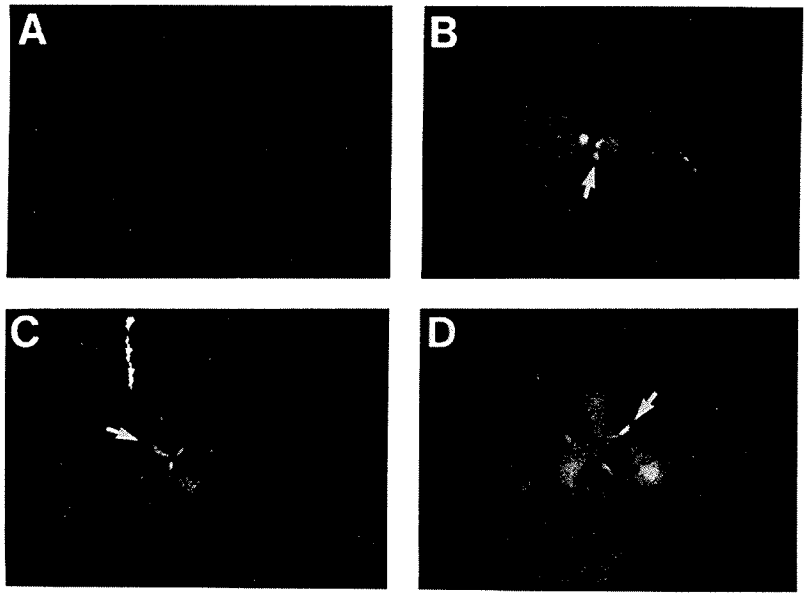

FIGURE 2. Localization of wild-type and mutant Cx32 in transfected communication-deficient HeLa cells by indirect immunofluorescence. HeLa cells were transfected with the expression vector pREP9 containing either the coding region for wild-type (WT) Cx32, for F235C mutant Cx32, or no insert. After expression, the cells were immunostained with monoclonal antibody 7C6.C7, which is directed against the cytoplasmic tail of Cx32.

In cells expressing WT Cx32 (**B**), the protein is detected in plaques at the membrane surface between apposed cells, presumably forming gap junction complexes. Staining is also often seen in a punctate pattern, or sometimes more diffusely distributed in the cytoplasm. The pattern in cells expressing the F235C mutant protein (**C, D**) was similar to that seen in cells with the WT protein. The protein was clearly seen in plaques between adjoining cells, as well as in the cytoplasm. Cells transfected with the pREP9 control plasmid showed no expression by immunostaining (**A**). *Arrows* indicate Cx32-containing plaques between cells.

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(pp. 3398) MNVS A. P. SEEMAN

Charcot-Marie-Tooth 1A: Heterozygous T118M Mutation over a CMT1A Duplication Has No Influence on the Phenotype

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INTRODUCTION

A tandem duplication of 1.5 Mb in chromosome 17p11.2-12 comprising the peripheral myelin protein gene (*PMP22*) is found in about 70% of Charcot-Marie-Tooth type 1 (CMT1) disease patients.[1] A reciprocal deletion of the same region is found in 86% of patients with hereditary neuropathy with liability to pressure palsies (HNPP).[1] Pathogenic point mutations of a dominant character were also described in *PMP22*,[2] as well as in other myelin genes: *Cx32*,[3] P_0,[4] or EGR2.[5] Roa *et al.* First in 1993[6] and later Bathke *et al.*[7] reported the T118M mutation in *PMP22* and assumed its recessive character because it didn´t produce any signs of neuropathy in heterozygous status over a wild-type allele, but it was found in a severely affected CMT1 member of a HNPP family over a HNPP deletion. Later reports by Nelis *et al.*[8] showed that the T118M is more likely a polymorphism than a pathogenic mutation.

We report here a CMT1A family where the typical 17p duplication was confirmed in three generations by a PCR-based method using seven microsatellite markers from the critical region as well as by Southern hybridization with probe pLR 7.8. Except for one female member of the youngest generation (Nr. 53), all five other members of this family (Nr. 51, 52, 54, 206, and 141) carrying a duplication have a very mild phenotype. To clear the severe phenotype of this individual, we sequenced the *PMP*22 gene in this family. Surprisingly, we did not find the T118M mutation in the patient (Nr. 53), as expected, but found it in her subjectively and clinically only very mildly affected mother (Nr. 51), carrying a CMT1A duplication on her paternal chromosome 17. T118M was inherited from her mother (Nr. 141) and also detected in one other healthy member of the family (Nr. 219).

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RESULTS

We investigated 11 persons in three generations of a Charcot-Marie-Tooth 1A family on the molecular genetic level. Eight of them were also examined in detail on the clinical neurological level and by electromyography (EMG). Six persons (Nr. 140, 51, 52, 53, 54, and 206) (FIG. 1) in three generations were found to carry a typical CMT1A duplication. The duplications were confirmed by two independent methods using a set of seven polymorphic microsatellite markers from the critical CMT1A/HNPP region as well as Southern blot analysis after *EcoRI/SacI* restriction digestion. All duplication carriers and only duplication carriers showed a severely decreased nerve conduction velocity (NCV) of under 25 m/sec.

Because of the more severe phenotype of a member of the youngest generation (Nr. 53), the *PMP22* gene was sequenced. A previously reported mutation T118M in the heterozygous state was found in three members of oldest and middle generations (Nr. 141, 219, and 51) (FIG. 2). Twice the mutation was detected over a normal wild-type "C" allele (Nr. 141 and 219) but once over a CMT1A duplication (Nr. 51). Both carriers of only the T118M (Nr. 141 and 219) didn't complain about any subjective neurological problems. Neurological examination of person Nr. 141 as well as an EMG didn't show any signs of polyneuropathy. The second confirmed carrier of only T118M (Nr. 219) didn't agree with the EMG examination. The carrier of the T118M over a CMT1A duplication (Nr. 51) didn't differ in clinical or EMG findings from her sister (Nr. 206), who carried only a duplication without T118M. Both these women are clinically almost unaffected and didn't complain about any subjective neurological problems, but both were found to have NCVs that were severely decreased to the same level of 21–23 m/sec.

Our observation shows further evidence that T118M "exchange" is not a real recessive mutation, but rather a polymorphism that has neither influence on clinical nor on electrophysiological phenotype in CMT.

DISCUSSION

We identified an unusual family, which demostrates segregation of two different mutations involving the same gene: a previously reported point mutation in the *PMP22* gene and a CMT1A duplication. Both mutations were confirmed by two independent detection techniques.

At least three members (Nr. 51, 141, 219) of a CMT1A family carry a point mutation T118M in the *PMP22* gene. The CMT I phenotype segregated together with the CMT1A duplication (Nr. 140, 51, 52, 53, 54, and 206) but not with the T118M pointmutation. The heterozygous carriers of a single T118M mutation over a wild-type, nonduplicated allele (Nr. 141 and 219) didn't show any signs of peripheral neuropathy even at the higher age of 74 years. This observation is consistent with previous reports by Nelis and Bathke, which both have found the T118M in HNPP families. There are no reports about any cases with T118M over wild-type allele that showed any signs of peripheral neuropathy up to now.

Individual number 51 carries a T118M over a CMT1A duplication. Neither phenotypically nor electrophysiologically have we found any positive or negative effect of T118M over the CMT1A duplication in comparision to those who carry only a duplication (Nr. 51 versus Nr. 206). The T118M alone over a wild-type, nonduplicated allele have not resulted in any signs of peripheral neuropathy in any of the carriers of our family. It is very likely that there are many more T118M carriers in this family because one female carrier (Nr. 219) has five adult children who themselves have further children. But there are no clinical signs of neuropathy observed or reported in that part of family, a finding that contributes to the theory of a harmless character for T118M.

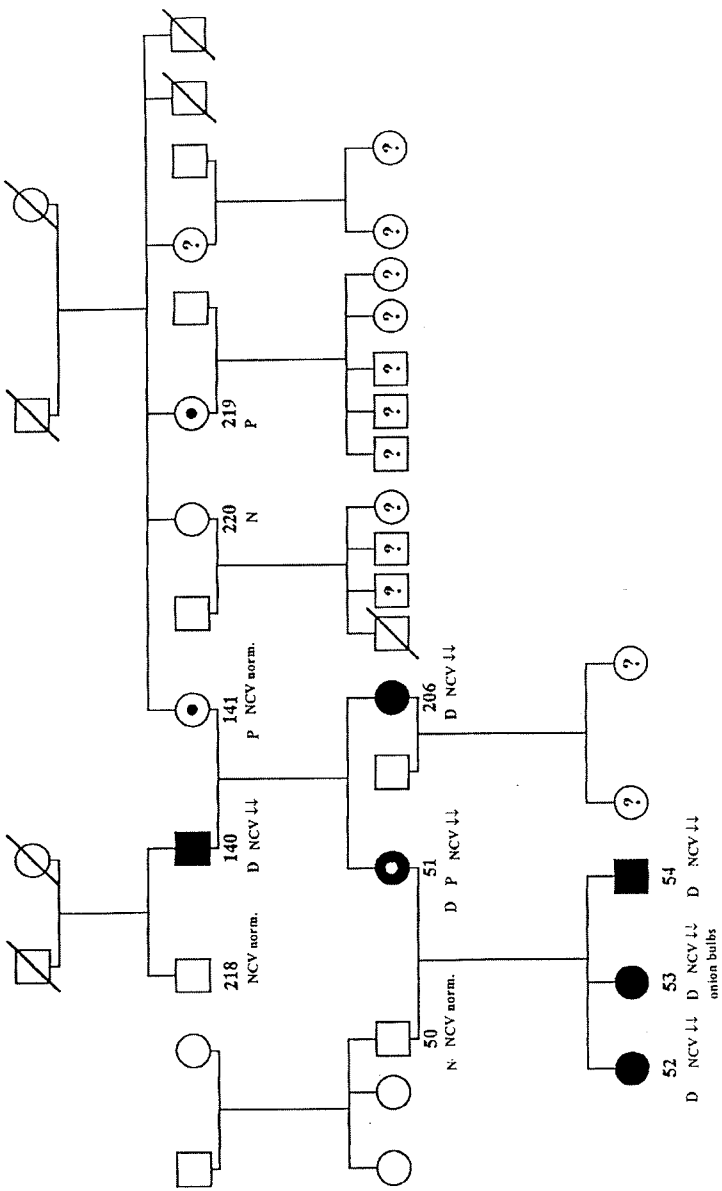

FIGURE 1. Pedigree of reported family. D, CMT1A duplication at 17p.11.2-12; P, point mutation T118M in *PMP22* gene, exon 4; N, normal—no point mutation, no duplication; NCV↓↓, severely decreased nerve conduction velocity (under 25 m/sec). Numbered individuals were investigated on the molecular genetic level.

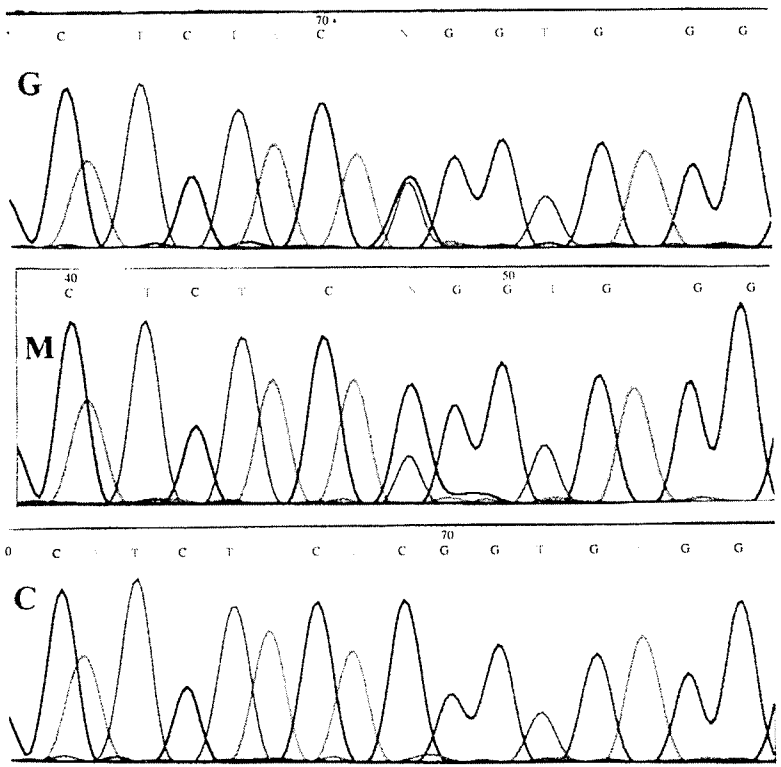

FIGURE 2. Sequence analysis of *PMP22* exon 4 region in persons 141, 51, and 53. Note the heterozygous signal "C / T" of the same intensity in the only T118M carrier, 141 (G), in contrast to case 51 with T118M over the CMT1A duplication (M), where the "C" signal is of double intensity compared to the "T" signal. This is because of the 2 : 1 ratio due to the duplication. Individual Nr. 53 (C), who carries only the duplication, has the homozygous, wild-type "C" signal.

The speculation that T118M over the duplication could make the phenotype milder by means of altering a third, supernummerary copy of *PMP22* could not be confirmed.

Should a genetic counselor recommend genotyping for T118M in the partner of a T118M heterozygous carrier if the effect on the phenotype of T118M in the homozygous state is not known? Only the identification of a T118M homozygous individual, who can be confrimed to be either healthy or affected may reliably solve the nature of this mutation and this discussion.

ACKNOWLEDGMENTS

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[*Note added in proof*: Recently, the youngest sister of individuals 141, 220, and 219 (indicated by ? in FIG. 2) was also found to carry the T118M mutation.]