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Molecular Analyses of Unrelated Charcot-Marie-Tooth (CMT) Disease Patients Suggest a High Frequency of the CMT1A Duplication

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Summary

Charcot-Marie-Tooth disease (CMT) is the most common inherited peripheral neuropathy. One form of CMT, CMT type 1A, is characterized by uniformly decreased nerve conduction velocities, usually shows autosomal dominant inheritance, and is associated with a large submicroscopic duplication of the p11.2-p12 region of chromosome 17. A cohort of 75 unrelated patients diagnosed clinically with CMT and evaluated by electrophysiological methods were analyzed molecularly for the presence of the CMT1A DNA duplication. Three methodologies were used to assess the duplication: measurement of dosage differences between RFLP alleles, analysis of polymorphic $(GT)_n$ repeats, and detection of a junction fragment by pulsed-field gel electrophoresis. The CMT1A duplication was found in 68% of the 63 unrelated CMT patients with electrophysiological studies consistent with CMT type 1 (CMT1). The CMT1A duplication was detected as a de novo event in two CMT1 families. Twelve CMT patients who did not have decreased nerve conduction velocities consistent with a diagnosis of CMT type 2 (CMT2) were found not to have the CMT1A duplication. The most informative molecular method was the detection of the CMT1A duplication–specific junction fragment. Given the high frequency of the CMT1A duplication in CMT patients and the high frequency of new mutations, we conclude that a molecular test for the CMT1A DNA duplication is very useful in the differential diagnosis of patients with peripheral neuropathies.

Introduction

Charcot-Marie-Tooth polyneuropathy syndrome (CMT) is the most common inherited peripheral neuropathy, with an estimated prevalence of 1 in 2,500 (Skre 1974). CMT is both clinically and genetically heterogeneous. It is characterized clinically by progressive weakness of distal muscles, particularly those innervated by the peroneal nerve. Clinical features are variable and may include steppage or equine gait, pes cavus deformity, loss of muscle stretch reflexes, and atrophy of intrinsic hand muscles (Charcot and Marie 1886; Tooth 1886; Dyck and Lambert 1968*a*, 1968*b*; Lupski et al. 1991*a*; Vance 1991; Dyck et al. 1992). Age at onset of these symptoms is 12.2 ± 7.3 years (Bird and Kraft 1978). Pathology and electrophysiology enable distinction of two major types of CMT patients: type 1 (CMT1), with severe slowing of nerve conduction velocities (NCVs) and, in some patients, visible or palpable enlarged nerves, and type 2 (CMT2), with normal to near normal NCVs, no enlarged nerves, and normal muscle stretch reflexes. The term "CMT1" has been used interchangeably with "hereditary motor and sen-

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sory neuropathy I" (HMSN I); likewise, "CMT2" has been used interchangeably with "HMSN II" (Lupski et al. 1991*a;* Vance 1991; Dyck et al. 1992). Decreased (<40 m/s) NCVs are present in all patients with CMT1 and appear to be a 100% penetrant phenotype virtually independent of age (Kimura 1989; Nicholson 1991; Kaku et al., in press-*a*). The conduction defect is uniform nerve to nerve and segment to segment in the same nerves, without evidence of conduction block (Dyck et al. 1992; Kaku et al., in press-*b*). Peripheral nerves of patients with CMT1 show onion-bulb formation and a decrease in the number of myelinated fibers, while onion bulbs are occasionally seen in nerves of CMT2 patients (Lupski et al. 1991*a;* Dyck et al. 1992; Lupski and Garcia 1992). However, it should be noted that, clinically, CMT1 and CMT2 are virtually indistinguishable in the absence of NCV information (Lupski et al. 1991*a;* Vance 1991).

Several modes of inheritance of CMT have been described, and sporadic cases of CMT have also been noted (McKusick 1992). In one form the disease locus is linked to the pericentromeric region of chromosome X (Bergoffen et al. 1993) and, in some X-linked recessive families, to a different region in Xp22.3 (Ionasescu et al. 1991). An autosomal recessive form has also been described, but no linkage has been established (Vance 1991). Linkage analysis in CMT1 families has delineated at least two autosomal dominant forms. Linkage to the Duffy locus on chromosome 1 (Bird et al. 1982; Lebo et al. 1991) has been demonstrated in a small number of families, but in the majority of families the disease locus is linked to the proximal region of the short arm of chromosome 17 (Raeymaekers et al. 1989, 1991, 1992; Vance et al. 1989, 1991; Chance et al. 1990; McAlpine et al. 1990; Middleton-Price et al. 1990; Patel et al. 1990*a,* 1990*b;* Timmerman et al. 1990; Lupski et al. 1991*a*). These two autosomal dominant forms are called "CMT1B" and "CMT1A," respectively. A third distinct locus for autosomal dominant CMT1 has also been implicated (Chance et al. 1990, 1992*b*).

CMT1A is associated with a large submicroscopic duplication in 17p11.2-p12 (Lupski et al. 1991*b,* 1993; Raeymaekers et al. 1991, 1992; Bellone et al. 1992; Brice et al. 1992; Chance et al. 1992*b;* Hallam et al. 1992; Hoogendijk et al. 1992; MacMillan et al. 1992) in the majority of patients examined to date. Both the identification of patients with cytogenetically visible partial (Lupski et al. 1992; Upadhyaya et al. 1993; Roa et al., in press) or complete (Chance et al. 1992*a*) trisomy 17p and electrophysiological studies consistent with CMT1 support a gene-dosage model as the cause of autosomal dominant CMT in patients with the CMT1A duplication. A gene which is highly expressed in peripheral nerve has been mapped within the CMT1A duplication. This gene, *PMP22* (*p*eripheral *my*elin *p*rotein, 22 kD), has been proposed as a candidate gene for CMT1A (Matsunami et al. 1992; Patel et al. 1992; Timmerman et al. 1992; Valentijn et al. 1992). The identification of *PMP22* point mutations in nonduplication CMT1A patients strongly suggests that this gene has a critical role in the phenotypic effects of DNA duplication associated with CMT1A (Valentijn et al. 1992; Roa et al. 1993).

Experiments described in this study were designed to determine whether a molecular test to detect the CMT1A duplication could be a practical first step in the diagnosis of patients having clinical symptoms of CMT. Clinical and molecular analyses of the CMT1A region in a large cohort of unrelated patients were undertaken to determine the frequency with which the CMT1A duplication is detected in patients with the CMT clinical phenotype. NCVs were measured for each patient. To determine the presence or absence of the CMT1A duplication, DNA from each patient was subjected to three molecular methodologies: analysis of $(GT)_n$ repeat polymorphisms, measurement of dosage differences between RFLP alleles by Southern analysis, and detection of a junction fragment by pulsed-field gel electrophoresis (PFGE). The informativeness of each of these methods, as well as the frequency with which this duplication is found in clinically diagnosed CMT patients, were determined. These analyses were also critical in identifying those patients who might have a mutation other than duplication, possibly mutation of the *PMP22* gene, as a cause of their disease. In addition, the mechanism and parental origin of de novo CMT1A duplication was investigated in two families. The results support the conclusion that the CMT1A duplication is a common mechanism for CMT and suggest that molecular analysis may be the most suitable first test in the diagnostic evaluation of patients with a clinical examination consistent with CMT.

Subjects, Material, and Methods

Patient Profiles

Blood samples and NCVs were obtained with informed consent from patients in clinics throughout the United States. NCVs were measured by physicians at the referring institutions. Approximately one-half of the patients were evaluated, and samples were col-

lected, at the Muscular Dystrophy Association clinics in the Louisiana cities of Lafayette, Baton Rouge, and New Orleans. All patients exhibited signs and symptoms consistent with a clinical diagnosis of CMT. Although the complete ethnic background of each patient was not determined, the group as a whole was quite diverse, including individuals of European, French-Acadian, African-American, and Lebanese descent. Patients presenting clinical evidence, as well as abnormal (<40 m/s) NCVs (Kimura 1989) without conduction blocks, were diagnosed as having CMT1, whereas those with normal or near normal NCVs were diagnosed as CMT2 patients. The CMT1 patients included 37 females and 26 males. Average NCVs and their SDs were calculated for CMT1 patients with and without the duplication and for CMT2 patients.

Molecular Analyses

Probes pVAW409R3a, pVAW409R1b, and pEW401HE have been described elsewhere (Wright et al. 1990; Raeymaekers et al. 1991) and were provided by Dr. Christine Van Broeckhoven. p132-G8R1, a cloned portion of the *PMP22* gene, was used as a probe to detect polymorphic *Hin*cII alleles (Patel et al. 1992). Southern blotting was performed as described elsewhere (Lupski et al. 1991*b*). Dosage differences between heterozygous RFLP alleles were assessed by visually comparing the intensity of the hybridization signal of one polymorphic allele versus another allele detected by the same probe. Dosage analysis was conducted by at least three different individuals who were unaware of the diagnosis. PFGE was performed as described elsewhere (Lupski et al. 1991*b*). In brief, patient lymphocyte DNA was prepared in agarose plugs. After digestion with *Sac*II, the DNA was separated by PFGE, transferred to nylon membrane, and hybridized to pVAW409R3a.

For $(GT)_n$ genotype analysis at the D17S122 locus (RM11-GT), 0.3 μg of genomic DNA from each patient was amplified with primers IMG 3342 (GT strand) and IMG 3343 (CA strand) as described elsewhere (Lupski et al. 1991*b;* Guzzetta et al. 1992), with the exceptions that (*a*) IMG 3343 was end labeled instead of IMG 3342 and (*b*) annealing was at 65°C instead of 55°C. A 2-μl portion of the 25-μl PCR reaction was mixed with 4 μl of loading dye (0.1% each of xylene cyanol and bromophenol blue, 0.01 M EDTA in deionized formamide), heated at 100°C for 5 min, and immediately loaded on a 6% Long-Ranger acrylamide/6 M urea gel. The gel was exposed, without drying, to Kodak XAR-5 film for 12–14 h. No intensifying screen was used.

DNA typing for paternity testing was performed by Southern analysis of DNA of HOU191 family members 741, 742, and 752 and HOU218 family members 771, 770, and 669 by using the single-locus probes D2S44, D4S139, D14S13, and D16S85, which provided a combined average 99.93% power of exclusion. Probability of paternity was calculated on the basis of a prior probability of .5 and Caucasian population frequency data compiled by the Baylor College of Medicine Kleberg DNA Diagnostic Laboratory, where the testing was performed.

Results

NCVs

NCVs of median, ulnar, or peroneal nerves from the 42 apparently unrelated CMT1 patients who possessed the CMT1A duplication (see below) and from the 21 in whom the duplication was not detected are depicted in figure 1. Normal values for NCVs are age dependent and can have a range of 40–60 m/s (Kimura 1989). The average median and ulnar motor NCVs of patients demonstrated to have the CMT1A DNA duplication were 20.8 ± 6.0 m/s and 19.9 ± 7.2 m/s, respectively. These values were not significantly different from those for CMT1 patients without the CMT1A duplication, for whom the average value for median motor NCV was 20.9 ± 7.4 m/s and the average ulnar motor NCV was 20.2 ± 7.9 m/s. For the 12 patients diagnosed with CMT2, the average value for median motor NCV was 49.1 ± 7.7 m/s, and that for ulnar motor NCV was 50.9 ± 10.6 m/s.

Detection of the Duplication by RFLP and Simple Sequence Repeat (SSR) Analyses

Probe VAW409R3a (D17S122) detects polymorphic *Msp*I alleles of 2.8 kb, 2.7 kb, and 1.9 kb (Wright et al. 1990; Raeymaekers et al. 1992). Either a dosage difference between two different alleles or, in rare cases, the appearance of three alleles can be detected in patients with the CMT1A duplication (Lupski et al. 1991*b;* Raeymaekers et al. 1991, 1992). Probe pVAW409R3a (D17S122) was used to assess dosage in CMT1 patients and was the most informative marker, with a heterozygosity of 70% (44/63). In 8% (5/63) of the CMT1 patients, all three VAW409R3a (D17S122) alleles were detected. Dosage was also scored between polymorphic *Eco*RI/*Hin*cII alleles (11 kb and 9.6 kb) detected by probe p132-G8R1 (a genomic subclone of the CMT1A candidate gene *PMP22*), because of a *Hin*cII site polymorphism (Patel et al. 1992). This marker was

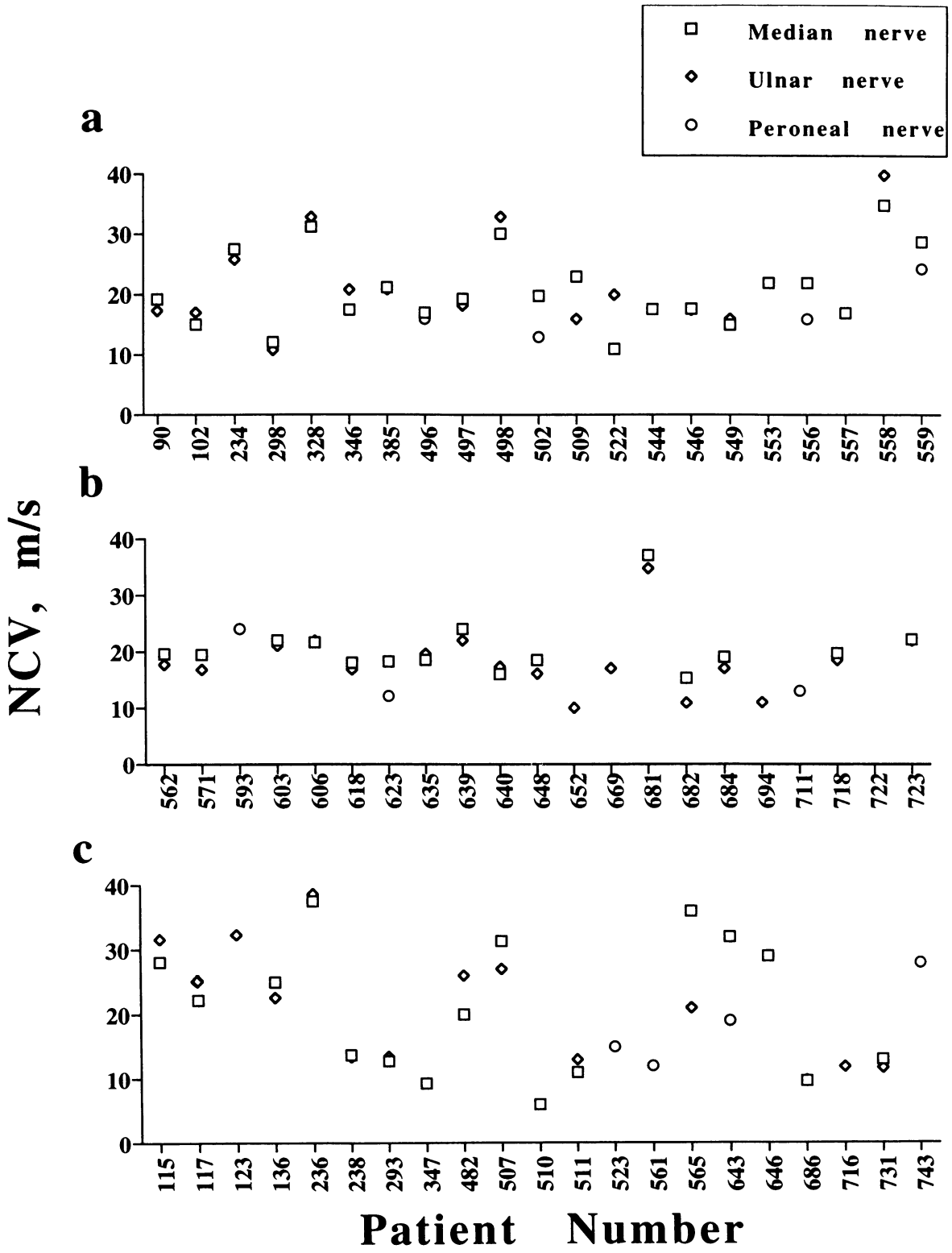

Figure 1 NCVs of unrelated CMT1 patients. Patient numbers are given below, while motor NCVs for median, ulnar, or peroneal nerves for each patient are given in meters per second. Electrophysiological studies were performed at the referring institutions. *a* and *b*, NCVs of CMT1 patients having the CMT1A duplication. NCVs for patient 722 were not recordable. *c*, NCVs of CMT1 patients not having the CMT1A duplication.

the second most informative, with 62% (39/63) patients heterozygous, including four patients (HOU97-385, HOU131-511, HOU160-565, and HOU230-694) not informative by the other RFLP analyses. In addition, dosage between polymorphic *Msp*I alleles detected by probes pVAW409R1b (D17S122) (5.3 kb and 2.7+2.6 kb) and pEW401HE (D17S61) (5.5 kb and 4.4 kb) was scored. pVAW409R1b (D17S122) and pEW401HE (D17S61) detected heterozygous alleles in 44% and 41% of the cases, respectively (data not shown).

Patients were also genotyped for RM11-GT, a $(GT)_n$ SSR previously identified from within the CMT1A duplicated region (Lupski et al. 1991*b*). Two or three alleles were detected in 80% of individuals, and in 46% of CMT1A duplication cases three alleles were detected, yielding a fully informative analysis. In all cases the results were consistent with the results from the other methods (data not shown). CMT2 patients were also subjected to RFLP and RM11-GT analyses. No evidence of a duplication was detected in any informative cases (data not shown).

Detection of a Junction Fragment by PFGE

It has been demonstrated in patients harboring the CMT1A duplication that a unique 500-kb junction fragment is detected in DNA digested with *Sac*II, fractionated by PFGE, and hybridized to pVAW409R3a (D17S122) (Lupski et al. 1991*b;* Raeymaekers et al. 1992). Data shown in figure 2 are representative of the PFGE analyses from all 63 CMT1 patients. The junction fragment detected in all CMT1A duplication patients appeared to be the same size. The presence of both 600-kb and 550-kb fragments in normal control individuals, as well as the variation in intensities between these bands from individual to individual, have been attributed to differential methylation (Lupski et al. 1991*b;* Pentao et al. 1992). Of those patients having decreased NCVs, 68% (43/63) possessed the junction fragment. Conversely, 100% of patients having the CMT1A duplication, as evidenced by the presence of the 500-kb *Sac*II junction fragment, also exhibited decreased NCVs. In contrast, no junction fragment was detected for any of the CMT2 patients (data not shown). In all cases where RFLP analysis or RM11-GT genotyping was informative, the results were consistent with PFGE detection of the junction fragment.

New-Mutation CMT1A Duplication

Raeymaekers et al. (1991) first reported new-mutation CMT1A duplication associated with the onset of

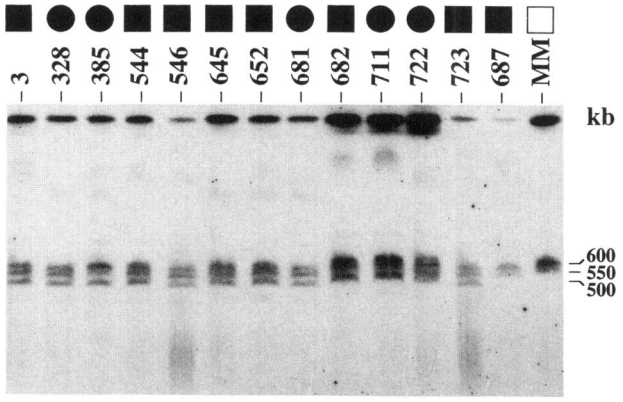

Figure 2 Unrelated CMT1 patients with the CMT1A duplication, who have apparently identical junction fragments. Lane 1 shows a control CMT1A duplication patient (HOU1-3; Lupski et al. 1991*b*), while lanes 2–12 represent 11 unrelated CMT1A duplication patients, with the numbers at the top of the figure indicating patient identification numbers. Note that all patients with the CMT1A duplication appear to have the same-size (i.e., 500-kb) junction fragment. The asymmetrical intensities of the 600-kb and 550-kb bands most likely reflect methylation differences (Lupski et al 1991*b;* Pentao et al. 1992). Lane MM represents an unaffected control individual. Patient 687 represents a CMT1 case who was subsequently shown to contain a point mutation in *PMP22* (Roa et al. 1993). Patients were of varied ethnic origins, including African-American (patient 546), Lebanese (patient 544), and French-Acadian (patient 3).

the CMT1 phenotype. The CMT1A duplication may account for as much as 90% of sporadic CMT1, according to one estimate (Hoogendijk et al. 1992). Two families in the present study, HOU191 and HOU218, were demonstrated to have a de novo CMT1A duplication. The new-mutation patients HOU191-752 and HOU218-669 appeared to have the same 500-kb *Sac*II junction fragment (fig. 3) as did the unrelated patients from CMT1A families segregating the disease phenotype in an autosomal dominant manner (fig. 2). The de novo duplication in pedigree HOU191 was determined to be paternal in origin, by analysis of both segregation of polymorphic $(GT)_n$ alleles at the D17S122 locus (data not shown) and dosage differences for *Msp*I RFLP alleles detected by pEW401HE at the D17S61 locus (fig. 4). RFLP analysis was fully informative for marker VAW409R3a (D17S122) in pedigree HOU218 and also showed a de novo duplication of paternal origin (fig. 4). DNA typing was consistent with paternity of (*a*) new-onset CMT1 patient 752 by the unaffected male 741, at a probability of >99.89%, and (*b*) CMT1 patient 669 by the unaffected male 771, at a probability of >99.99%.

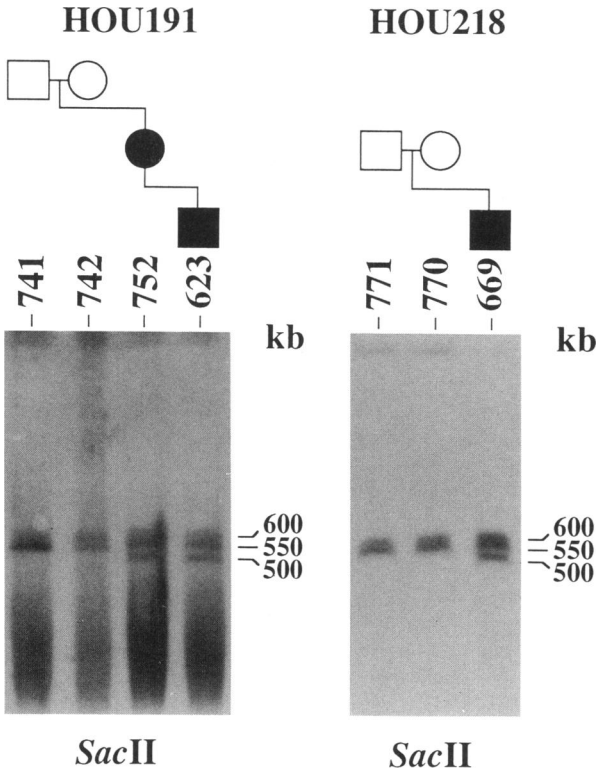

Figure 3 New-mutation CMT1A duplication patients, who appear to have the same-size junction fragments. PFGE was performed on lymphocytes from the new-mutation families HOU191 and HOU218, as in fig. 2. Standard pedigree symbols are used, and the patient numbers are given at the top of the gel. Patient HOU191-752 has four clinically unaffected brothers and one clinically unaffected sister, while patient HOU218-669 has two clinically unaffected brothers. Note that the new-mutation-duplication patients appear to have the same 500-kb *Sac*II junction fragment as was observed in unrelated patients (fig. 2 and Lupski et al. 1991*b*, 1993) and in CMT1A families with dominant inheritance (Lupski et al. 1991*b*).

Discussion

CMT1A is associated with a large submicroscopic duplication of the proximal short arm of chromosome 17 in affected individuals within several large families. The CMT1A duplication has also been observed in a number of unrelated individuals (Lupski et al. 1991*b*, 1993; Raeymaekers et al. 1991, 1992; Bellone et al. 1992; Brice et al. 1992; Chance et al. 1992*b*; Hallam et al. 1992; Hoogendijk et al. 1992; MacMillan et al. 1992). It was important to know whether this association is specific and common in a large population of unrelated CMT patients, in order to determine whether a molecular test for the duplication would be a useful diagnostic tool. Only patients with clearly documented clinical findings and nerve conduction studies were included in this study, so as to firmly establish the diagnosis of CMT. The results indicate that 57% of the 75 unrelated patients having clinical symptoms of CMT have the CMT1A duplication and that 68% of the 63 patients with decreased NCVs, as well as clinical symptoms of CMT or CMT1, harbor the CMT1A duplication. Although half of the patients came from one geographic area in southern Louisiana, and although some could have been distantly related, 68% of this subset of 31 CMT1 patients were found to harbor the CMT1A duplication. The similarity of this frequency to that

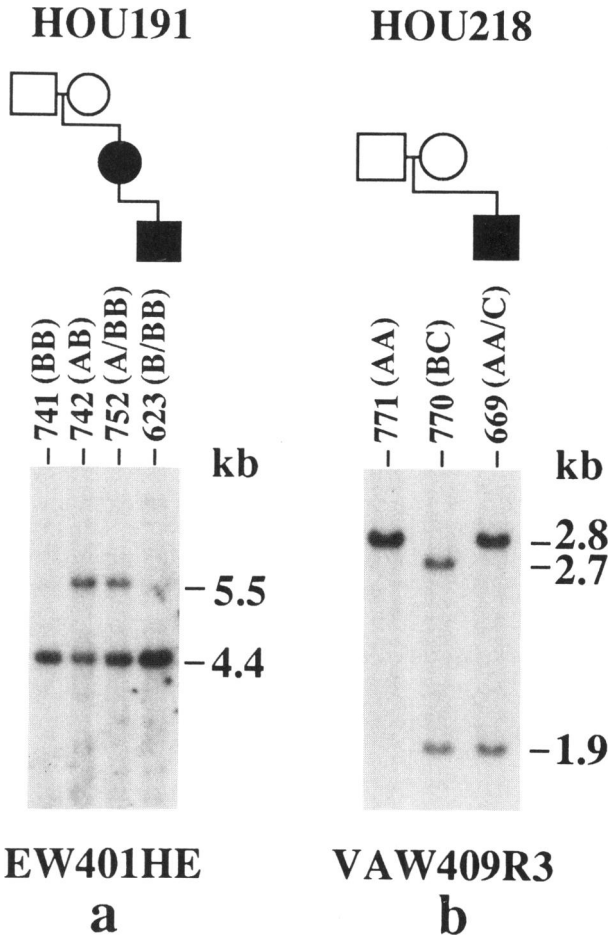

Figure 4 Parental origin of new-mutation CMT1 duplication. *a*, RFLP analysis of new-mutation family HOU191, which demonstrates dosage differences for *Msp*I alleles detected by pEW401HE (D17S61) in the new-mutation patient (lane 3) and confirms the paternal origin of the CMT1A duplication. *b*, RFLP analysis of new-mutation family HOU218, which is fully informative for D17S122 detected by pVAW409R3a and demonstrates a de novo duplication (lane 3) of paternal origin.

seen for all the patients suggests that this subgroup reflects the true frequency in an unrelated CMT1 patient population.

RFLP analyses in CMT patients were performed to determine the overall informativeness of each marker and to provide multiple lines of evidence for detection of the CMT1A duplication in each patient. RM11-GT genotyping was also performed, for the same reasons. VAW409R3a (D17S122), p132-G8R1 (*PMP22*), VAW409R1b (D17S122), and EW401HE (D17S61) were 70%, 62%, 44%, and 41% heterozygous, respectively. The calculated heterozygosities of VAW409R3a and VAW409R1b at the D17S122 locus were higher than the published heterozygosities of 55% and 24%, respectively (Barker et al. 1989); however, the 42% heterozygosity calculated for EW401HE (D17S61) was similar to the published heterozygosity of 43.5% (Barker et al. 1987). The SSR RM11-GT at the D17S122 locus had a heterozygosity (80%) slightly higher than that previously published (74%) (Lupski et al. 1991*b*), although only 46% (20/43) of CMT1A duplication patients had three distinguishable alleles. This method was considered informative only in the latter case, because the shadow bands inherent in the procedure made it difficult to assess dosage between two alleles. Although the $(GT)_n$ polymorphism analysis was not as useful as PFGE or standard RFLP analysis, for detecting the CMT1A duplication, the high heterozygosity of this marker is very useful for monitoring the origin of the duplication as well as segregation of this chromosomal region. In this study and others (Lupski et al. 1991*b;* Raeymaekers et al. 1991, 1992), no preferential association of a particular haplotype with the CMT1A duplication has been observed. This, combined with the evidence of de novo duplication mutations, suggests several independent origins of the duplication.

In this study the only consistently unambiguous method for scoring the CMT1A duplication was detection of a junction fragment by PFGE followed by hybridization to the appropriate probes. However, PFGE is the most laborious and time-consuming methodology of the three used. We suggest that measurement of dosage differences between RFLPs within the CMT1A region might be one of the most efficient molecular tests in most cases of CMT. Indeed, only five CMT1 patients (117, 136, 509, 558, and 711) were uninformative by RFLP analysis, and cumulative RFLP analysis with probes VAW409R3a (D17S122) and p132-G8R1 (*PMP22*) was informative for the presence of the CMT1A duplication in 86% of CMT1 patients. Thus, RFLP analysis with the latter probes may be the most efficient first test for detecting the CMT1A duplication. However, care must be exercised when this approach is used, since partial restriction-enzyme digestion or degradation of the genomic DNA can lead to spurious interpretations. Cases where neither probe detects heterozygous alleles could then be resolved by PFGE.

It is striking that the same-size junction fragment, at least at the resolution of PFGE, has been detected in all duplication patients examined to date (Lupski et al. 1991*b,* 1993; Raeymaekers et al. 1992). In Duchenne muscular dystrophy, intragenic duplication appears to be a causative mechanism in 10% of cases; however, these duplications are of varying sizes (Hu et al. 1990). The present study demonstrated that the de novo CMT1A duplication was associated with a 500-kb *Sac*II junction fragment, as observed in the other unrelated patients. The fact that multiple unrelated CMT1A patients, as well as de novo CMT1A duplication patients, appeared to have a similar junction fragment (Lupski et al. 1991*b,* 1993; Raeymaekers et al. 1992; present paper, figs. 2 and 3) suggests that a precise recombination mechanism is involved in generating the CMT1A duplication. Physical mapping of the region has revealed that the CMT1A duplication is a 3-Mb tandem duplication of a 1.5-Mb monomer unit flanked on the normal chromosome by a large (>17-kb) repeat sequence (CMT1A-REP) in the same orientation. This repeat sequence was found in two copies on the normal chromosome and in an additional copy on the CMT1A chromosome, as predicted by the duplication mechanisms described above (Pentao et al. 1992). According to models of interchromosomal unequal crossing-over, two different products should be generated: one chromosome with an interstitial duplication and the other with a deletion. The corresponding deleted chromosome appears to have been identified recently in three families exhibiting hereditary neuropathy with liability to pressure palsies (McKusick 1992; MIM 162500) (Chance et al. 1993).

Both new-mutation CMT1A duplications described in this study, as well as the first de novo duplication identified (Raeymaekers et al. 1991), appear to have arisen by unequal crossing-over between paternal chromosome 17 homologues. In one report, 9 of 10 unrelated sporadic CMT1 patients analyzed for the CMT1A duplication by either RFLP or SSR analysis at the D17S122 locus were identified as new mutations (Hoogendijk et al. 1992); however, the parental origin of the duplication was not determined. Isolated cases

of CMT have been assumed to be autosomal recessive (Harding and Thomas 1980), but, according to the above data, a significant number may be de novo duplications. Thus, it appears that the high frequency of the CMT1A duplication is due not only to familial inheritance but also to recurrence of the original molecular event. In light of this, family history of the disease should not be a required criterion for diagnosis of CMT1.

The nosology of the inherited peripheral neuropathies is complex and may include several overlapping disease entities. Molecular analysis will be useful in determining which inherited neuropathies result from variable expression of a single mutation, allelic heterogeneity, or nonallelic heterogeneity (Suthers and Davies 1992). We have documented here that the CMT clinical phenotype, including electrophysiological measurements of motor NCV, can vary tremendously in unrelated patients with the CMT1A duplication (fig. 1). We have previously documented variable expression of the clinical phenotype in members of the same family (Lupski et al. 1991*a*) and even in identical twins (Garcia et al. 1990). The CMT1 electrophysiological phenotype can also vary within a family where all affected members have the CMT1A DNA duplication (Kaku et al., in press-*a*). The CMT1A duplication encompasses a 1.5-Mb region (Pentao et al. 1992) likely to contain many genes. Recombination within a segment this large would be expected to occur with a frequency of $\geqslant 1\%$. Indeed, recombination within the duplicated region has been demonstrated (Hallam et al. 1992; Raeymaekers et al. 1992), limiting the application of haplotype analysis in linkage disequilibrium studies. Therefore, patients within a family can have the same-size duplication but could contain different alleles of the genes within the duplication (Lupski 1992). In addition, gene dosage appears to be an underlying mechanism in CMT1A-duplication patients (Chance et al. 1992*a;* Lupski 1992; Lupski et al. 1992; Roa et al., in press), and we speculate that modifier genes, environment, or stochastic factors could play an even more prominent role than they do in disorders not resulting from dosage effects.

Allelic heterogeneity was demonstrated in patient HOU226-686, who presented clinical and electrophysiological features similar to those of the other CMT1 patients but who did not have evidence of the CMT1A duplication. Instead, this patient was recently shown to have a point mutation in the *PMP22* gene (Roa et al. 1993). A number of other patients likewise exhibited the decreased NCVs consistent with a diagnosis of CMT1 but appeared to have neither the CMT1A duplication nor mutation within the *PMP22* coding region (authors' unpublished observations). Since linkage data were not available, some of these latter patients may represent either (*a*) CMT1B, which has been linked to chromosome 1, (*b*) CMT1C, which is linked neither to chromosome 1 nor to chromosome 17 (Chance et al. 1990, 1992*b*), or (*c*) X-linked CMT.

Other patients may represent nonallelic heterogeneity or coinheritance of two separate disorders. For example, patient HOU91-247 appeared clinically to have a Dejerine-Sottas phenotype (Dejerine and Sottas 1893), which has been classified as a more severe form of CMT that is inherited recessively, but did not have the CMT1A duplication. Alternatively, patient HOU230-694, who was shown to have the CMT1A duplication, has CMT1 in addition to hearing loss, listed as a separate genetic entity (McKusick 1992). We previously demonstrated that two patients with CMT1 and NF1 had the CMT1A duplication and likely inherited these two separate disorders by chance (Lupski et al. 1993) Other phenotypes associated with CMT may reflect the high frequency of this disorder and coinheritance of two different genetic disorders.

We propose that, in light of both the frequency with which the CMT1A duplication is detected in this population of CMT patients and the frequency of de novo duplication in sporadic patients (Hoogendijk et al. 1992), a molecular analysis for the CMT1A duplication should be considered as a primary test in differential diagnosis of peripheral neuropathy patients, as well as for presymptomatic and prenatal diagnosis in CMT1 families. We are currently screening the CMT1A region for polymorphic SSRs that could be amplified by the PCR (Litt and Luty 1989; Weber and May 1989) in a multiplex strategy, in order to assemble enough distinguishable alleles to be consistently informative. We are also exploring the use of fluorescence in situ hybridization to interphase lymphocyte chromosomes to detect duplication of probes within the CMT1A region (Lupski et al. 1991*b*). Both are promising strategies for rapid molecular detection of the CMT1A duplication.

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