finally, we gratefully acknowledge Généthon, Evry, France, for its technical service and assistance. The financial contribution of Telethon-Italy (grant no.598) and of NIH grant HG00008 is gratefully acknowledged. V.B. is supported by a fellowship of the Italian Telethon.

REFERENCES