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SHORT COMMUNICATION

Charcot-Marie-Tooth (CMT) 1a duplication at 17p11.2 in Italian families

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Charcot-Marie-Tooth disease type 1 (CMT1) constitutes a group of genetically heterogeneous neuropathies and different chromosomal locations have been described for the genes related to the disease. The majority of families show tight linkage with markers on chromosome 17 at 17p11.2 (CMT1a).[1-4] In a few families, linkage has been established between the gene for CMT and the Duffy blood group, close to the FcgammaRII gene on chromosome 1 at 1q22-q23. (CMT1b).[5,6] Finally, two families have been described without linkage to either of these loci.

Recently, Raeymaekers *et al*[7] and Lupski *et al*[8] showed that a duplication in chromosome 17p11.2, as shown by probe pVAW409R3, segregates with the disease, with no recombinants detected. One de novo mutation has also been described, with both electrophysiological and DNA evidence, thus suggesting that this structural mutation is directly related to the disease.

Owing to the genetic heterogeneity of HMSNI, we studied whether the 17p11.2 duplication could be confirmed in a sample of Italian families: this finding would allow an estimate of the heterogeneity and would confirm the applicability of a direct individual diagnosis in a large number of patients, including sporadic cases.

Fourteen families (137 subjects) were ascertained through the Departments of Neurology and Medical Genetics of the University of Genova, and the Istituto Neurologico Besta in Milano. In all families a complete clinical and electrophysiological evaluation was carried out. The patients, after informed consent was obtained, were selected on the basis of the following criteria: a positive family history of CMT disease, with clinical confirmation of the disease in at least three generations, and the clinical diagnosis confirmed by electromyography and nerve conduction velocity in all subjects and sural nerve biopsy in at least one patient from each family. Of these families, eight were amenable to linkage analysis because of size and structure, while in the remaining six, although family history confirmed the inheritance of the disease, only some affected subjects and some of their normal relatives could be tested, but no linkage analysis could be performed. Two cases with severe clinical and electrophysiological symptoms but no family history were also tested: in both cases the parents were unaffected on clinical examination and on electromyography. Paternity was tested and confirmed.

Two point lod score between the duplication detected by pVAW409R3a and CMT.

θ	0·000	0·001	0·010	0·050	0·100
cmt2	0·000	0·000	0·000	0·000	0·000
cmt3	0·851	0·849	0·830	0·744	0·635
cmt4	0·000	0·000	0·000	0·000	0·000
cmt6	0·301	0·300	0·289	0·244	0·191
cmt8	0·602	0·601	0·593	0·558	0·511
cmt10	0·903	0·901	0·886	0·814	0·721
cmt12	0·371	0·369	0·354	0·286	0·206
cmt15	0·787	0·785	0·771	0·704	0·620
	3·815	3·806	3·723	3·350	2·884

Lod scores were calculated with the LINKAGE 5·03 program in CMT families. No liability classes were used.

DNA was extracted and processed as previously described.[9] In summary, phenol extracted DNA was digested with *Msp*I, separated by gel electrophoresis on 0·8% agarose gel, and transferred to nylon membranes by the method of Southern. Probe pVAW409R3 was labelled with ^{32}P by the random oligonucleotide primer method.[10] This probe, which recognises the *D17S122* locus, is localised in 17p11.2 and detects on *Msp*I three alleles with sizes of 2·8, 2·7, and 1·9 kb. In affected subjects the duplication is shown by the presence of all three *Msp*I alleles or by a combination of the *Msp*I alleles differing in density on hybridisation.

The duplication was present in all 25 affected subjects tested, including two cases with severe symptoms but no family history. Two affected subjects were homozygous for the 2·8 kb duplication (family CMT4) and one carried three alleles (figure), while the remaining 12 families were heterozygous for the 2·8 and 2·7 kb alleles. Six were 2·8/2·8/2·7 and six were 2·8/2·7/2·7, their duplication being detected because of different band intensity. The allele frequencies, estimated on 35 unrelated subjects, were 2·8 kb = 0·46, 2·7 kb = 0·51; and 1·9 kb = 0·03.

Two point linkage analysis between the duplication and the disease was performed with the LINKAGE 5·03 program.[11] Only eight families could be tested for linkage.

The table shows the lod scores for linkage between the duplication, as detected by probe

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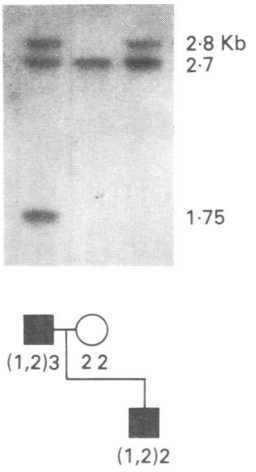

Autoradiogram of an MspI Southern blot hybridised with probe pVAW409R3. The affected father has all three MspI alleles (1, 2, and 3). He transmitted the duplicated allele of 2·7 and 2·8 kb to his son who also received the 2·7 kb allele from his mother, thus resulting in a double intensity band of 2·7 kb in the affected son.

pVAW409R3, and the disease. Although the family size was rather small, a significant lod score was obtained (3·815 at $\theta = 0$). Two families (CMT2 and CMT4) had 0 values, in CMT2 because of family size (only one affected son and his affected father available), and in CMT4 because both affected subjects were homozygous for the 2·8 kb allele.

Our data therefore confirm, in a sample of Italian families, that the duplication at 17p11.2 is found in all patients studied with a positive family history for CMT and in two cases without a family history, thus confirming the widespread occurrence of the duplication in different populations. No recombinants were found between the duplication, as detected by probe pVAW409R3, and the disease in any of the families tested.

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