*Am. J. Hum. Genet.* 67:14–22, 2000

# Additional Copies of the Proteolipid Protein Gene Causing Pelizaeus-Merzbacher Disease Arise by Separate Integration into the X Chromosome

M. E. Hodes,[1] Karen Woodward,[2] Nancy B. Spinner,[3] Beverly S. Emanuel,[3] Agnes Enrico-Simon,[3,*] John Kamholz,[3,†] Dwight Stambolian,[4] Elaine H. Zackai,[3] Victoria M. Pratt,[1,‡] I. T. Thomas,[5,§] Kerry Crandall,[6,‖] Stephen R. Dlouhy,[1] and Sue Malcolm[2]

[1]Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis; [2]Clinical and Molecular Genetics Unit, Institute of Child Health, London; [3]Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, and [4]Department of Ophthalmology, University of Pennsylvania School of Medicine, Philadelphia; [5]Bowman Gray School of Medicine, Winston-Salem, North Carolina; and [6]Division of Maternal and Child Health, North Carolina Department of Environment, Health and Natural Resources, Black Mountain, NC

**The proteolipid protein gene (*PLP*) is normally present at chromosome Xq22. Mutations and duplications of this gene are associated with Pelizaeus-Merzbacher disease (PMD). Here we describe two new families in which males affected with PMD were found to have a copy of *PLP* on the short arm of the X chromosome, in addition to a normal copy on Xq22. In the first family, the extra copy was first detected by the presence of heterozygosity of the *Aba*II dimorphism within the *PLP* gene. The results of FISH analysis showed an additional copy of *PLP* in Xp22.1, although no chromosomal rearrangements could be detected by standard karyotype analysis. Another three affected males from the family had similar findings. In a second unrelated family with signs of PMD, cytogenetic analysis showed a pericentric inversion of the X chromosome. In the inv(X) carried by several affected family members, FISH showed *PLP* signals at Xp11.4 and Xq22. A third family has previously been reported, in which affected members had an extra copy of the *PLP* gene detected at Xq26 in a chromosome with an otherwise normal banding pattern. The identification of three separate families in which *PLP* is duplicated at a noncontiguous site suggests that such duplications could be a relatively common but previously undetected cause of genetic disorders.**

## Introduction

Pelizaeus-Merzbacher disease (PMD [MIM 312080]) is a rare X-linked dysmyelinating disorder of the CNS. Clinical features of PMD include nystagmus, psychomotor developmental delay, spasticity, and ataxia, all of which may also be present—but in a milder form—in X-linked spastic paraplegia (SPG2 [MIM 312920]) (see Hodes et al. 1993; Hodes 1998). Both conditions may be caused by alterations in the coding region of *PLP* (Hudson et al. 1989; Trofatter et al. 1989). These changes include missense, deletion/insertion, and splice-site mutations (reviewed in Hodes [1998]; Hodes et al. 1993). Complete gene deletions (Raskind et al. 1991) also cause the disorder. However, a considerable number of affected boys, even those with clear X-linked family pedigrees, appear to have no structural alterations of *PLP*. Change in gene dosage has been shown to be an additional and common cause of the disorder. Complete duplications of *PLP* have been observed in a number of affected males (Ellis and Malcolm 1994; Harding et al. 1995; Inoue et al. 1996; Nave and Boespflug-Tanguy 1996; Woodward et al. 1998). In fact, duplication of *PLP* appears to be the single most common cause of PMD/SPG2 (Sistermans et al. 1998; Woodward et al. 1998; Inoue et al. 1999).

The clinical findings associated with mutations of the *PLP* gene are spread over a wide spectrum extending from the very severe connatal PMD to the relatively mild, later-onset SPG2. This has led us to designate the condition as "PMD/SPG2" (Hodes 1998). The clinical phenotype for patients with duplications may generally be milder than that of patients with point mutations in *PLP* (Sistermans et al. 1998; Inoue et al. 1999), whereas patients with complete loss of *PLP* appear to be less severely affected (Raskind et al. 1991).

In the present study, we report three families with unusual duplications of *PLP* in which the additional

Received March 8, 2000; accepted for publication May 8, 2000; electronically published May 25, 2000.

Address for correspondence and reprints: Dr. M. E. Hodes, Department of Medical and Molecular Genetics, Indiana University School of Medicine, 975 West Walnut Street, IB130, Indianapolis, IN 46202-5251. E-mail: mhodes@iupui.edu

[*] Present affiliation: Harpers Ferry Family Medicine, Harpers Ferry, WV.

[†] Present affiliation: Department of Neurology, Wayne State University School of Medicine, Detroit.

[‡] Present affiliation: LabCorp, Research Triangle Park, NC.

[§] Present affiliation: Department of Pediatrics, Children's Hospital, Louisiana State University Health Sciences Center, New Orleans.

[‖] Present affiliation: Fullerton Genetic Center, Asheville, NC.


0002-9297/2000/6701-0006$02.00

copy is aberrantly located on the X chromosome. The first family was discovered when a male with clinical and radiological findings of PMD was found to be heterozygous for the *Aha*II dimorphism of exon 4 of *PLP*. The results of examination by FISH showed copies of *PLP* in chromosomes Xq22 and Xp22.1. The second family was found after the propositus's karyotype suggested that an inversion might disrupt the gene, but copies were found at both ends of the inversion by FISH. The third family was discovered by Woodward et al. (1998).

## Patients and Methods

### *Patients*

Appropriate informed consent was obtained from all subjects.

*Family 1.*—The propositus (patient IV-1; fig. 1*A*) was a 6-year-old male who, at age 6 mo, was referred to an ophthalmologist for congenital horizontal nystagmus with fine, rapid eye movements that were equal in all fields of gaze. Slight unsteadiness of the head, which was not typical of the bobbing seen with spasmus nutans, was noted. A CT scan was normal. The diagnosis seemed to rest between congenital nystagmus and, less likely, spasmus nutans. At age 10 mo, an elevated $\alpha$-fetoprotein level prompted concern about ataxia-telangiectasia, and the infant was referred to a neurologist. The neurologist noted that no attempts at crawling or weight-bearing were made when the infant was placed in a standing position, an inability to maintain a sitting position for more than 5 s, truncal ataxia, no ankle clonus, deep tendon reflexes that were 3–4 plus, and a normal sensory examination. Magnetic-resonance imaging (MRI) showed a high T2 signal throughout the white matter. Electroencephalography results were abnormal due to left temporal and occipital slowing, but no definite seizure activity was noted. At age 15 mo, the child could walk with holding. At age 19 mo, nystagmus, wobbling, and truncal ataxia were still noted but were ameliorating. Results of laboratory examinations included a normal karyotype with no evidence of fragile X syndrome, normal thyroid function, and normal serum and urine amino acid screenings.

At age 8 years, height, weight, and occipitofrontal circumference were all at <5th percentile. Speech was deliberate. Nystagmus was intermittent, and clonus and increasing contractures of the knees occurred. The patient could walk behind his wheelchair, with scissoring. His vision seemed to be worsening. He played video games and also played with LEGOs™. He was repeating the second grade and was "doing well."

The paternal family history was not remarkable. On the maternal side, there were numerous cases of mental retardation and "cerebral palsy." Epilepsy and, probably, congenital nystagmus were also noted, only in males. The mother's half brother (patient III-7; fig. 1*A*) was seen at age 31 years. He had severe visual impairment, incoordination with tremor, and spastic paraplegia. He had been graduated from a special high school.

*Family 2.*—The propositus (patient II-3; fig. 1*B*) presented shortly after birth with features (dysmorphic facial features, microcephaly, right ventricular outflow-tract obstruction, and ventricular septal defect) that were suggestive of Down syndrome. At age 2 mo, he had bilateral horizontal nystagmus superimposed on pendular nystagmus. He had bilateral dysmetria and decreased tone reflexes. At age 6 mo, he had an episode of apnea with asystole. At age 3 years, he was unable to sit alone or to support his weight on his feet, and his speech was limited. At age 7 years, he could not crawl or walk. He had a marked increase in extensor tone in the lower extremities and tightness of hip flexors, hamstrings, and heel cords. The patient is, at present, of age 16.5 years and is wheelchair-bound.

The family history is outlined in fig. 1*B*. The father (patient I-1; fig. 1*B*) and the mother (patient I-2) were normal. A maternal uncle (patient I-6) has "cerebral palsy," the details of which are unknown. Maternal grandparents were reported to be normal. One of the propositus's brothers (patient II-1) presented with nystagmus at age 3 mo. Early motor milestones were normal, but, by 18 mo, he had an unsteady gait. The results of ophthalmologic examination were consistent with congenital idiopathic nystagmus without retinopathy. At age 4 years, the patient walked by holding on but was largely wheelchair-dependent. Speech was severely delayed. At age 8 years, he had pendular nystagmus at rest and no marked titubation. Tone was slightly decreased in the left arm, and he was bilaterally dysmetric (in the left arm more than in the right arm). He walked with support and had a wide-based gait. His reflexes were increased in the lower extremities (in the left extremity more than in the right extremity). The results of MRI studies were consistent with a diffuse dysmyelinating disorder. Another brother (patient II-2) was normal neurologically and developmentally at age 6 years. A third brother (patient II-4) had nystagmus at age 2 mo. He did not achieve normal milestones. By age 18 mo, his gait was unsteady, and the results of ophthalmologic examination were consistent with congenital idiopathic nystagmus without retinopathy. By age 4 years, he was largely wheelchair-dependent, and speech was severely delayed. The results of MRI studies done at age 8 years were consistent with a diffuse dysmyelinating disorder. A sister (patient II-5) was normal neurologically at age 5 wk. She is, at present, of age 12.5 years and is well.

Families 1 and 2 have disparate karyotypic findings.

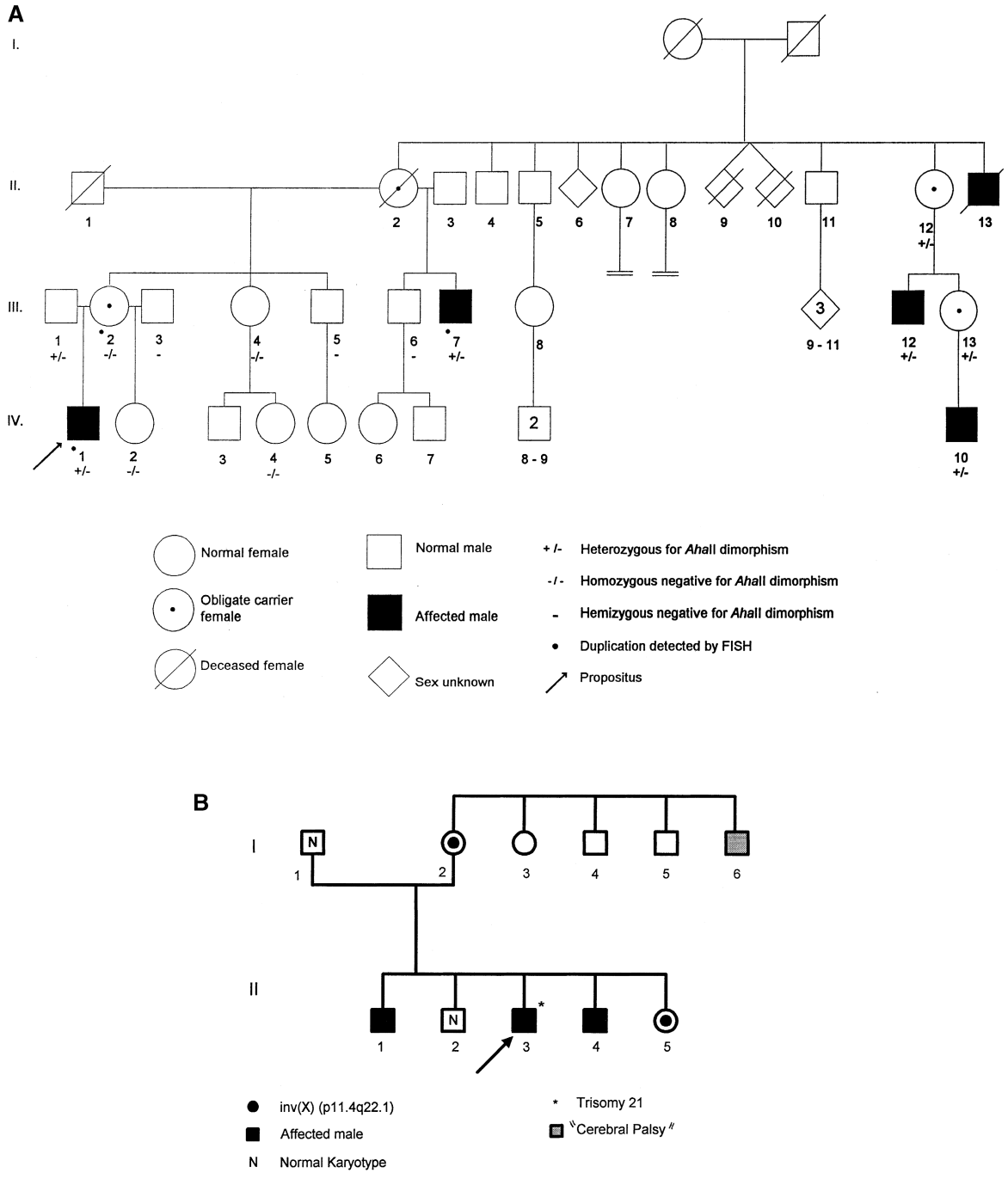


**Figure 1** Pedigrees of families 1 (*A*) and 2 (*B*)

It is also unlikely that they are distantly related, since they have different racial origins.

*Family 3.*—Family 3 was previously described by Woodward et al. (1998). There was no family history of note. In the propositus, onset of nystagmus at age 2 mo and hypermetropia were noted. By age 1 year, the propositus had head titubation and an upper-limb intention tremor develop. By age 4 years, signs of spastic paraparesis and mild scoliosis had developed. The patient has had no seizures. He sat unsupported at age 7 mo, crawled at 10 mo, pulled to stand at 14 mo, and walked with aid at age 2 years, 3 mo. He had a vocabulary of 50 words at age 2 years and was using 5-word sentences by 4 years, and he follows the normal curric-

ulum at school. The results of MRI of his brain, done at 13 mo and 24 mo, showed high signal throughout the supratentorial white matter on T2-weighted imaging and a normal signal on T1-weighted imaging, which were consistent with dysmyelination.

### *Molecular Studies*

The following tests have previously been described: SSCP analysis (Orita et al. 1989); DNA-sequence analysis (Trofatter et al. 1989); tests for the *Aha*II dimorphism (Trofatter et al. 1991); the CA-dinucleotide repeat in the first intron of *PLP* (Mimault et al. 1995); the *Mva*I polymorphism in exon 2 (Osaka et al. 1995); digestion with restriction enzymes; and Southern blotting (Sambrook et al. 1989).

### *Cytogenetic Analysis*

Cytogenetic studies were performed on short-term lymphocyte cultures or lymphoblastoid cell lines, by use of standard techniques.

### *FISH*

*Families 1 and 3*.—FISH was performed, as previously described, with a cosmid (cU125A1) containing the *PLP* gene and a control cosmid (cU144A10) mapping ~850 kb distal to the *PLP* gene (Woodward et al. 1998). A digoxigenin-labeled X chromosome–specific centromeric probe was included to confirm hybridization efficiency and X chromosome number. The cosmid probes were detected with fluorescein isothiocyanate conjugated with avidin, and the centromeric probe was detected with rhodamine conjugated to antidigoxigenin. Cells were counterstained with 4,6-diamidino-2-phenylindole and were viewed with a Zeiss Axiophot fluorescent microscope with a triple-band pass filter. Ten metaphase chromosomes and 50 interphase nuclei were analyzed per slide for both cosmid probes.

*Family 2*.—Three yeast artificial chromosomes (YACs [122e11, 226c9, and 526g7]) containing the *PLP* gene were identified by a computer search of the Human Genome Sequencing Center, Baylor College of Medicine database. DNA was purified by standard techniques and was labeled with biotin-16-dUTP by means of nick translation (Oncor). Genomic probes (kindly provided by Dr. Lynn Hudson) containing portions of the *PLP* gene were also labeled with biotin-16-dUTP, for FISH studies. Probe pJB008 contains a 9-kb *Bam*HI fragment of the human *PLP* gene that includes a portion of exon 1 and all of exon 2, and probe pJC102 contains a 9-kb *Bam*HI fragment that includes exons 5–7. FISH studies were done by use of standard techniques.

## Results

### *Family 1*

*Duplication of the* Aha*II dimorphic site and molecular analyses*.—Routine SSCP screening of the coding region of *PLP* of the propositus (patient IV-1; fig. 1*A*) showed a pattern consistent with heterozygosity for the *Aha*II dimorphism—a result that is unique in our experience studying several hundred males, many of whom have a diagnosis of PMD. DNA from a second blood sample yielded identical results (fig. 2). DNA from three other affected males (patients III-7, III-12, and IV-10; fig. 1*A*) was also found to be heterozygous for the dimorphism. The findings were confirmed by amplification of exon 4, followed by sequencing of the exon, and by the presence, in the propositus, of the dimorphism in amplified fragments containing exons 3B–4, 4–5, and 4–6 (data not shown). The presence of heterozygosity of the *Aha*II dimorphism implied duplication of the gene, and this was consistent with results from multiplex PCR analysis with *PLP* exon 4 primers and with primers for the prion protein gene *PRNP*. Additional findings were as follows: (*a*) Southern blotting of genomic DNA digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, and *Xba*I and probed with exons 4–5 showed only fragments of normal (expected) size, and (*b*) probing (with exons 4–5) of pulsed-field gels of *Not*I-digested DNA showed a single intense fragment (not shown). Taken together, these results suggest that both copies of *PLP* are intact and that a duplication encompasses both *Not*I sites.

*Identification of the* PLP *duplication by FISH*.—Figure 3*A* and *B* shows the duplicated signals for a cosmid containing *PLP,* detected by FISH, in the propositus (patient IV-1; fig. 1). The hybridization signals are shown on chromosome Xq22 in the region expected for the *PLP* gene (Willard and Riordan 1985; Mattei et al. 1986) and also on chromosome Xp22. A single *PLP* signal is shown in figure 3*D*.

The *PLP* duplication was confirmed in interphase nuclei (fig. 3*B*) by the presence of two clearly resolvable yellow signals. The control cosmid gave one signal, thereby indicating that the DNA detected by that probe was not duplicated. The duplication was also found by FISH in both the patient's mother and an uncle.

No inversion or any other rearrangement of the X chromosome was found in the karyotype. Inversion was further ruled out by FISH with a commercially available probe to the *XIST* gene (results not shown). Characterization of the duplication by means of interphase FISH showed that the proximal breakpoint lies ⩾350 kb from the *PLP* gene (data not presented). This suggests that both copies are intact.

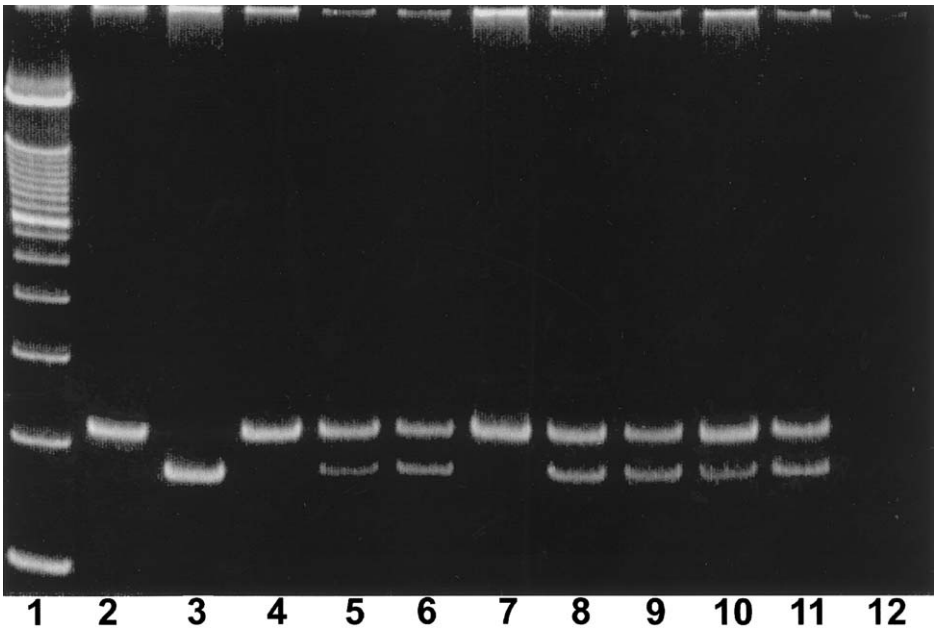


**Figure 2** Separation of fragments of genomic DNA from family 1, after digestion by *Aha*II. Fragments of genomic DNA obtained by PCR amplification of exon 4 were digested by *Aha*II. Electrophoresis of the digest was performed in a 12% polyacrylamide gel. Lane 1 denotes a 100-bp ladder; lane 2, a male control who was *Aha*II negative; lane 3, a male control who was *Aha*II positive; lane 4, patient III-1; lane 5, patient III-2; lane 6, patient IV-1; lane 7, patient IV-2; lane 8, patient IV-10; lane 9, patient III-12; lane 10, patient II-12; lane 11, a female control who was heterozygous; and lane 12, distilled water.

*Family 2*

*Cytogenetic analysis.*—The initial cytogenetic analysis performed on the propositus demonstrated a pericentric inversion of the X chromosome (46,XY, inv(X)(p11.4q22.1). The results of studies performed on additional family members showed the same inversion in the phenotypically normal mother (patient I-2; fig. 1*B*) of the propositus, in an unaffected sister (patient II-5), and in two affected brothers (patients II-1 and II-4).

Molecular cytogenetic (FISH) studies were performed with three *PLP*-containing YACs, to identify their position with respect to the inversion breakpoints. A signal from each of the YACs was present on both the short and long arms of the inverted X chromosome near the breakpoints (fig. 3*C*). This finding suggested either disruption or duplication of the YACs. On FISH with plasmids containing the 5′ and 3′ ends of the *PLP* gene, signals were observed on both arms of the inverted X chromosome. This is consistent with a duplication of the *PLP* gene near the inversion breakpoints.

*Molecular analysis.*—*PLP* genomic organization was investigated by Southern blotting with *PLP* cDNA and genomic probes, after digestions with multiple enzymes and standard and pulsed-field gel analysis. Probes derived from the upstream region and from within the *PLP* gene did not demonstrate any abnormal pattern. Additionally, sequencing of the gene showed no mutations within the coding sequences. The patient and his mother were homozygous for the *Aha*II dimorphism of exon 4, the CA-dinucleotide repeat of the first intron, and the *Mva*I polymorphism of exon 2 (data not shown).

*Family 3*

*Cytogenetic analysis.*—The FISH result was reported in Woodward et al. (1998). In addition to the normal copy of *PLP* in Xq22, a copy was found in Xq26. The patient's mother also carried this change; however, she was a somatic chromosomal mosaic. Approximately half her cells contained two X chromosomes that were cytogenetically normal, but one chromosome had a single copy of *PLP* at Xq22, and the other chromosome had copies of the gene at Xq22 and Xq26. The other half of the cells contained a cytogenetically normal X chromosome with a single copy of *PLP* at Xq22 and a partially deleted X chromosome in which the PLP gene was missing. Therefore, the mother's cells contained either three copies of *PLP* or one copy of the gene. The maternal grandmother's chromosomes were normal by karyotype and FISH analysis.

## Discussion

*Nature of Sequences and Event*

There is an increasing awareness that genomic instability arises from homologous recombination that

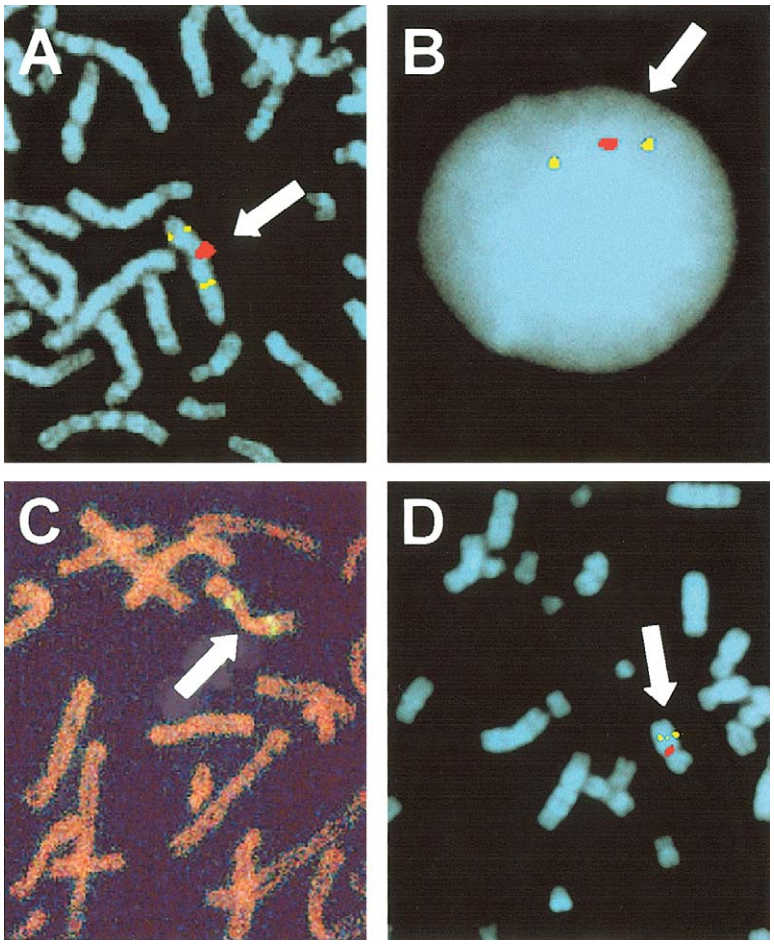


**Figure 3** FISH analysis of the families. Metaphase chromosome spread (*A*) and interphase nucleus (*B*) from the propositus in figure 1*A*, as hybridized with a *PLP* cosmid probe (*yellow*) and an X chromosome centromeric probe (*red*). The signal on chromosome Xq22 shows the normal *PLP* gene, and the signal on Xp22.1 shows the *PLP* duplication. The two clearly resolvable yellow signals in the interphase nucleus show the *PLP* duplication on either side of the red centromeric signal. *C,* Metaphase chromosome spread from the propositus in family 2. Hybridization was with the YAC 122e11 probe. *D,* Single *PLP* signal from control metaphase spread.

occurs at low-copy-number repeats. This has been shown to give rise to a number of genetic disorders. The majority of these disorders—including the Prader-Willi/Angelman, DiGeorge/velo-cardio-facial, and Williams syndromes and hereditary liability to pressure palsies—result from deletions of the regions between the repeats. In contrast, Charcot-Marie-Tooth type 1A (CMT1A) is a well-characterized example of duplication leading to a genetic disorder (as reviewed in Harding [1995]). There are many points of similarity between the duplications in the CNS neuropathy (PMD) and those in the peripheral neuropathy (CMT1A). However, the results presented here show that the duplications that result in the additional copy of the *PLP* gene can arise by a mechanism different from homologous recombination that is mediated by low-level repeats. The finding, obtained by metaphase FISH analysis of the duplicated sequences in several different X chromosome bands, must involve excision from the X chromosome. Such a mechanism would require at least three DNA breaks. Although the sequence in and around *PLP* is being determined (GenBank Genetic Sequence Database and The Sanger Centre), to date nothing is known about the nature of the DNA sequences at the site of insertions.

### *Origin of the Extra Copy*

Available evidence suggests that the duplication in PMD arises most commonly as the result of an intrachromosomal event. Heterozygosity of the *Aha*II dimorphism in an affected male has been observed only in the family reported here, despite the fact that the average heterozygosity of the marker is 0.38 and that ⩾20 affected males from different families have been studied. The finding of *Aha*II heterozygosity in family 1

could have two explanations: either there was a duplication event (intrachromosomal), followed by mutation at the site, or the dimorphism arose from two separate copies of the gene, each of which carried a different *Aha*II variant. Analysis of four multiallelic markers within the duplicated region shows homozygosity throughout the region for most patients (Woodward et al. 1998); however, the propositus was also heterozygous for the CA repeat within the *PLP* gene and for DXS8096 within the duplication. Such heterozygosity for multiple markers is more consistent with an interchromosomal duplication event (i.e., between unlike chromosomes) than with an intrachromosomal event followed by mutation at the *Aha*II site. Thus, it is our contention that the more likely explanation is that the *Aha*II heterozygosity arose as a result of recombination between two X chromosomes in a female, with each chromosome carrying a different form of the dimorphism. Direct evidence that the extra copy can be acquired from the other female X chromosome is available in family 3 (Woodward et al. 1998). This was the only case, among the series of duplications reported, in which multiallelic polymorphic markers from within the duplication were found to be heterozygous. The duplication arose de novo in the mother of the propositus (unpublished results).

The propositus (patient IV-1) in family 1 is a member of a four-generation family with proved PMD in each of the last three generations. In a normal situation, recombination between the gene copies on the long and short arms would be expected. Comparison of the haplotypes of markers along the X chromosome in the propositus and his affected uncle show that there have indeed been recombinants (data not presented). Each affected male member of the family is also heterozygous for the polymorphism. This suggests that the "extra copy" (on Xp) carries the less common (frequency .25) *Aha*II + variant, whereas the "normal" copy (on Xq) is most likely the common (frequency .75) *Aha*II allele.

In family 2, the propositus and his presumed carrier mother were homozygous for the *Aha*II dimorphism in exon 4, the *Mva*I polymorphism in exon 2, and the CA-dinucleotide repeat in the first intron of the *PLP* gene. Although this is consistent with an intrachromosomal origin of the duplication, an interchromosomal exchange cannot be ruled out.

At this time, the mechanism by which the duplicated copies of *PLP* arose is not understood. Although several disorders are known to involve deletion or duplication based on low-copy-number repeats, the investigations of mechanism are new. Such is the current status of *PLP* duplications.

### *Stability of the Resulting Chromosome*

The origin of the X chromosome carrying the duplication in family 2 is unknown, although examination of the pedigree indicates stable transmission of the inv(X) from the mother (patient I-2; fig. 1*B*) to four of her five children. The pedigree also provides circumstantial evidence that an abnormal chromosome is carried by at least three generations, since there is a history of "cerebral palsy" in the maternal uncle of the propositus. However, samples have not been available for chromosome analysis of the mother's brother or mother.

### *Wider Implications*

It is entirely unclear why the additional copy should be located, in each case, on the X chromosome. There is no known tendency for pairing between Xq22 and the three regions involved. If the additional copy were located on an autosome, there would be an equal chance of its affecting a female. It may be that, since females have not been routinely tested for the *PLP* duplication, such cases, which are expected to be rare, have been missed.

As far as we know, the situation in which patients have a noncontiguous duplication, as observed in the present study, is unprecedented. Questions arise as to how commonly such undetected submicroscopic transpositions occur and what clinical outcomes might be expected. Several factors have led to the fortuitous observation of these *PLP* duplications. First, *PLP* is one of only a very small number of known genes that are dosage-sensitive to an extra copy, and this was examined only because of analogies with the *PMP-22* gene in hereditary motor and sensory neuropathy type 1 (Ellis and Malcolm 1994). CMT1A, which is a peripheral neuropathy that results in demyelination, is usually caused by duplication of the *PMP-22* gene, which lies in the center of the 1.5-Mb duplicated segment. The duplication results in an additional (third) copy of the *PMP-22* gene, which is disease causing. Second, extra copies are easier to detect on the X chromosome than on an autosome. Third, not only must the gene be subject to dosage sensitivity, but it must lie within a region of the genome where there are factors operating at the chromosomal level that drive the formation of a duplication. For *CMT1A,* on chromosome 17, the breakpoints lie well outside the *PMP-22* coding region, and not only are they detectable by pulsed-field gel electrophoresis (Raeymaekers et al. 1992), but they have been shown to lie within a hotspot for recombination (Kiyosawa et al. 1995; Lopes et al. 1998; Reiter et al. 1998). The rearrangement leads to tandem repeats only. The only exceptions have been cytogenetically visible duplications in patients with multiple abnormalities, including signs of Charcot-Marie-Tooth disease (Chance et al. 1992; Upadhyaya et al. 1993; Pellegrino et al. 1996), analogous to the published example involving PMD (Cremers

et al. 1987). No case in which the 1.5-Mb segment is translocated to a distant locus has been reported.

It is possible that other examples exist in which such submicroscopic rearrangements have occurred but have remained undetected because the favorable factors of gene-dosage sensitivity and chromosomal environment have not occurred together. One way in which insertions might be found is during large-scale linkage projects that use closely linked markers. International collaborations to map the *CMT1A* gene foundered for some time, because of apparent inconsistency of marker order, until the existence of the duplication was appreciated (Nicholson et al. 1992). Such insertions have not been reported, but there may be underreporting of "unexplained" findings. Transposed sequences could be detected if they inserted, by chance, into a coding region of a structural gene that was under intense analysis because of its known role in genetic disease. Examples, although rare, were reported for *Alu* (Oldridge et al. 1999 [and references therein]) and LINE (Kazazian 1998 [and references therein]) repeats. It is likely that other examples of the insertions described here are less common but may occur. A further possibility is that insertions may occur but may not contain dosage-sensitive genes. They may exert an effect by disruption of sequences at the insertion point or by a position effect (Kleinjan and van Heyingen 1998). With the exception of patient II-3 (see fig. 1*B*), who has Down syndrome in addition to PMD, the clinical features in the patients with PMD who are presented here were within the usual range. Thus, it is likely that no important sequences were disrupted. This may not always be the case.

## Acknowledgments

We thank Dr. Mark Pettinati, for the karyotype of the propositus in family 1. S.M. and K.W. thank the Wellcome Trust, for generous support, and Dr. R. Surtees, for assistance. N.B.S., B.S.E., and E.H.Z. thank Ray Colliton, for technical assistance. M.E.H. and S.R.D. thank the Rheta A. Sosland Charitable Trust Fund and the Tillery and Skelly families, for aid with this work. The work was supported in part by funds from the National Institutes of Health (HD26979 [to B.S.E.]).

## Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

GenBank Genetic Sequence Database, http://helix.nih.gov/science/genbank.html

Human Genome Sequencing Center, Baylor College of Medicine, http://www.hgsc.bcm.tmc.edu/index.html (for three YACs [122e11, 226c9, and 526g7] containing the *PLP* gene)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for PMD [MIM 312080] and X-linked SPG2 [MIM 312920])

Sanger Centre, The, http://www.sanger.ac.uk/HGP/ChrX/

## References

Chance PF, Bird TD, Matsunami N, Lensch MW, Brothman AR, Feldman GM (1992) Trisomy 17p associated with Charcot-Marie-Tooth neuropathy type 1A phenotype: evidence for gene dosage as a mechanism in CMT1A. Neurology 42:2295-2299

Cremers FPM, Pfeiffer RA, van de Pol TJR, Hofker MH, Kruse TA, Wieringa B, Ropers HH (1987) An interstitial duplication of the X chromosome in a male allows physical fine mapping of probes from the Xq13-q22 region. Hum Genet 77:23–27

Ellis D, Malcolm S (1994) Proteolipid protein gene dosage effect in Pelizaeus-Merzbacher disease. Nat Genet 6:333–334

Harding AE (1995) From the syndrome of Charcot, Marie and Tooth to disorders of peripheral myelin proteins. Brain 118: 809–818

Harding B, Ellis D, Malcolm S (1995) A case of Pelizaeus-Merzbacher disease showing increased dosage of the proteolipid protein gene. Neuropathol Appl Neurobiol 21:111–115

Hodes ME (1998) Pelizaeus-Merzbacher disease. In: Gilman S, Goldstein GW, Waxman SG (eds) Neurobase, 4th ed. Arbor Publishing, La Jolla, CA

Hodes ME, Pratt VM, Dlouhy SR (1993) The genetics of Pelizaeus-Merzbacher disease. Dev Neurosci 15:383–394

Hudson LD, Puckett C, Berndt J, Chan J, Gencic S (1989) Mutation of the proteolipid protein gene *PLP* in a human X chromosome–linked myelin disorder. Proc Natl Acad Sci USA 86:8128–8131

Inoue K, Osaka H, Imaizumi K, Nezu A, Takanashi J, Arii J, Murayama K, et al (1999) Proteolipid protein gene duplications causing Pelizaeus-Merzbacher disease: molecular mechanism and phenotypic manifestations. Ann Neurol 45: 624–632

Inoue K, Osaka H, Sugiyama N, Kawanishi C, Onishi H, Nezu A, Kimura K, et al (1996) A duplicated *PLP* gene causing Pelizaeus-Merzbacher disease detected by comparative multiplex PCR. Am J Hum Genet 59:32–39

Kazazian HH Jr (1998) Mobile elements and disease. Curr Opin Genet Dev 8:343–350

Kiyosawa H, Lensch MW, Chance PF (1995) Analysis of the CMT1A-REP repeat: mapping crossover breakpoints in CMT1A and HNPP. Hum Mol Genet 4:2327–2334

Kleinjan DJ, van Heyningen V (1998) Position effect in human genetic disease. Hum Mol Genet 7:1611–1618

Lopes J, Ravise N, Vandenberghe A, Palau F, Ionasescu V, Mayer M, Levy N, et al (1998) Fine mapping of de novo CMT1A and HNPP rearrangements within CMT1A-REPs evidences two distinct sex-dependent mechanisms and candidate sequences involved in recombination. Hum Mol Genet 7:141–148

Mattei MG, Alleil PM, Dautigny A, Passage E, Pham-Dinh DD, Mattei JF, Jolles P (1986) The gene encoding for the

major brain proteolipid (*PLP*) maps on the q22 band of the human X chromosome. Hum Genet 72:352–353

Mimault C, Cailloux F, Giraud G, Dastugue B, Boespflug-Tanguy O (1995) Dinucleotide repeat polymorphism in the proteolipoprotein (*PLP*) gene. Hum Genet 96:236

Nave KA, Boespflug-Tanguy O (1996) X-linked developmental defects of myelination: from mouse mutants to human genetic diseases. Neuroscientist 2:33–43

Nicholson GA, Kennerson ML, Keats BJ, Mesterovic N, Churcher W, Barker D, Ross DA (1992) Charcot-Marie-Tooth neuropathy type 1A mutation: apparent crossovers with D17S122 are due to a duplication. Am J Med Genet 44:455–460

Oldridge M, Zackai EH, McDonald-McGinn DM, Iseki S, Morriss-Kay GM, Twigg SR, Johnson D, et al (1999) De novo *Alu*-element insertions in *FGFR2* identify a distinct pathological basis for Apert syndrome. Am J Hum Genet 64:446–461

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5: 874–879

Osaka H, Inoue K, Kawanishi C, Yamada Y, Onishi H, Sugiyama N, Suzuki K, et al (1995) *Mva*I polymorphism in the proteolipid protein (*PLP*) gene. Hum Genet 95:461

Pellegrino JE, Pellegrino L, Spinner NB, Sladky J, Chance PF, Zackai EH (1996) Developmental profile in a patient with monosomy 10q and dup(17p) associated with a peripheral neuropathy. Am J Med Genet 61:377–381

Raeymaekers P, Timmerman V, Nelis E, Van Hul W, De Jonghe P, Martin JJ, Van Broeckhoven C (1992) Estimation of the size of the chromosome 17p11.2 duplication in Charcot-Marie-Tooth neuropathy type 1a (CMT1a). HMSN Collaborative Research Group. J Med Genet 29:5–11

Raskind WH, Williams CA, Hudson LD, Bird TD (1991) Complete deletion of the proteolipid protein gene (*PLP*) in a family with X-linked Pelizaeus-Merzbacher disease. Am J Hum Genet 49:1355–1360

Reiter LT, Hastings PJ, Nelis E, De Jonghe P, Van Broeckhoven C, Lupski JR (1998) Human meiotic recombination products revealed by sequencing a hotspot for homologous strand exchange in multiple HNPP deletion patients. Am J Hum Genet 62:1023–1033

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Sistermans EA, de Coo RFM, de Wijs IJ, van Oost BA (1998) Duplication of the proteolipid protein gene is the major cause of Pelizaeus-Merzbacher disease. Neurology 50:1749–1754

Trofatter JA, Dlouhy SR, DeMyer W, Conneally PM, Hodes ME (1989) Pelizaeus-Merzbacher disease: tight linkage to proteolipid protein gene exon variant. Proc Natl Acad Sci USA 86:9427–9430

Trofatter JA, Pratt VM, Dlouhy SR, Hodes ME (1991) *Aba*II polymorphism in human X-linked proteolipid protein gene (*PLP*). Nucleic Acids Res 19:6057

Upadhyaya M, Roberts SH, Farnham J, MacMillan JC, Clarke A, Heath JP, Hodges IC, et al (1993) Charcot-Marie-Tooth disease 1A (CMT1A) associated with a maternal duplication of chromosome 17p11.2→12. Hum Genet 91:392–394

Willard HF, Riordan JR (1985) Assignment of the gene for myelin proteolipid protein to the X chromosome: implications for X-linked myelin disorders. Science 230:940–942

Woodward K, Kendall E, Vetrie D, Malcolm S (1998) Pelizaeus-Merzbacher disease: identification of Xq22 proteolipid-protein duplications and characterization of breakpoints by interphase FISH. Am J Hum Genet 63:207–217