PMP22 Thr(118)Met: recessive CMT1 mutation or polymorphism?

Sir— Charcot-Marie-Tooth disease type 1 (CMT1)[1] is a genetically heterogeneous peripheral neuropathy involving mutations in the genes encoding peripheral myelin protein 22 (*PMP22*), myelin protein zero (*MPZ*) and connexin 32 (*Cx32*) on chromosomes 17p11.2, 1q22–23 and Xq13.1, respectively. The most common

Fig. 1 Pedigrees of CMT1 families with the Thr(118)Met mutation. Open rectangle: CMT1A- REP sequences[3]; filled rectangle: PMP22; *: Thr(118)Met mutation.

mutation in CMT1 is a 1.5-Mb duplication in 17p11.2 comprising the *PMP22* gene[2–4]. Hereditary neuropathy with liability to pressure palsies (HNPP)[5] is mostly associated with a 1.5–Mb deletion in 17p11.2, the same region that is duplicated in CMT1. Four distinct point mutations in *PMP22* have been associated with dominant CMT1[6–9]. In one CMT1 patient, there is evidence for a recessive *PMP22* mutation[10]; the CMT1 patient is a compound heterozygote for the Thr(118)Met point mutation, and the 1.5-Mb deletion in 17p11.2. One son, heterozygous for the *PMP22* point mutation has no signs of neuropathy while two other sons with the 17p11.2 deletion have an HNPP phenotype. Recently, a second CMT1 patient with the Thr(118)Met mutation and the 17p11.2 deletion was identified[11].

We analysed the three CMT1 myelin genes for mutations by single-strand conformation polymorphism (SSCP) analysis and direct PCR sequencing in 17 nonduplicated CMT1 patients and 14 unaffected relatives from northern Sweden[12]. In twelve sporadic CMT1 cases, the

parents were not affected, based on clinical and electrophysiological examination. The other patients belong to three pedigrees suggesting autosomal recessive inheritance[12]. In one isolated CMT1 patient (Fig. 1, pedigree 19) we identified a heterozygous Thr(118)Met mutation in *PMP22* exon 4. SSCP analysis of the 3 other coding *PMP22* exons did not reveal evidence for sequence alterations making it unlikely that a second *PMP22* mutation was present on the heterologous chromosome[13]. Also, no altered SSCP patterns were detected in the coding regions of *MPZ* and *Cx32*, likely excluding the involvement of at least these two loci in a possible digenic model of inheritance[14]. DNA analysis of the unaffected parents showed that the *PMP22* mutation was transmitted by the father. We also detected the mutation in the unaffected father of a second isolated CMT1 patient, who herself did not carry the mutation (Fig. 1, pedigree 50). These results suggested that the Thr(118)Met mutation is not a CMT1 disease mutation but a *PMP22* polymorphism that is frequent in the Swedish population. To test this possibility, we analysed 262 control individuals living in northern Sweden by SSCP analysis and *Nla*III restriction digestion[10] of the *PMP22* exon 4 PCR fragment. The Thr(118)Met mutation was found in a heterozygous state in ten persons, resulting in a Thr(118)Met allele frequency of 1.9%.

There are two possible explanations for

our results. The Thr(118)Met mutation is a *PMP22* polymorphism not associated with the CMT1 phenotype either in the heterozygous state, as demonstrated in this report, or in the homozygous or hemizygous state. Under this hypothesis, the severe clinical phenotype described for the CMT1 patients hemizygous for the Thr(118)Met mutation[10,11] can be explained by variable clinical expression of the HNPP phenotype[5]. Before the molecular defects were known, HNPP and CMT1 were considered distinct clinical entities[1,5]. Later, when deletion/duplication mutations were identified, molecular genetic analyses of large cohorts of patients identified the HNPP deletion in 4% of patients clinically diagnosed with CMT1[15]. The latter observation indicated that the clinical phenotype of severe HNPP patients may overlap with that of CMT1. In our study, we did not find the Thr(118)Met mutation in the homozygous state in CMT1 patients or in controls, therefore it cannot be formally excluded that it is a recessive mutation. If the Thr(118)Met mutation is a recessive CMT1 mutation, one would expect a prevalence of CMT1 of at least 1 in 2746, the latter corresponding to the expected number of homozygotes calculated from the allele frequency. However, in an epidemiological study in northern Sweden the prevalence of CMT1 was calculated as 1 in 6200[16]. Since the CMT1A duplication frequency in northern Sweden is 37.5%[4], the prevalence of nonduplicated CMT1 patients is even less. Although there is a greater incidence of recessive CMT1 in northern Sweden[12], none of the patients in the three recessive CMT1 families carried the Thr(118)Met mutation. On the other hand, overexpression of the Thr(118)Met mutation in NIH-3T3 cells suggested that this mutation could be a recessive CMT1 mutation. Expression of the Thr(118)Met mutation created an apoptotic-like phenotype similar to that of the dominant *PMP22* mutations, however, the normal phenotype was restored when Thr(118)Met was expressed together with wildtype *PMP22* cDNA[17]. The question of whether Thr(118)Met is a polymorphism or a recessive mutation will only be unambiguously solved when a recessive CMT1 patient or an unaffected individual homozygous for Thr(118)Met is identified.
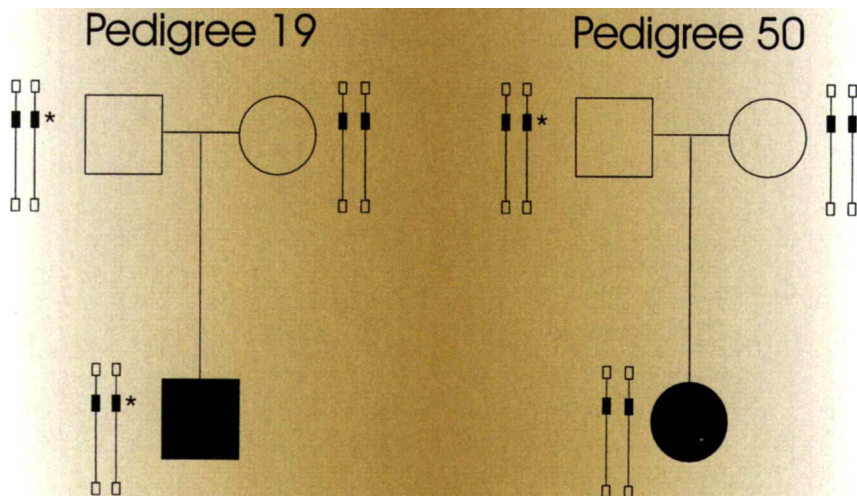
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A breast/ovarian cancer patient with germline mutations in both *BRCA1* and *BRCA2*

Sir — Hungarian breast/ovarian cancer families were screened for germline mutations in *BRCA1*[1,2] and *BRCA2*[3,4], genes that predispose towards breast cancer. One patient with breast and ovarian cancer was found to have truncating mutations in both the *BRCA1* and *BRCA2* genes. This individual carried the 185delAG mutation in *BRCA1* as well as the 6174delT mutation in *BRCA2*. Both of these mutations are common in Ashkenazi Jewish breast cancer patients[5,6,7]. Each mutation has been shown to have a frequency of approximately 1% in the Ashkenazi Jewish population[8,9,10].

Although this patient was not recorded as having a Jewish origin, the alleles at *D17S1320*, *D17S1322*, *D17S1323*, *D17S1327* and *D17S855* were consistent with the known Jewish haplotype associated with the 185delAG mutation in *BRCA1*[11]. Three polymorphic markers near *BRCA2* — *D13S1695*, *D13S1697* and *D13S1701*[12] — were used to examine whether the 6174delT mutation in this individual was also of Jewish origin. The patient was compared to 7 Ashkenazi Jew-

ish cancer patients with the 6174delT mutation in *BRCA2*. All 8 patients shared an allele at each marker, consistent with the presence of a common haplotype. This result suggests that there may be a common Jewish founder for 6174delT, as is the case for 185delAG in *BRCA1*.

This individual had a maternal family history of breast cancer and the paternal family history was unknown (Fig. 1). The patient had no developmental abnormalities and was diagnosed with breast cancer at 48 and ovarian cancer at 50 years of age respectively. The breast tumour was a grade III infiltrating ductal carcinoma with a prominent inflammatory infiltrate both within and around the tumour. The ovarian tumour was a poorly differentiated papillary/solid adenocarcinoma with features suggestive of serous origin. The ages of diagnosis and the tumour types were not different from those of patients with either *BRCA1* or *BRCA2* mutations.

DNA samples were not available from any other individuals in the family. It was therefore not possible to determine whether each mutation was inherited

from a different parent or if both were inherited from one or the other. The mother had endometrial cancer at the age of 55 and lung cancer. To control for possible contamination, the patient's genomic DNA was tested for 9 highly polymorphic microsatellite repeats and was found to have two alleles for each marker, indicating that this sample was derived from a single individual. In addition, both mutations were present in 3 different samples from the patient: breast tumour, ovarian tumour and lymphocyte DNA. There was no evidence of loss of heterozygosity on either chromosome 13q12 or 17q12–q21, which may be due to contamination of the tumour preparations by normal cells.

Our observation raises two points. The

Fig. 1 The proband, indicated by an arrow, was diagnosed with breast cancer at 48 y and ovarian cancer at 50 y. Incidence of breast cancer is indicated by shading on the right half of the circle, ovarian cancer by shading on the left half, and endometrial cancer by a stippled circle. *BRCA1* and *BRCA2* were screened for mutations from lymphocyte DNA using the protein truncation test (PTT) or combined single strand conformational polymorphism/heteroduplex analysis (SSCP/HA). A deletion of 2 bp at nt 185 (185delAG) in *BRCA1* and a deletion of a single bp at nt 6174 (6174delT) in *BRCA2* were identified by direct sequencing of PCR products and confirmed in both breast tumour and ovarian tumour DNA from the proband.