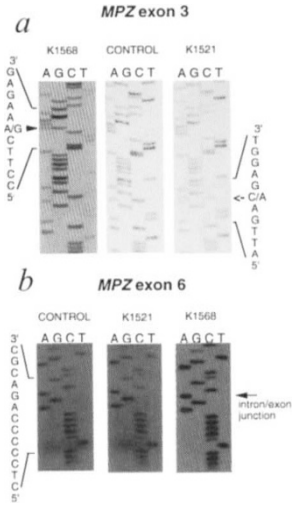

Fig. 1 Direct DNA sequencing of PCR-amplified products from *MPZ* exons 3 and 6. *a*, Sense strand sequences from *MPZ* exon 3. A CMT1B patient from family 1568 is heterozygous for an A to G transition (solid arrow) producing a Lys96Glu substitution. An affected individual from family 1521, (or pedigree 2), is heterozygous for a C to A transversion (thin arrow), that predicts an Asp90Glu substitution. Normal sequence from a non-CMT1B individual is shown in between the two patients for comparison. *b*, Sense strand sequences of the *MPZ* intron 5–exon 6 junction (solid arrow). No mutations in this region are detected in either family 1568, 1521, or in the control. Direct sequencing was performed using single stranded biotinylated PCR products isolated on streptavidin beads (Dynal corp.) and sequenced by standard dideoxy chain termination methods (Sequenase).

in a masked fashion. DNA sequence for both the sense and antisense strands of *MPZ* exons 3 and 6 were determined for one patient from each family, as well as a non-CMT1B control. First, we confirmed the A^{286} to G transition in family K1568 (Fig. 1*a*). We also confirmed this mutation by restriction analysis of *MPZ* exon 3 PCR products with *Bst*BI, as done by Su *et al.*[7], and with *Mbo*II, whose restriction site is also created by the A^{286} to G mutation (data not shown).

More importantly, we have also confirmed the presence of the C^{270} to A transversion reported by Hayasaka *et al.*[3] by sequence analysis. A thin arrow in Fig. 1*a* indicates the location of the mutation in K1521 (pedigree 2). The C^{270} to A mutation was also confirmed by restriction analysis of *MPZ* exon 3 PCR products with *Mbo*II (data not shown)[3]. We found no evidence of the *MPZ* intron 5–exon 6 substitutions reported by Su *et al.*[7] (Fig. 1b).

Our data indicate that CMT1B families K1568/pedigree 1 and K1521/pedigree 2 contain the respective missense point mutations originally reported by Hayasaka *et al.*[3]. The location of these mutations is consistent with the majority of published *MPZ* mutations, which are largely missense amino acid substitutions that reside in the extracellular domain and theoretically disrupt the protein's homophilic adhesion properties[8].

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In reply — Hayasaka *et al.*[3] and Su *et al.*[7] reported abnormal *MPZ* sequences in two pedigrees with the CMT1B locus linked to the *FcgRII* gene[9]. Both groups showed that a Lys96Glu mutation in family 1568 - which we had traced to a couple born in Pennsylvania in 1820 and their 500 offspring (termed pedigree 1) (ref. 9) and provided samples to Hayasaka *et al.* - segregated with the CMT1B phenotype in this family[3,7].

The situation with family 1521 (pedigree 2), which we had traced to a couple living in Belfast in the 1820s, is more complicated. Hayasaka *et al.*[3] found that a conservative Asp90Glu mutation cosegregated with CMT1B in all family members tested. In contrast, in duplicate experiments four months apart, we found a mutation at the splice site in exon 6 in 3/3 affected patients' samples (patients III-1, III-9 and IV-2) (ref. 9)from Dr. Phillip Chance's laboratory[7]. Our results were obtained using one primer derived from the murine *Mpz* sequence and one from the human *MPZ* sequence. We also found no evidence of the codon 90 mutation in exon 3 using human PCR primers; Isolated *MPZ* subclones from patient III-9 had normal exon 3 sequences, consistent with reported and subsequent results (see below).

Our recent tests of the same three K1521 samples using human primers from a different part of *MPZ* have revealed the Asp90Glu nucleotide change[3], but only in patients III-1 and IV-2. However, *MPZ* primers did not amplify an allele with the exon 6 splicing mutation. We think there are at least two explanations: (i) the conservative nucleotide substitution[3] does not result in CMT1B whereas the abnormal *MPZ* allele amplifies poorly under normal stringency in the region of a more severe mutation; or (ii) an homologous pseudo-*MPZ* gene with an exon 6 splice site mutation or another homologous sequence originally gave us abnormal results only in patient DNAs from family 1521 using mouse primers. In our other tests of *MPZ* in 30 CMT1 patients without the CMT1A mutation, we found many single nucleotide substitutions resulting in conservative amino acid changes. Whether these substitutions are pathogenic or merely normal variants is uncertain.

In conclusion, we did not originally find the codon 90 substitution[3] because we used different PCR primers and amplification conditions. Based upon all available data, we believe it is unclear whether this mutation results in CMT1B in family 1521.

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