11 The CMT1A Duplication and HNPP Deletion

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CONTENTS

INTRODUCTION

The Charcot-Marie-Tooth type 1A (CMT1A) duplication was the first recurrent, large (>1 Mb), submicroscopic DNA duplication rearrangement found to be associated with a common autosomal dominant trait. Mechanistic studies of the CMT1A duplication have set the paradigm for genomic disorders. The CMT1A-REP low-copy repeats (LCRs) were among the first identified nongenic genomic architectural features that could act as substrates for nonallelic homologous recombination (NAHR). Identification of the predicted reciprocal recombination product, the hereditary neuropathy with liability to pressure palsies (HNPP) deletion, resulted in a model for reciprocal duplication/deletion genomic disorders.

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CLINICAL ENTITIES

CMT disease is a common inherited neuropathy with two major forms distinguished by whether the myelin (type 1 [CMT1]) or the axon (type 2 [CMT2]) is primarily affected. Both CMT1 and CMT2 are genetically heterogeneous with some 35 linked loci, and 25 of the corresponding genes having been identified (http://www.molgen.ua.ac.be/CMTMutations). CMT is characterized by symmetric slowly progressive length-dependent neuropathy, which manifests as a distal weakness of the legs progressing proximally. CMT1 can be distinguished from CMT2 by electrophysiological studies that reveal slowed nerve conduction velocities (NCVs) and neuropathology showing "onion bulbs." The most common form of CMT1 is that associated with the CMT1A locus mapping to 17p12. HNPP is an episodic, asymmetric neuropathy, which may be preceded by an antecedent event, such as motor nerve compression or trauma. Presentations may include pressure palsies or focal neuropathy and sometimes carpal tunnel syndrome. The electrophysiology may show conduction blocks, whereas the neuropathology reveals tomacula—sausage-like thickening of the nerve myelin sheath. CMT1A and HNPP are, therefore, both myelinopathies, but clinically distinguishable entities *(1,2)*.

THE CMT1A DUPLICATION

The CMT1A duplication was identified independently in Europe *(3)* and in the United States *(4)*. Multiple molecular methods including: (1) the presence of three informative alleles revealed by polymorphic simple sequence repeats and restriction fragment length polymorphism (RFLP) analysis in affected individuals; (2) the identification of a 500-kb patient-specific junction fragment by pulsed-field gel electrophoresis (PFGE); and (3) duplication of probes detected by interphase fluorescence *in situ* hybridization (FISH) revealed the duplication (Fig. 1).

The CMT1A duplication was identified as a prominent cause for CMT1A and confirmed in many world populations *(5–13)*. In population-based studies, the CMT1A duplication was shown to be responsible for about 70% of CMT1 *(13,14)*.

DE NOVO DUPLICATION

Remarkably, the CMT1A duplication was also shown to frequently occur *de novo (14–18)* and, in fact, causes 76–90% of sporadic CMT1 *(13,15)*. Intriguingly, the *de novo* duplication occurs preferentially during male meiosis *(19)*. The first molecular demonstration of a *de novo* duplication was shown by informative polymorphic alleles in an European family wherein the segregation of marker genotypes revealed evidence for unequal crossing over during meiosis *(3)*. This finding stimulated a search for genomic architectural features, such as LCRs that might act as recombination substrates. Recombination mediated by paralogous copies (i.e., NAHR) could result in duplication or deletion of the unique sequence located between the LCRs.

CMT1A-REP: AN LCR-MEDIATING NAHR

Physical analysis revealed proximal 17p region-specific LCR (CMT1A-REP) that flank the genomic region duplicated in the CMT1A duplication *(20)*. The CMT1A-REP LCR was not found in early mammals (mouse and hamster) *(20)*, but instead shown to have evolved during primate speciation. CMT1A-REP segmental duplication occurred after the divergence of gorilla and chimpanzee because there were two copies noted in the chimpanzee genome, whereas only

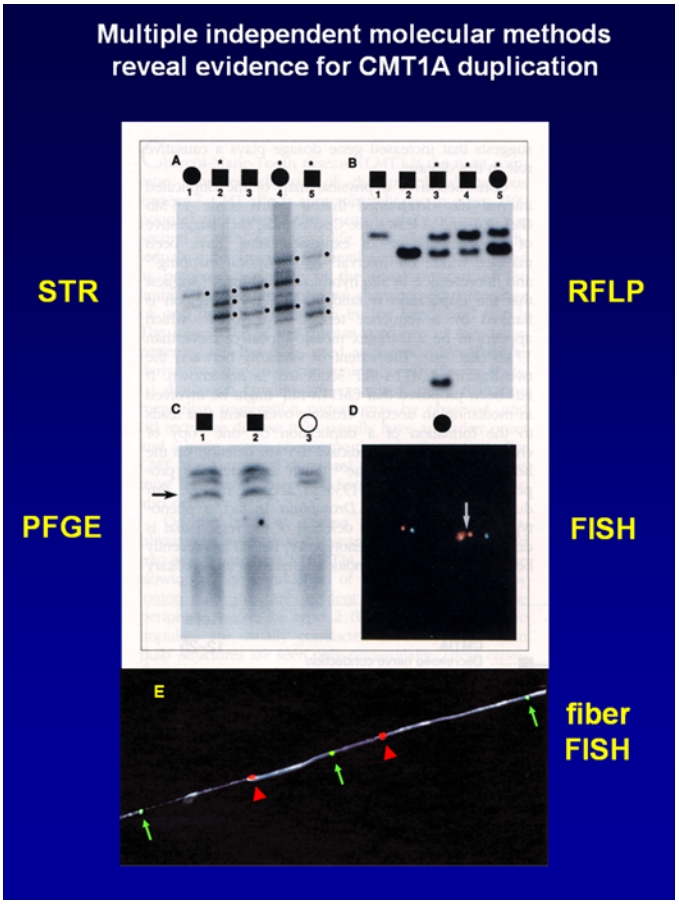

Fig. 1. Multiple methods reveal evidence for duplication. Shown are four separate methods (A–D) that originally enabled visualization of the duplication *(74)*. Simple sequence repeat or short tandem repeat analysis showed three alleles in informative affected individuals. Restriction fragment length polymorphism (RFLP) analysis showed a dosage difference in heterozygous affected individuals, whereas some patients with the CMT1A duplication were fully informative with three different RFLP alleles observed. Pulsed-field gel electrophoresis identified a patient-specific junction fragment of approx 500 kb in size. Fluorescence *in situ* hybridization (FISH) showed the duplicated segment (two adjacent red dots) only in interphase (this is not resolved in metaphase nuclei) where the control probe (green dots) was not replicated enabling one to distinguish duplication from replication. Note the duplicated chromosome has two red signals and one green signal compared to the normal (one red and one green) control chromosome 17 homolog. (E) Fiber FISH (bottom) reveals the predicted three copies of CMT1A-REP (green) and two copies of *PMP22* (red) in the CMT1A duplication bearing chromosome *(75)*.

one copy was present in the gorilla genome *(21–23)*. Segmental duplications occurring during primate genome evolution are a recurrent theme in genome architectural features associated with rearrangements causing genomic disorders *(24)*.The proximal CMT1A-REP and distal CMT1A-REP share about 24,000 bp of approx 99% DNA sequence identity *(23)*. The proximal (centromeric) copy of CMT1A-REP derived from the distal (telomeric) copy. The *COX10* gene, encoding the hemeA:farnesyltransferase that farnesylates the hemeA group that is incorporated into cytochrome oxidase, spans the distal CMT1-REP. Proximal CMT1A-REP represents a segmental

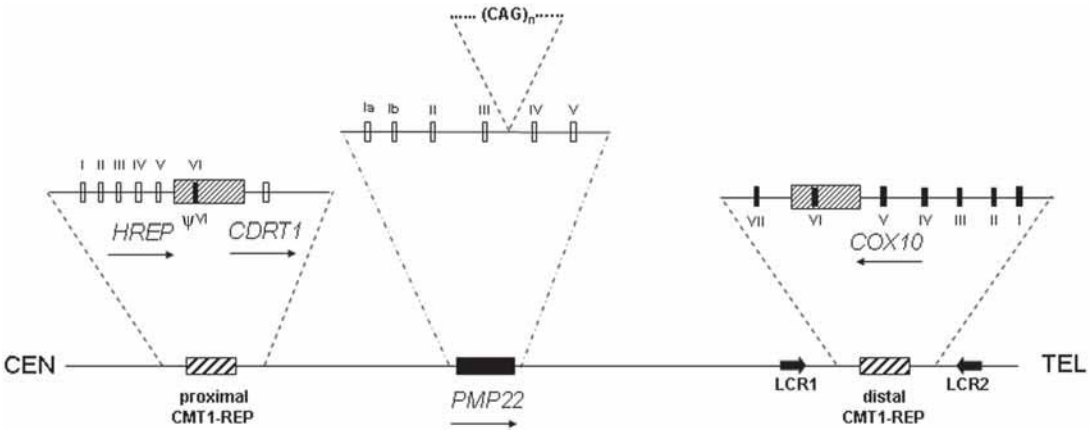

Fig. 2. Partial genome structure of the 1.4-Mb region duplicated in CMT1A and deleted in HNPP. The genomic region is flanked by proximal and distal CMT1A-REP low copy repeats (hatched rectangles). The *COX10* gene (exons depicted as filled boxes) spans the distal CMT1A-REP. Proximal CMT1A-REP was generated by segmental duplication of *COX10* exon VI and surrounding intronic sequences. This insertional event created two new genes, *HREP* through exon accretion (its exon VI is formed from the complementary strand of the segmentally duplicated *COX10* pseudoexon (Ψ)VI) and *CDRT1*, that are transcribed in different tissues. Distal CMT1A-REP is flanked by low-copy repeats (LCR)1 and LCR2. The dosage sensitive *PMP22* gene, with exons shown as open boxes, is located within the genomic interval that is rearranged. It contains an intronic polymorphic CAG trinucleotide repeat between exons III and IV.

duplication of *COX10* exon VI plus 24 kb of surrounding intronic sequences (i.e., distal CMT1A-REP) *(23,25)* (Fig. 2). After the segmental duplication event, the complementary strand of the duplicated *COX10* exon VI ("pseudoexon VI") is transcribed *(23,26)* as part of a new gene created through exon accretion *(27)*. In fact, the segmental duplication that created proximal CMT1A-REP from the distal copy actually created two new genes, *HREP* and *CDRT1*; (Fig. 2), with differing expression profiles *(27,28)*. Exon accretion and the creation of new genes associated with segmental duplication appears to be a common occurrence *(29)*.

MECHANISM FOR THE CMT1A DUPLICATION

The approx 3.0-Mb CMT1A duplication, consisting of two tandem 1.4-Mb copies, results from NAHR, whereby the proximal CMT1A-REP and distal CMT1A-REP, that are separated by 1.4 Mb of genomic DNA *(27)*, recombine resulting in a duplication (Fig. 3). This mechanism was supported by genetic evidence that showed the *de novo* duplication was accompanied by unequal crossover of flanking markers *(3)*. Interestingly, as anticipated, the duplication chromosome has three copies of CMT1A-REP *(20)* (Fig. 1). Moreover, the CMT1A duplication was shown to be a tandem or direct duplication, and not inverted, by both PFGE and FISH *(20,30)*. The mechanism of NAHR using flanking CMT1A-REP repeats as substrates for recombination predicted the existence of a reciprocal recombination product that would result in a deletion of the same genomic region that is duplicated in the CMT1A duplication *(20)*.

THE RECIPROCAL HNPP DELETION

HNPP mapped to the same region as CMT1A and all 17p12 genetic markers known to be duplicated in CMT1A were shown to be deleted in HNPP *(31)*. The HNPP deletion breakpoints

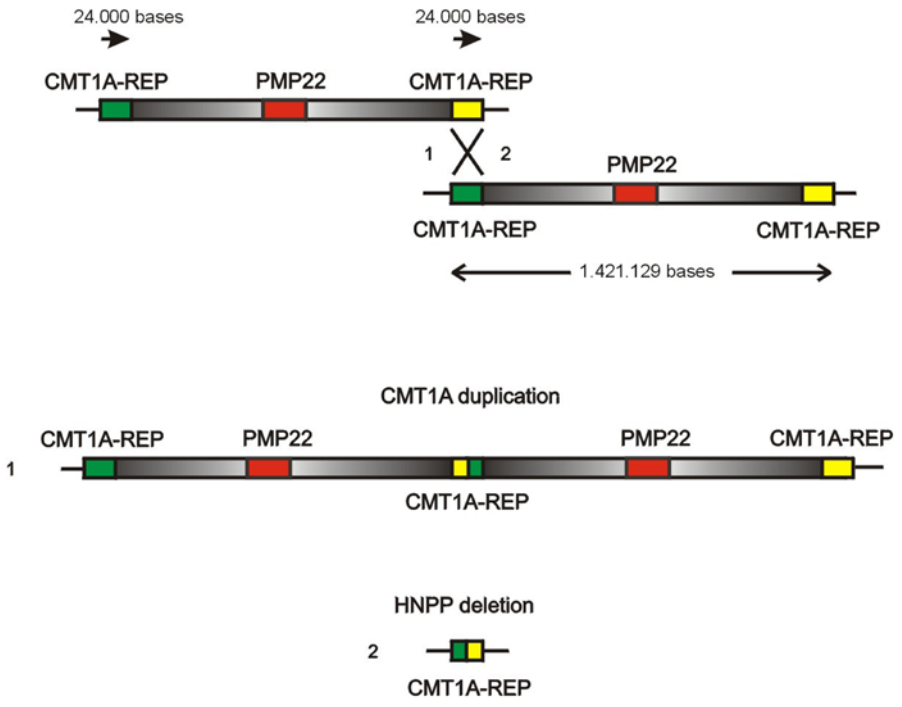

Fig. 3. Nonallelic homologous recombination (NAHR) generates the reciprocal CMT1A duplication and HNPP deletion. Schematic representation of the unequal crossover resulting from misalignment of the low-copy repeat elements, the proximal CMT1A-REP (green) and the distal CMT1A-REP (yellow) sizes of the CMT1A region and CMT1A-REPs are indicated in number of nucleotides or bases. The CMT1A-REPs share 99% of DNA sequence identity. The CMT1A-REP copies can act as substrates for homologous recombination with reciprocal crossovers (numbered 1 and 2) resulting in either the CMT1A tandem duplication or the reciprocal HNPP deletion as alternate products of NAHR.

mapped to the same intervals as those in the CMT1A duplication. The 1.4-Mb HNPP deletion was demonstrated to be the reciprocal recombination product to the CMT1A duplication by the identification of predicted PFGE junction fragments and by showing the expected one copy of CMT1A-REP on the recombinant deleted chromosome (Fig. 3) *(32,33)*. Thus, the HNPP deletion and CMT1A duplication represent the products of a reciprocal NAHR involving CMT1A-REP.

RECOMBINATION HOTSPOT IN CMT1A-REP

Observations regarding the dosage of CMT1A-REP-specific restriction fragments suggested that the CMT1A duplication and HNPP deletion arose from recombination events within a limited region of CMT1A-REP (33). The CMT1A-REP copies are approx 99% identical. The sequence differences between the two paralogous copies, which resulted in different restriction endonuclease recognition sites, were exploited for the mapping of the strand exchanges (i.e., crossovers) within CMT1A-REP. Such paralogous sequence variations, or differences between the two copies located on the same chromosome homolog, are referred to as *cis*-morphisms to distinguish them from polymorphisms-variations of allelic copies on homologous chromosomes *(34)*. The majority of crossovers occurred within a limited region

of CMT1A-REP defining a recombination hotspot. This was the first NAHR hotspot defined-initially in a US cohort *(35,36)* and confirmed in a European cohort *(37)*. The CMT1A-REP recombination hotspot was also found in the French *(38)*, Japanese *(39)*, and Chinese *(40)* populations. Such NAHR recombination hotspots have been identified in all genomic disorders in which the strand exchanges have been studied at the nucleotide sequence level *(41)*.

Examination of the products of recombination in the recombinant CMT1A-REP from both patients with the HNPP deletion *(42)* and the CMT1A duplication *(43)* revealed evidence for gene conversion. The products of recombination are best explained by the double-strand break model for homologous recombination. Although *cis* acting recombinogenic sequences that might stimulate double strand breaks have been postulated *(41–43)* none have been either verified experimentally or shown to be common among different hotspots.

CONSEQUENCES OF DUPLICATION/DELETION MUTATIONS ON SEGREGATION OF MARKER GENOTYPES AND LINKAGE ANALYSIS

The failure to recognize a molecular duplication can lead to misinterpretation of marker genotypes for affected individuals, the identification of false recombinants, and incorrect localization of the disease locus *(4,44)*. This occurs because the usual biallelic locus is now triallelic as a result of the duplication *(34)*. By contrast, the deletion results in a lack of transmission of alleles to affected offspring such that the failure to recognize the deletion may lead to an erroneous exclusion of paternity or maternity *(31)*.

PERIPHERAL MYELIN PROTEIN 22 (*PMP22*) GENE DOSAGE ABNORMALITIES CAUSE NEUROPATHY

The initial hypothesis that gene dosage may be mechanistically important with respect to the CMT disease phenotype came from the observation of a more severe neuropathy phenotype in a patient homozygous for the CMT1A duplication *(4)*. Subsequent studies of patients with cytogenetically visible duplications of 17p revealed that as long as the 17p12 region containing the CMT1A locus was duplicated, then part of the complex phenotype observed in the patient would include the decreased NCV diagnostic of CMT1 *(1,45)* consistent with a gene dosage effect causing the disease.

After the identification of the CMT1A duplication in the majority of CMT1 families the peripheral myelin protein 22 (*PMP22*) gene was mapped in the middle of the CMT1A region *(30, 46–48)*. Important is that the rare smaller duplications and deletions that cause neuropathy still contained the *PMP22* gene within the rearrangement interval *(19,49,50)*, supporting a *PMP22* gene dosage effect as the disease mechanism *(51)*.

The fact that the phenotype in CMT1A duplication and HNPP deletion patients results from abnormal *PMP22* gene dosage was supported by multiple lines of independent experimental evidence including: (1) quantitative *PMP22* mRNA and (2) PMP22 protein studies in the peripheral nerves from patients with the duplication and deletion rearrangement *(52–59)*. Moreover, rodent models that overexpress *PMP22*, or disrupt it by gene targeting or antisense, recapitulated the respective CMT1A and HNPP phenotypes *(60–65)*. Strategies aimed at normalizing the *PMP22* gene dosage may provide therapeutic approaches as recently demonstrated in rodent models *(66–68)*.

STRUCTURE OF THE 1.4-MB CMT1A DUPLICATION/HNPP DELETION GENOMIC REGION

The genomic interval that is duplicated in the CMT1A duplication and deleted in the HNPP deletion spans 1.4 Mb and is flanked by the 24-kb CMT1A-REP copies (Fig. 2). This interval contains 21 genes and 6 pseudogenes *(27)*. Additional architectural features include two copies of an 11-kb LCR (LCR1 and LCR2), that are inverted with respect to one another and flank the distal CMT1A-REP *(27)*. No evidence could be found for inversion of the intervening sequences using LCR1 and LCR2 as NAHR substrates after evaluating dozens of Caucasian individuals (data not shown) *(27)*. The 1.4 Mb interval also contains 53 simple sequence repeats with more than 11 repeating units *(27,69)*. Interestingly, one of these is a CAG trinucleotide repeat located within the largest (20 kb) intron of *PMP22* between exons III and IV (Fig. 2). We hypothesized that in rare families with CMT accompanied by anticipation, the anticipation may be related to an expanded allele of this triplet repeat. We could not find any data to support this hypothesis in one CMT1A family manifesting anticipation that was examined for expansion of this allele *(27,70)*. It is possible that variation at this *PMP22* intronic polymorphic trinucleotide repeat may affect *PMP22* expression and severity of disease in patients with the CMT1A duplication or HNPP deletion.With the exception of a few rare cases, the CMT1A duplication and HNPP deletion appear to almost always have the same size (i.e., 1.4 Mb), suggesting that a precise recurrent recombination mediated by the CMT1A-REP repeat sequences in this region is the predominant cause for DNA rearrangement. A patient, mosaic for the CMT1A duplication was reported to have a reversion of the 1.4-Mb CMT1A duplication in several somatic tissues *(71)*.

SUMMARY

CMT1A and HNPP are common autosomal dominant traits that result from a 1.4 Mb reciprocal duplication/deletion. The CMT1A duplication and HNPP deletion rearrangements occur via NAHR using flanking LCRs (LCRs termed proximal and distal CMT1A-REP) as the recombination substrates, setting the paradigm for genomic disorders *(72,73)*. A hotspot for strand exchanges, that is associated with the unequal crossover, is located within CMT1A-REP. The CMT1A-REP evolved by segmental duplication of *COX10* exon VI and surrounding intronic sequences during primate genome evolution and speciation. This segmental duplication created two new genes with different tissue expression profiles. The CMT1A duplication has consequences for the interpretation of marker genotypes because it creates a triallelic locus. Finally, the phenotype associated with CMT1A duplication and HNPP deletion results because of an abnormal *PMP22* gene dosage effect. Therapeutic strategies should be directed at correcting the dosage of *PMP22* or ameliorating the consequences of its abnormal expression.

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