Prevalence and Origin of De Novo Duplications in Charcot-Marie-Tooth Disease Type 1A: First Report of a De Novo Duplication with a Maternal Origin

Ian P. Blair, Janet Nash, Melissa J. Gordon, and Garth A. Nicholson

Molecular Medicine Laboratory, University of Sydney, Sydney, and Concord Hospital, New South Wales

Summary

Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy. Sporadic cases of CMT have been described since the earliest reports of the disease. The most frequent form of the disorder, CMT1A, is associated with a 1.5-Mb DNA duplication on chromosome 17p11.2, which segregates with the disease. In order to investigate the prevalence of de novo CMT1A duplications, this study examined 118 duplication-positive CMT1A families. In 10 of these families it was demonstrated that the disease had arisen as the result of a de novo mutation. By taking into account the ascertainment of families, it can be estimated that ≥10% of autosomal dominant CMT1 families are due to de novo duplications. The CMT1A duplication is thought to be the product of unequal crossing over between parental chromosome 17 homologues during meiosis. Polymorphic markers from within the duplicated region were used to determine the parental origin of these de novo duplications in eight informative families. Seven were of paternal and one of maternal origin. This study represents the first report of a de novo duplication with a maternal origin and indicates that it is not a phenomenon associated solely with male meioses. Recombination fractions for the region duplicated in CMT1A are larger in females than in males. That suggests that oogenesis may be afforded greater protection from misalignment during synapsis, and/or that there may be lower activity of those factors or mechanisms that lead to unequal crossing over at the CMT1A locus.

Introduction

Charcot-Marie-Tooth disease (CMT), also known as hereditary motor and sensory neuropathy, is the most common inherited peripheral neuropathy (Skre 1974). It is a clinically and genetically heterogeneous disorder, involving both motor and sensory nerves. Autosomal dominant, autosomal recessive, X-linked, and sporadic cases have been described (Lupski et al. 1991*b;* McKusick 1992). CMT1 is the most common subtype of CMT and is distinguished from other forms of the disease by severely slowed nerve-conduction velocities. Other clinical characteristics include distal muscle atrophy and weakness, pes cavus, mild sensory impairment, and demyelination observed in sural nerve biopsy (Dyck et al. 1993).

At least three genetic loci have been identified for CMT1. The most frequent form, CMT1A, is autosomal dominant with a locus on chromosome 17p11.2 (Vance et al. 1991). CMT1B has a genetic locus on chromosome 1q21.2-q25, and an X-linked dominant form maps to Xq11.2-q21.1 (Lebo et al. 1991; Bergoffen et al. 1993). In the majority of CMT1 cases, a 1.5-Mb DNA duplication is present on chromosome 17p11.2, which segregates with the disease (Lupski et al. 1991*a;* Raeymaekers et al. 1991; Pentao et al. 1992; Wise et al. 1993). This duplication encompasses the gene *PMP22,* which encodes a peripheral myelin protein. Point mutations in *PMP22* have been identified in rare CMT1A patients and families that do not carry the duplication (Patel et al. 1992; Valentijn et al. 1992; Roa et al. 1993*a,* 1993*b*), implicating *PMP22* as the crucial gene involved in the pathogenesis of CMT1A. Increased gene dosage or structural alteration of *PMP22* may be the mechanisms that produce the CMT1A phenotype. Other unidentified genes in the 1.5-Mb duplication may also play a role in the disease phenotype.

Sporadic cases of CMT have been described since the earliest reports of the disease. Friedreich included a sporadic case in his paper on progressive muscular atrophy in 1873. Three more apparent sporadic cases were reported by Charcot and Marie in 1886. Sainton recorded sporadic cases in a review of Paris cases in his 1899 thesis. Many other early reports of CMT included apparent sporadic cases (Marie 1906; Hoffmann 1912; Archard and Thiers 1924; Schaller and Newman 1935; Tarassiéwitch and Michéjew 1935; Krücke 1942; Isaacs 1960). Sporadic cases of CMT1 may represent instances

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Address for correspondence and reprints: Ian P. Blair, Molecular Medicine Laboratory, University of Sydney, Clinical Sciences Building, Concord Hospital, New South Wales 2139, Australia. E-mail: ianb@med.su.oz.au

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of autosomal recessive or X-linked CMT, variable penetrance, phenocopies, or de novo mutations. Patel et al. (1990) suggested that a de novo mutation was present in a woman several generations back in a large French-Arcadian kindred, since she had nine unaffected siblings. This woman's subsequent CMT lineage was the first family to be shown to carry the CMT1A DNA duplication. Hoogendijk et al. (1992) estimated that de novo duplications were responsible for 9 of 10 sporadic CMT1 cases.

In order to determine the prevalence of de novo CMT1A duplications, we investigated 118 duplication-positive CMT1A families. We have shown that in 10 of these families the disease has arisen as the result of a de novo mutation. We have also shown that the de novo duplication may have either a paternal or a maternal origin.

Families and Methods

CMT1A Family Selection and Diagnosis

Patients with CMT were referred to us by neurologists and geneticists because of our interest in CMT. The appropriate informed consent was obtained from all subjects. One hundred and eighteen CMT1A families were used in this study.

CMT1 was diagnosed by the combination of a CMT syndrome (distal wasting and weakness, mild sensory loss, and pes cavus) in a patient with slow median nerve-conduction velocities (Harding and Thomas 1980). No CMT1A individuals had median conduction velocities faster than 42 m/s (Nicholson 1991).

All 118 families had been shown to be positive for the CMT1A DNA duplication, by establishing linkage (Nicholson et al. 1992) and/or by one of the four tests described below. Paternity was tested by genotyping with a set of microsatellite polymorphisms that included Apo C2, D21S167, MCT112, D17S793, D17S921, D17S1356, D17S1357, D17S1358, and RM11-GT (Weber and May 1989; Guo et al. 1990; Wallis et al. 1990; Lupski et al. 1991*a;* Gyapay et al. 1994; Blair et al. 1995). Observed genotypes were consistent with the stated paternity.

De novo mutation families were initially ascertained by identifying those kindreds in which the parents of the first affected individual had no clinical or electrophysiological abnormalities. Each proband showed a CMT1 syndrome when examined clinically and electrophysiologically. Normal nerve-conduction velocities were obtained from remaining healthy family members.

CMT1A Duplication Analysis

Pulsed-field gel electrophoresis (PFGE) tests for the CMT1A duplication were carried out according to the procedures described by Lupski et al. (1991*a*) and Kennerson et al. (1995). Agarose plugs containing high-molecular-weight patient DNA were prepared for PFGE from fresh lymphocytes. DNA was digested with *Sac*II or *Eag*I according to the suppliers' instructions (Boehringer Mannheim) and electrophoresed in a CHEF-Mapper PFGE apparatus (Bio-Rad) using reported conditions. DNA was transferred to nylon membrane (Hybond N+, Amersham) and hybridized either with the probe VAW409R3 (Lupski et al. 1991*a*) or cosH1 (Kennerson et al. 1995). These probes reveal novel DNA fragments in patients carrying the CMT1A duplication. VAW409R3 identifies a novel 500-kb fragment, and cosH1 reveals a novel 150-kb fragment.

A PCR-based test for the CMT1A duplication was carried out on genomic DNA according to the procedure described by Blair et al. (1995). DNA was extracted from whole blood as described elsewhere (Griffiths et al. 1988). Genotyping was carried out for the polymorphic microsatellite markers AFM165zd4 (D17S793), AFM191xh2 (D17S921) (Gyapay et al. 1994), RM11-GT (D17S122) (Lupski et al. 1991*a*), 103B11ac1 (D17S1357), and 133C4ac1 (D17S1358) (Blair et al. 1995), using reported oligonucleotide primers and conditions.

A DNA dosage test for the CMT1A duplication locus D17S122 was carried out on patient genomic DNA. Patients were genotyped for the duplication by using RFLP probe VAW409R3, according to the procedure described by Raeymaekers et al. (1991) and Lupski et al. (1991*a*). Comparison of hybridization signals from each allele allows CMT1A duplication status to be determined on those patients who are heterozygous for that locus.

Results

Detection of De Novo Duplications in CMT1A Families

CMT1A duplication analysis identified a de novo duplication in 10 of the 118 CMT1A families. This number was determined by demonstrating the presence of the CMT1A duplication in the proband while demonstrating its absence in all parents (fig. 1).

Parental Origin of De Novo Duplications

The 10 de novo duplication families were genotyped for the duplication microsatellite markers D17S793, D17S921, RM11-GT, D17S1357, and D17S1358, and the RFLP VAW409. Informative genotypes are shown in figure 1. Allele segregation allowed the parental origin of the disease chromosome to be determined. In probands from families 1 and 4–9, the CMT1A chromosome had a paternal origin, while the proband from family 2 showed a duplication of maternal origin (see, e.g., fig. 2). Families 3 and 10 were not informative for

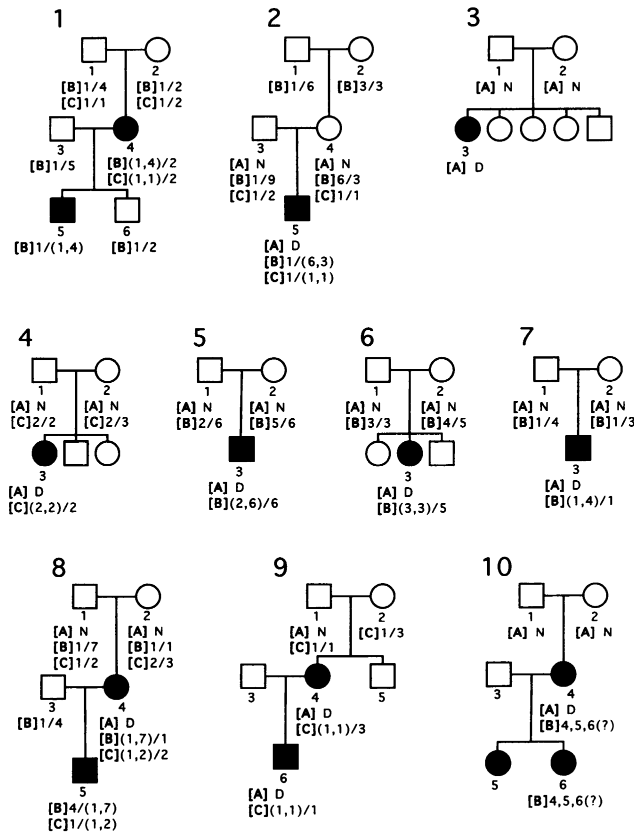

Figure 1 CMT1A duplication analysis in 10 de novo duplication families identified from 118 duplication-positive CMT1A families. Data following "[A]" represents CMT1A duplication status determined by PFGE analysis. N = normal; D = duplication. "(?)" = phase could not be determined. Data following "[B]" represents informative genotypes for polymorphic AC repeat markers from the CMT1A duplication. For family 1, the genotypes are for the marker D17S1357; for family 2 the data are for marker D17S921; for families 5 and 10 the data are for marker RM11-GT; for families 6 and 7 the data are for marker D17S1358; and for family 8 the data are for marker D17S793. Data following "[C]" represents informative genotypes for RFLP VAW409 from the CMT1A duplication.

these duplication markers, and the duplication origin could not be determined.

Discussion

We have identified 10 CMT1A families which have de novo duplications shown by molecular means. These constitute 8.5% of the total 118 families investigated. This represents the largest investigation to date of the prevalence of de novo duplications. Previous studies by Raeymaekers et al. (1991), Wise et al. (1993), and Hertz et al. (1994) observed de novo duplications in 8.3% (1/12), 16.7% (1/6), and 4.7% (2/43) of CMT1A families, respectively. The results of this and previous studies are probably underestimates due to the means of ascertainment. There would have been an initial selection bias for families for linkage studies; de novo mutation families were selected/identified only where parents were available; and sporadic cases may not be recognized as having CMT. This would suggest that ⩾10% of autosomal dominant CMT1 families are due to de novo duplications.

Skre (1974) found 10 (27%) isolated sporadic cases in a study of 37 index families in Norway. All but one were males, which he assumed were instances of X-linked CMT. Harding and Thomas (1980) reported 26 (38%) sporadic cases in 69 CMT type I families. By segregation analysis, 70% of these cases were determined to be recessive CMT. With this correction, CMT in 11% of Harding and Thomas's families may be due to de novo mutations, which is in agreement with our lower estimate.

To account for this high new mutation rate while the incidence of CMT1A is assumed to be not increasing, these de novo CMT1A mutations must be balanced either by back mutations or by decreased fitness. Harding and Thomas (1980) estimated that biological fitness is unimpaired in CMT. Therefore, de novo CMT1A mutations are likely to be balanced by the presence of back mutations. Back mutations are found in the Bar duplication of *Drosophila* (Tsubota et al. 1989).

Sporadic CMT1 cases that do not have a duplication are more difficult to define. These may be sporadic cases

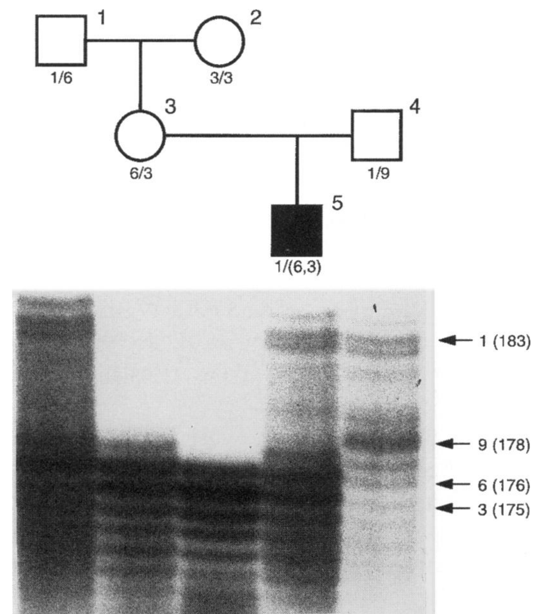

Figure 2 Maternal origin of a de novo CMT1A duplication demonstrated by allele segregation in family 2. Genotypes for the polymorphic microsatellite marker D17S921 are shown. Arrows indicate designated alleles; allele sizes (in base pairs) are in parentheses. Allele segregation for RFLP VAW409 also demonstrated the maternal origin (not shown).

of X-linked CMT, CMT1B, recessive CMT I, or Dejerine-Sottas disease.

Physical mapping of the region duplicated in CMT1A has shown that it is flanked by a complex binary repeat element known as CMT1A-REP. The CMT1A duplication is thought to be the product of unequal crossing over between two parental chromosome 17 homologues during meiosis, probably due to misalignment of these CMT1A-REP elements (Pentao et al. 1992). A paternal origin of the de novo duplication has been demonstrated in all 13 sporadic cases reported prior to this study (Raemaekers et al. 1991; Palau et al. 1993; Wise et al. 1993; Hertz et al. 1994). This report presents the first evidence of a de novo duplication with a maternal origin. Recombination fractions for the region duplicated in CMT1A are significantly larger in females than in males (CHLC World Wide Web database), eliminating higher male recombination as a possible explanation for this disparity in paternal/maternal origin. Palau et al. (1993) suggested that male-specific factors may operate during spermatogenesis to help form the duplication and/or stabilize the duplicated chromosome. The identification of a de novo duplication with a maternal origin indicates that this is not a phenomenon associated solely with male meioses. There may be greater protection from misalignment during synapsis, and/or lower activity of those factors or mechanisms which lead to unequal crossing over at the CMT1A locus during oogenesis.

Another neuropathy, hereditary neuropathy with liability to pressure palsies (HNPP), has been linked to the same genetic locus as CMT1A and is attributed in most cases to a 1.5-Mb DNA deletion. The region deleted corresponds exactly to the region duplicated in most CMT1 patients and is thought to be the reciprocal product of the unequal crossing over caused by misalignment of the CMT1A-REP elements (Pentao et al. 1992). A de novo deletion of maternal origin has been reported in a patient with HNPP (Reisecker et al. 1994).

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