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Linkage of a New Mutation in the Proteolipid Protein (PLP) Gene to Pelizaeus-Merzbacher Disease (PMD) in a Large Finnish Kindred

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Summary

The purpose of this study was to confirm linkage of the proteolipid protein gene (PLP) and Pelizaeus-Merzbacher disease (PMD). A T→A transversion in nucleotide pair 35 of exon 4 of PLP was found in a large Finnish kindred with PMD. This mutation results in the substitution Val^{165}→Glu^{165}. We used a combination of single-strand conformational polymorphism and PCR primer extension to determine the presence or absence of the point mutation in family members. A lod score of 2.6 ($\theta = 0$) was found for linkage of the gene and the disease. We examined 101 unrelated X chromosomes and found none with the transversion. This is the second report of linkage of PMD to a missense mutation in PLP. These findings support the hypothesis that PMD in this family is a result of the missense mutation present in exon 4 of PLP.

Introduction

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked recessive dysmyelinating disorder of the central nervous system (CNS). In individuals affected by PMD all myelin proteins, but especially proteolipid protein (PLP), the major component of CNS myelin, are markedly decreased. Several cases of PMD have been reported in which a defect in *PLP* is the probable cause (Gencic et al. 1989; Hudson et al. 1989*b;* Trofatter et al. 1989; Pham-Dinh et al. 1991; Pratt et al. 1991, 1992; Raskind et al. 1991; Doll et al. 1992; Strautnieks et al. 1992). In only one family (Trofatter et al. 1989) was tight linkage between a missense mutation and PMD established. We report here molecular and linkage analyses of a missense mutation in exon 4 of *PLP* in a large Finnish family (Mäenpää et al. 1990) with the classical form of PMD.

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Material and Methods

The family was described by Mäenpää et al. (1990), and the pedigree is shown in figure 1 (our family 22). Human genomic DNA samples were obtained from affected and unaffected individuals.

Amplification and Sequencing of Genomic DNA

The seven exon-containing regions of the 7.8-kb *PLP* gene were individually amplified and sequenced as described elsewhere (Pratt et al. 1991).

Single-Strand Conformational Polymorphism (SSCP)

SSCP was performed according to a modification of the protocol of Orita et al. (1989). The 10-μl SSCP reaction mixture contained approximately 5 ng of previously PCR-amplified DNA, 0.56 μl (0.125 mM each) dNTP mixture, 0.2 μl ^{32}P dATP (3,000 Ci/mmol), 30 ng of each primer, and 0.25 μl *Taq* polymerase (Perkin Elmer). The samples were amplified in a Perkin Elmer Thermocycler for 35 cycles. One microliter of the reaction mixture was added to 50 μl of dilution buffer (0.1% SDS, 10 mM EDTA). Two microliters of diluted sample were added to 2 μl of sequencing stop solution (98% formamide, 0.05% bromophenol blue, 0.05% xy-

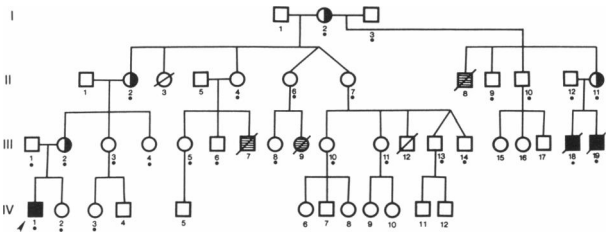

Figure 1 Pedigree of family 22. Blackened symbols indicate affected individuals. Striped symbols indicate individuals with uncertain diagnosis.

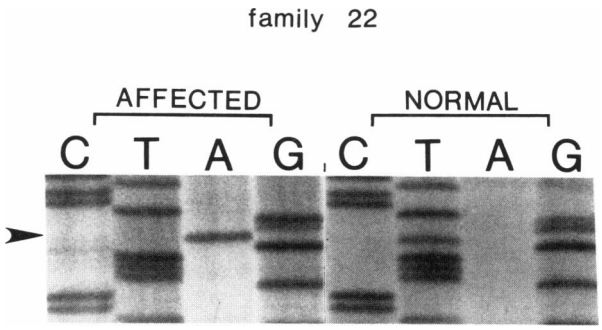

Figure 2 Partial sequence of exon 4 of the PLP gene in a PMD-affected and a normal male. An arrowhead indicates the T→A transversion.

lene cyanol, and 20 mM EDTA). The entire sample was denatured at 98°C for 2 min, cooled quickly on ice, and separated electrophoretically on a 5% polyacrylamide:5% glycerol gel for 4 h at a constant 30 W. The gel was dried and exposed to autoradiographic film for approximately 6 h.

Primer Extension

Primer extension was performed according to the method described by Sokolov (1990), with modifications in amount of primer DNA, radioactive nucleotides, and DNA. The reaction mixture contained approximately 5 ng of PCR-amplified exon 4, 1 μCi of the respective alpha-labeled radionucleotide (dATP, dTTP, or dCTP), 300 ng of primer (5′-AGGCACAGCAGAGCAGGCAAAC-3′), 2.5 μl of 10× *Taq* polymerase buffer, and 0.125 μl of *Taq* polymerase (Perkin Elmer) in a total of 25 μl. Denaturation was for 1 min at 94°C, and extension was for 1 min at 70°C. The denaturation-extension sequence was repeated 20 times. One microliter of each primer extension sample was added to 20 μl of loading dye, and the diluted sample was separated electrophoretically on a 20% polyacrylamide:8 M urea gel for 8 h at 200 V.

Linkage Analysis

Linkage analysis was done with the MENDEL program (Lange et al. 1988).

Results

The seven exons of *PLP* were amplified from genomic DNA of one affected male and were sequenced. A T→A transversion was found at nucleotide 35 of exon 4 (fig. 2). The presence of the base change was confirmed after reamplification and resequencing of exon 4. No other change was found in the seven exons. The transversion results in a Glu (GAG) instead of a Val (GTG) at amino acid 165.

To detect the presence or absence of the point mutation in family members, we used two methods—SSCP and DNA primer extension. SSCP analysis of a 227-bp PCR fragment indicated that the T-to-A transversion in exon 4 caused an alteration in mobility (fig. 3). A fragment with this mobility was found in all affected males and obligate carrier females. This provided evidence that the mutation segregated concordantly with the disease. The results of the SSCP screen were confirmed by single-base DNA primer extension (fig. 4). For primer extension, presence of the specific T-to-A point

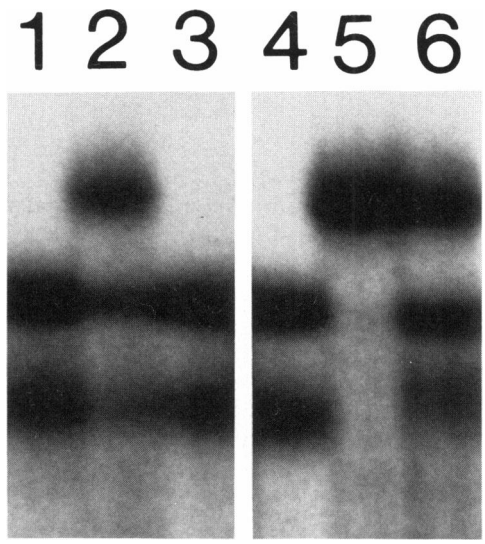

Figure 3 Single-strand conformational polymorphism analysis of family members. Lane 1, Normal male. Lane 2, Carrier female. Lane 3, Normal male. Lane 4, Normal female. Lane 5, Affected male. Lane 6, Carrier female. In lanes 2, 5, and 6, the mutation-containing allele runs more slowly.

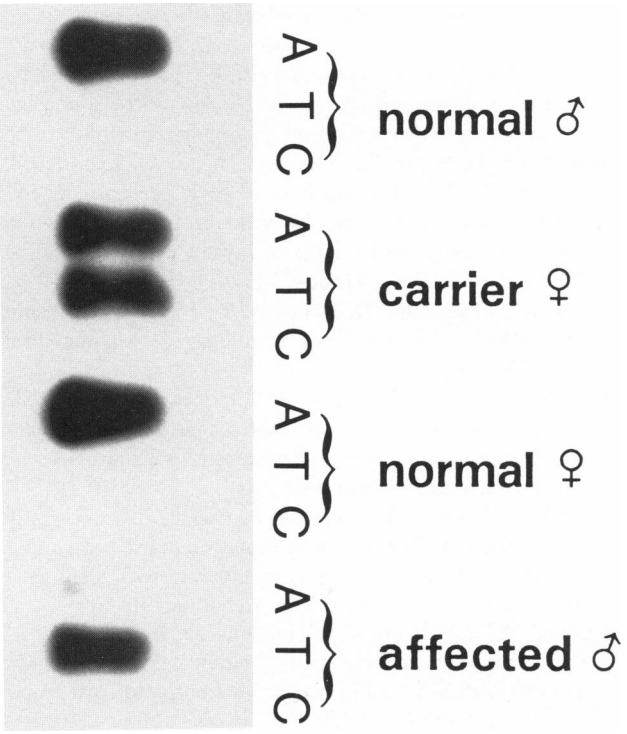

Figure 4 DNA primer extension applied to representative individuals of family 22. The presence of the T→A transversion is determined by the addition of radiolabeled dTTP. The extension primer sequence is that of the antisense strand. The normal allele is indicated by the addition of labeled dATP. Labeled dCTP served as negative control. All samples were separated in a 20% polyacrylamide:8 M urea gel at 200 V in a vertical Hoefer apparatus, with the anode at the left of the picture. The gel was wrapped wet and exposed to film for 4–8 h.

mutation was determined by the addition of radiolabeled dTTP to an antisense primer. The base specificity of the primer extension method made it an important complement to the SSCP analysis since, in theory, an alteration in mobility detected by SSCP might be caused by different mutations. Because different genotypes were obtained for twins II-6 and II-7, DNA fingerprinting was done on the samples to confirm dizygosity (not shown). The genotypes of twins III-13 and III-14 were identical, yet fingerprinting showed them to be fraternal twins.

To determine the population frequency of the substitution, 101 X chromosomes from unrelated males and females were examined by the primer extension technique, and none was found to contain the mutation. The frequency was conservatively estimated to be .01, although in fact it is probably much lower.

Linkage was performed between the missense mutation and PMD. A lod score of 2.6 ($\theta = 0$) was obtained. For these analyses, only those individuals previously identified as definitely affected (Mäenpää 1990) were considered in the calculation of lod scores. In an earlier report on this family, the diagnoses for individuals III-7 and III-9 were uncertain, but each did have some features consistent with PMD, even though III-9 was a female. If these individuals, whose mothers carry the mutation, were indeed affected, the lod score would rise to 3.5 at $\theta = 0$.

Discussion

PMD is a rare dysmyelinating disorder of the CNS. There is now much circumstantial evidence implicating mutations in the exons of *PLP* in the etiology of PMD. At the time of writing, 10 mutations had been published. However, there have been no laboratory studies that prove that one or another of these mutations can cause dysmyelination, and the only linkage data were from our laboratory (Trofatter et al. 1989). We now add linkage studies on this Finnish family and report that we find a combined lod score of 9.0 for our own published and unpublished families. This is very strong evidence that the mutations in *PLP* are causative of PMD.

It is of interest that each of the 10 mutations published to date is unique to an individual (or family), and none is found in exons 1, 6, or 7. Several attempts have been made to correlate the amino acid sequence of human *PLP* and the arrangement of the molecule at the cell membrane (Laursen et al. 1984; Stoffel et al. 1984; Hudson et al. 1989*a;* Popot et al. 1991). There is only a measure of accord among these models, but each of them does have the hydrophobic Val^{165}, the site of the mutation in this family, located in the middle of a transmembrane α-helical domain. It is conceivable that Glu, which is hydrophilic, disrupts the α helix. This may be the cause of dysmyelination in this family.

There is yet another aspect to the riddle of the etiology of PMD. Unlike the family reported here, three-fourths (authors' unpublished data; and P. Bridge, personal communication) of PMD patients do not have a defect in any of the seven known exons of *PLP*. This led McKusick to divide PMD into PMD with defects in the *PLP* gene (312080) and PMD-like with no defects in the *PLP* gene (311601) (McKusick 1992). By examining only the coding regions, we have avoided regions of *PLP* that might be the site of mutations (i.e., promoter region, branchpoint consensus sequence, distal regulatory sites, generation of cryptic splice sites, etc.) that

might result in PMD-like disorders. We are currently investigating other families with the classical form of PMD but who lack any mutation in the coding region of *PLP*.

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