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A Duplicated *PLP* Gene Causing Pelizaeus-Merzbacher Disease Detected by Comparative Multiplex PCR

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Summary

Pelizaeus-Merzbacher disease (PMD) is an X-linked dysmyelinating disorder caused by abnormalities in the proteolipid protein (PLP) gene, which is essential for oligodendrocyte differentiation and CNS myelin formation. Although linkage analysis has shown the homogeneity at the PLP locus in patients with PMD, exonic mutations in the *PLP* gene have been identified in only 10%–25% of all cases, which suggests the presence of other genetic aberrations, including gene duplication. In this study, we examined five families with PMD not carrying exonic mutations in *PLP* gene, using comparative multiplex PCR (CM-PCR) as a semiquantitative assay of gene dosage. *PLP* gene duplications were identified in four families by CM-PCR and confirmed in three families by densitometric RFLP analysis. Because a homologous myelin protein gene, *PMP22,* is duplicated in the majority of patients with Charcot-Marie-Tooth 1A, *PLP* gene overdosage may be a important genetic abnormality in PMD and affect myelin formation.

Introduction

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked dysmyelinating disorder of the CNS (Pelizaeus 1885) resulting from a reduced number of mature oligodendrocytes (Merzbacher 1910; Seitelberger 1970). Recent studies have demonstrated that proteolipid protein (PLP), the most abundant protein in CNS myelin, is involved in the pathogenesis of PMD. Functional mutations in the *PLP* gene have been identified in animal models (Boison and Stoffel 1989; Nave et al. 1989; Gencic and Hudson 1990; Nadon et al. 1990) and human patients with PMD (Hudson et al. 1989; Trofatter et al. 1989; Gencic et al. 1989; Pham-Dinh et al. 1991; Raskind et al. 1991; Doll et al. 1992; Iwaki et al. 1993; Kurosawa et al. 1993; Pham-Dinh et al. 1993; Pratt et al. 1993) and X-linked spastic paraplegia 2 (X-SPG2), an allelic disorder of PMD (Kobayashi and Hoffman 1994; Saugier-Verber et al. 1994; Osaka et al. 1995). It also has been reported that exonic mutations are present in only 10%–25% of PMD patients, although all PMD cases were linked to the *PLP* locus (Boespflug-Tanguy et al. 1994). In previous studies, we identified point mutations in some cases with PMD (authors' unpublished data) and X-SPG2 (Osaka et al. 1995), but we could not find any genetic defects in the majority of cases. These observation suggest the existence of *PLP* genetic abnormalities other than exonic mutations.

Recent studies have shown that an increased PLP dosage resulting from total duplication of the gene also causes PMD in transgenic mice (Readhead et al. 1994; Kagawa et al. 1994) and patients (Cremers et al. 1987; Ellis and Malcolm 1994). Carango et al. (1995) also reported PMD patients who showed overexpressed mRNA but have no *PLP* duplication. A similar phenomenon has also been reported in the majority of Charcot-Marie-Tooth (CMT) 1A patients with a duplicated *PMP22* gene (Lupski et al. 1991), which is expressed in Schwann cells of the peripheral nervous system. Considering the similarity between PMD and CMT1A, we hypothesized that PLP gene duplication may cause PMD.

In the present study, we examined five PMD families having no *PLP* exonic mutations, by using a PCR-based semiquantitative method, which we developed to screen for the *PLP* gene duplications. This method, comparative multiplex PCR (CM-PCR), is a rapid and reliable way to estimate gene dosage. We identified *PLP* gene duplications in four patients and found these to be inherited from their mothers. We confirmed these gene duplications in three of these families by densitometric RFLP (DRFLP) analysis of a polymorphic site in exon 4 of the *PLP* gene. Since the clinical manifestations of these cases were as severe as those of classic form of PMD due to exonic mutations, gene duplication may affect myelination in CNS as severe as exonic mutations in the *PLP* gene.

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Patients, Material, and Methods

Patients

Five patients with PMD from five independent Japanese families (PM1, PM2, PM3, PM4, and PM5) were examined. We previously screened them for *PLP* gene mutations by PCR amplification followed by heteroduplex analysis and DNA sequencing and did not find either point mutations, insertions, or deletions in any exons, splice junctions, or the 5′ promoter region. Clinical features of four of the families have been described elsewhere (Nezu 1995). All patients showed early-onset nystagmus, pyramidal tract signs, and cerebellar involvement. Magnetic resonance imaging showed a diffuse hyperintense white-matter signal in T2-weighted images. They were diagnosed as having the classic form of PMD (Boulloche and Aicardi 1986). Genomic DNA from peripheral blood was extracted and examined, for further analysis.

Comparative Multiplex PCR (CM-PCR)

Two pairs of primers for exon 1 (PM1A and PM1B) and exon 7 (PM7A and PM7B) of the *PLP* gene were used (Osaka et al. 1995). As an internal control to allow comparison of *PLP* signals, exon 2 of the prion protein gene (*PRNP*) on chromosome 20 was amplified with a pair of primers (PRP44, 5′-TCCCACTATCAGGAAGATGA-3′; PRP200A, 5′-ACTGCGTCAATATCACAATC-3′) in the same reaction tube. The cycle number was optimized by multiple control analysis. PCR was performed with 0.2 μmol of each primer, 200 μM of dNTPs, 10 ng of genomic DNA, standard PCR buffer, and 0.5 U of *Taq* polymerase in a 25-μl volume in a thermal cycler (J-2000, Perkin Elmer). Cycling conditions were 3 min at 94°C initial denaturation, followed by 25–35 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, followed by a final extension of 7 min at 72°C. Ten microliters of the amplified products were analyzed by electrophoresis on a 2% agarose gel and were photographed after ethidium bromide staining. The intensity of each band in the photograph was measured using a video densitometer (Bio-profil system, Vilber Lourmat), and the ratio of averaged intensities of two PLP bands to that of the internal control *PRNP* band was calculated (*PLP/PRNP* ratio). To verify that the ratio of amplified products reflects that of the original templates, control experiments with cloned templates of known amounts were performed. Each PCR fragment from *PLP* gene and *PRNP* gene was subcloned into a T-vector (TA cloning kit, Invitrogen) and transfected into *Escherichia coli* competent cells. The purified plasmids containing *PLP* gene and *PRNP* gene were mixed into ratios of normal male (single), normal female/duplication (double), or triplication (triple), which were utilized as templates for subsequent CM-PCR analysis. As control experiments with human genomic DNA, 39 normal controls (19 males and 20 females) were examined to obtain the reference range for normal males and females, who carry one and two PLP alleles, respectively. For each patient and his mother, CM-PCR analysis was performed more than five times.

Densitometric RFLP (DRFLP) Analysis of the AhaII *Polymorphism in Exon 4*

There is a highly heterogeneous *Aha*II polymorphism in exon 4 of the *PLP* gene (Trofatter et al. 1991). If duplicated *PLP* genes carry different polymorphisms, patient samples will show bands corresponding to both cut and uncut DNA, after *Aha*II digestion. Even if a homozygous pattern was observed in a patient, if his mother has heterozygous alleles, the ratio of the intensities of the uncut and cut bands may be 2:1 or 1:2 according to the genotype of the duplicated allele.

Exon 4 of the *PLP* gene was amplified with specific primers (PM4AN, 5′-GGATCTCCCAGTTTGTGTTTCT-3′; and PM4BN, 5′-AATCACCACCCTCCTTACACTA-3′) followed by digestion with *Bsa*HI, which is an isoschizomer of *Aha*II, for ≥16 h. Digested samples were analyzed by electrophoresis on 2% agarose gels and photographed after ethidium bromide staining. The intensities of a 333-bp band, reflecting absence of the *Aha*II site, and a 227-bp band, reflecting presence of the site, were estimated with the video densitometer (Bio-profil system, Vilber Lourmat) and the uncut/cut (U/C) ratios were calculated.

Results

Control CM-PCR Analysis Using Subcloned Plasmids and Normal Genomic DNA

The optimal cycle number for amplification was determined to be 28, which is the least number of cycles to obtain a high enough signal for densitometric analysis while avoiding a plateau effect due to excessive amplification (data not shown). CM-PCR analysis using subcloned vectors mixed into ratios of single, double, and triple showed clear separation between each of them, confirming that the *PLP/PRNP* ratio of the amplified products reflects that of the original templates (fig. 1*A*, *B*). The *PLP/PRNP* ratios between males and females differed significantly (fig. 1*A*, *C*). They were used as control reference for further comparative analysis. Some of the controls also were examined with various cycles of amplification, and *PLP/PRNP* ratios were calculated. They were distinctly separated between males and females at all cycles (fig. 2).

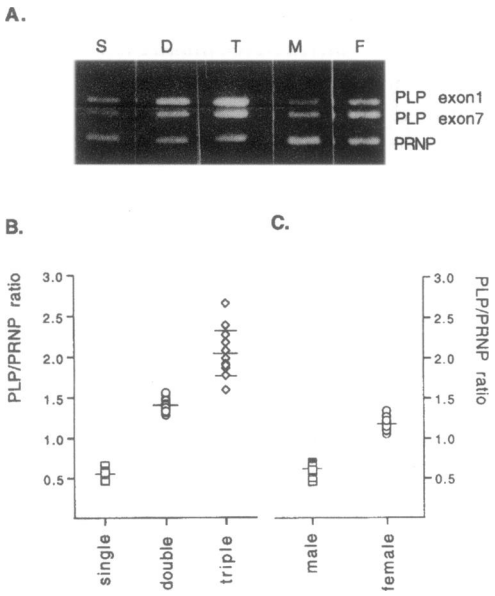

Figure 1 Control CM-PCR analyses. *A*, Amplified products from exon 1 (351 bp) and exon 7 (305 bp) of the *PLP* gene, in comparison with *PRNP* inner control (237 bp). S = single; D = double; T = triple; M = male; F = female. Note that both *PLP* bands show relatively higher intensities in triple and double than in single, as well as in female than in male. *B*, Control CM-PCR analysis by use of cloned templates. Each PCR product was subcloned (pPLP1, pPLP7, and pPRNP) and mixed into the following ratios: single, corresponding to normal male, contains 1×10^{-4} pmol of pPLP1 and pPLP7 with 2×10^{-4} pmol of pPRNP; double, corresponding to normal female and *PLP* duplication, contains twice the amount of pPLP1 and pPLP7 of single, with the same amount of pPRNP; triple, corresponding to female carrying *PLP* duplication, contains three times the amount of pPLP1 and pPLP7 of single, with the same amount of pPRNP. Fifteen samples were examined for each template with 28 cycles of amplification. Values from three groups differ significantly ($P < .001$ by one-way ANOVA followed by Student's *t*-test.) *C*, Control CM-PCR analysis by use of normal male and female genomic DNA samples after 28 cycles of amplification. Values in the female are significantly higher than in the male ($P < .001$ by Student's *t*-test). Note that *PLP/PRNP* ratios of the male and female are corresponding to those of single and double, respectively. Data are mean ± SEM (bars). The SEM is not indicated unless it is larger than the symbol.

Duplicated PLP Gene Identified by CM-PCR in PMD Patients

Patients from four families, PM1, PM2, PM3, and PM4 showed significantly higher *PLP/PRNP* ratios than male controls, which were within the female control range (fig. 3; table 1). The *PLP/PRNP* ratios of their mothers were higher than those of the female controls and identical to that of the triple in the control analysis using subcloned plasmids. These data strongly suggest that these patients and their mothers carry duplicated *PLP* genes. The patient and mother from PM5 showed the *PLP/PRNP* ratios within the male and female control ranges, suggesting they do not have *PLP* gene duplication. Two PMD and one X-SPG2 patients with identified *PLP* exonic mutations also showed normal male ratios. To neglect an asymmetrical amplification, we determined the *PLP/PRNP* ratio at various cycle numbers and compared it to the control references (fig. 4*A*). The ratios for these patients were within the female control range at all cycles. Their mothers revealed higher *PLP/PRNP* ratios than the female control range in the lower cycles (fig. 4*B*).

DRFLP Analysis

Eleven heterozygous female controls were examined, and their U/C ratios were approximately identical to the theoretical ratio (table 2). None of male controls showed

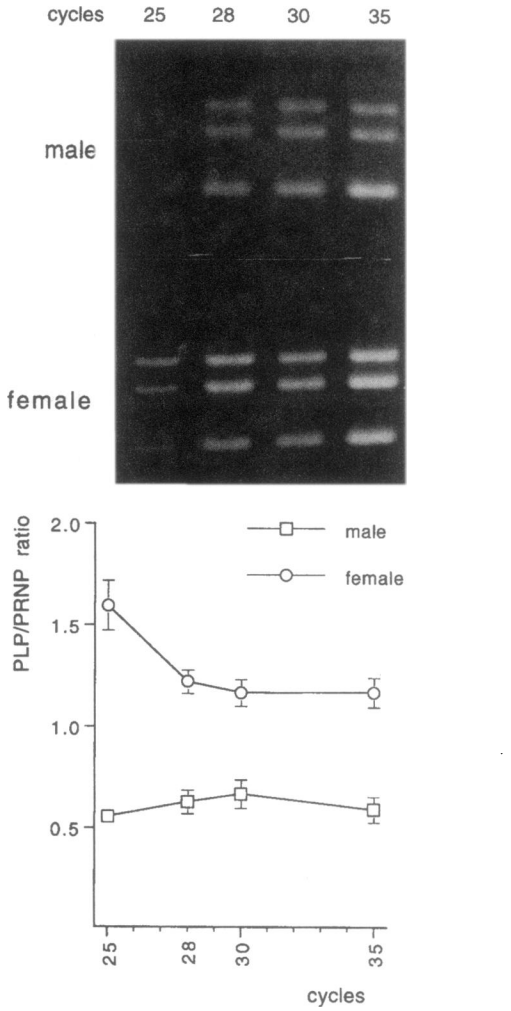

Figure 2 CM-PCR analysis by use of control genomic DNA with various cycle numbers. Male and female control DNA were amplified at 25, 28, 30, and 35 cycles. Data are mean ± SEM (bars) values from at least five experiments. The *PLP/PRNP* ratios are significantly different from each other at any cycle numbers ($P < .001$ by Student's *t*-test).

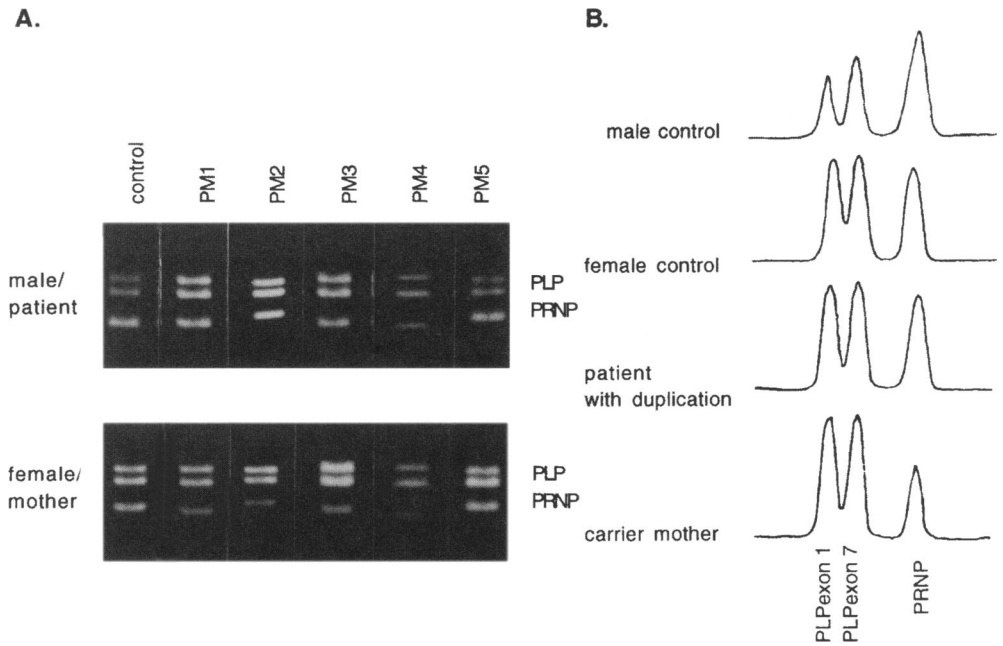

Figure 3 CM-PCR analysis of the PMD patients and their mothers. *A,* Both patients and mothers from PM1, PM2, PM3, and PM4, who represent higher intensities of *PLP* bands than male and female controls, respectively, in comparison with the *PRNP* bands. The patient and mother from PM5 show *PLP* intensities equal to the normal controls. *B,* Raw data of the intensities by densitometric analysis. The *PLP* signal peaks of the patient with duplication, which are higher than those in male control, are as high as those of the female control. The carrier mother showed prominent peaks compared to that of *PRNP* control.

a heterozygous pattern. Each patient from PM1, PM2, PM3, and PM4 showed a single uncut pattern, and the patient from PM5 showed a single cut pattern. Heterozygous patterns were not recognized in all patients. Four mothers from PM1, PM2, PM4, and PM5, however, represented heterozygous patterns (fig. 5). Densitometric analysis identified significantly higher U/C ratios in the mothers from PM1, PM2, and PM4 than the normal controls. Moreover, their U/C ratios absolutely consisted with theoretical calculation of duplication in the uncut allele (table 2). These indicate that the original dosages of the uncut alleles, which were carried by their sons, are twice that of the cut alleles. Therefore, *PLP* gene duplications were suggested in these three families, which were also suspected by CM-PCR analysis. The mother from PM5, in which *PLP* gene duplication was not identified by CM-PCR analysis, showed a normal U/C ratio, supporting the absence of abnormal *PLP* gene dosage. Although the duplication was suspected in PM3 by CM-PCR analysis, both patient and mother represented only an uncut pattern, yielding no additional information from this assay.

Table 1

CM-PCR Analysis in the Families with PMD

Sample	*PLP/PRNP* Ratio		*PLP* Exonic Mutation
Male control	.63 ± .06		...
Female control	1.18 ± .20		...
	Patients	Mother	
PM1	1.35 ± .13*	1.89 ± .24**	...
PM2	1.25 ± .17*	2.12 ± .23**	...
PM3	1.29 ± .14*	1.97 ± .21**	...
PM4	1.30 ± .07*	2.15 ± .18**	...
PM5	.61 ± .07	1.18 ± .20	...
PMDex1[a]	.54 ± .06	...	exon 5
PMDex2[a]	.66 ± .04	...	exon 5
X-SPG2	.67 ± .12	...	exon 3

[a] PMD carrying exonic mutation in the *PLP* gene.
* $P < .001$ by Student's *t*-test, in comparison with the male control.
** $P < .001$ by Student's *t*-test, in comparison with the female control.

Discussion

We reported here four families with PMD carrying duplicated *PLP* genes, which may cause an increase in the dosage of gene products. So far, exonic mutations in the *PLP* gene, such as point mutations and small insertions or deletions, have been found in only ~10%–25% PMD patients. A recent analysis of linkage be-

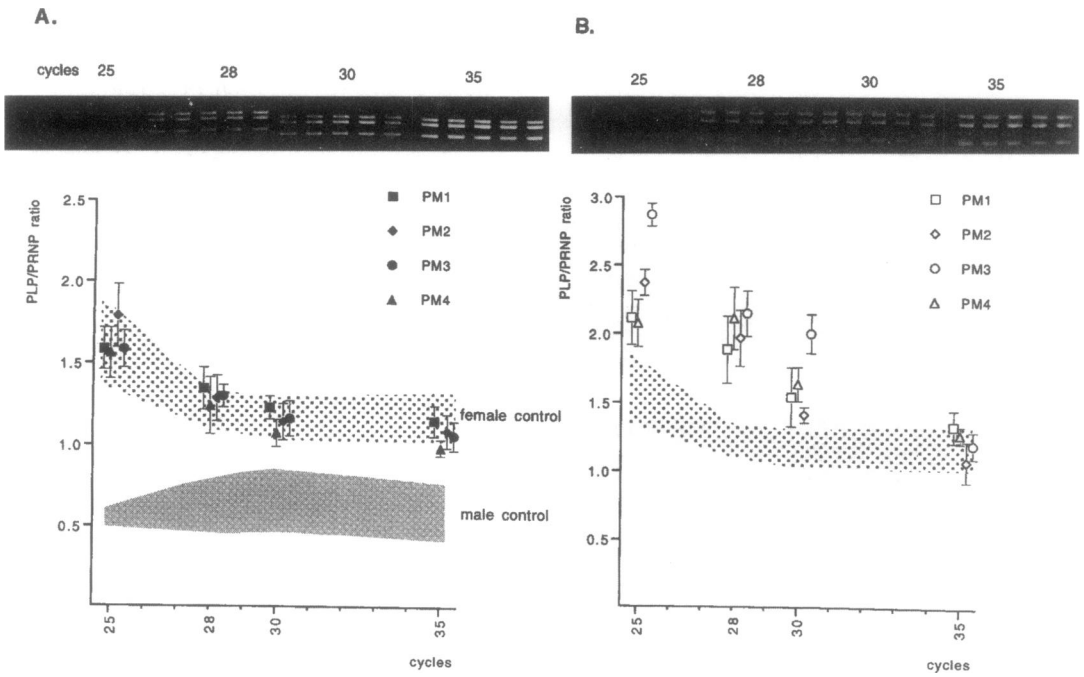

Figure 4 *A*, CM-PCR analysis on the patients having duplicated *PLP* gene with various cycles of amplification. The photograph indicates the CM-PCR analysis of PM1 patient with 25, 28, 30, and 35 cycles of amplifications. Ranges of the male and female controls are shown as shadows (mean ± 2 SEM). Note that all patients represent *PLP/PRNP* ratios identical to the female control range. *B*, CM-PCR analysis on the mothers carrying *PLP* duplications with a variety of amplification cycles. The photograph indicates the CM-PCR analysis of PM1 mother with 25–35 cycles of amplifications. Note that all samples show higher *PLP/PRNP* ratios than female controls at the low cycle numbers. Data were mean ± SEM (bars) values from five experiments.

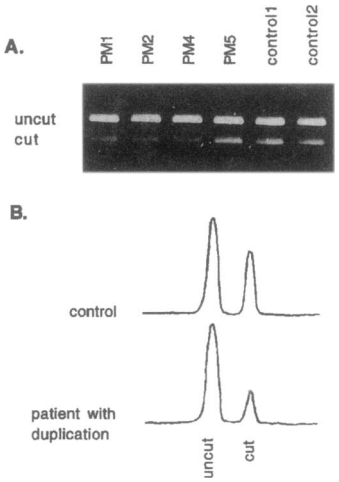

Figure 5 DRFLP analysis. *A*, Mothers from PM1, PM2, PM4, and PM5, shown with two heterozygous controls. Note that the intensities of cut bands in PM1, PM2, and PM4 are lower than those in normal controls in comparison with uncut bands. PM5 mother represent as high intensity as the controls. The other pieces of cut fragments, 106-bp bands, are not shown. *B*, Raw data of the densitometric analysis. The cut band of the patient with duplication revealed remarkably weak signal in comparison with that of the control.

tween the *PLP* locus and PMD in 16 families, including 14 lacking *PLP* gene mutations, demonstrated linkage to *PLP* locus in all cases, which suggests a homogeneous genetic basis for PMD (Boespflug-Tanguy et al. 1994). This has raised the question of what the genetic aberration is responsible for remaining PMD cases. In

Table 2

DRFLP Analysis in the Families with PMD

Sample	U/C Ratio (*n*)
PM1	2.86 ± .21 (5)
PM2	2.95 ± .28 (5)
PM4	3.08 ± .24 (5)
PM5	1.68 ± .26 (5)
Control 1[a]	1.50 ± .06 (5)
Control 2[a]	1.54 ± .08 (5)
Total controls[b]	1.51 ± .08 (11)

NOTE.—Data are mean ± SEM.

[a] Two female controls examined by five independent experiments.

[b] Data from one experiment of 11 independent female controls. The theoretical U/C ratio for a heterozygous female control is 1.47 (333 bp/227 bp), and that for a duplicated uncut allele is 2.93 (333 bp/227 bp).

CMT1A, a heritable disorder of dysmyelination limited to the peripheral nervous system, duplication of the *PMP22* gene is the most common genetic abnormality (Lupski et al. 1991). PMP22 and PLP show high structural homology in a hydrophilic region that spans the membrane four times and is found only in myelin (Suter et al. 1993). Considering reported cases with *PLP* gene duplication (Cremers et al. 1987; Ellis and Malcolm 1994), it is strongly suggested that the *PLP* duplication is another potential cause of PMD. Since duplicated *PLP* genes were identified in four of five PMD families examined, we proposed that *PLP* gene overdosage plays a major role in the pathogenesis of PMD in the absence of exonic mutations.

Initially, we performed Southern analyses of gene dosage. However, we could not obtain reproducible results in comparison with controls. We, therefore, developed CM-PCR to detect *PLP* gene duplication. CM-PCR is an semiquantitative assay for the *PLP* gene–dosage analysis by comparing intensities of the *PLP* gene with that of the *PRNP* internal control after multiplex PCR amplification in the same tube. The original ratio of *PLP* to *PRNP* in the template DNA is reflected by that of the amplified products, which is detected as the ratio of intensities of ethidium-stained bands after electrophoresis. Thus, a normal female with two *PLP* alleles gives a stronger signal than does a normal male with a single *PLP* allele. Therefore, male patients with duplicated genes represent a signal strength equal to that of a normal female. CM-PCR is an exceedingly rapid and simple technique with a number of advantages. First, a single PCR amplification allows all comparative analyses. Second, no radioisotope or other labeled reagents are necessary and simple agarose gel electrophoresis followed by ethidium bromide staining is sufficient for signal detection. Third, a small amount of DNA template ($\leq$0.1 mg) is sufficient for analysis. Finally, the whole procedure may be completed in 1 d. Thus, CM-PCR is a useful technique to screen for duplicated *PLP* genes.

In both of the control experiments using subcloned plasmids and genomic DNA, samples containing each number of *PLP* allele were successfully separated with statistical significance. Moreover, each subcloned template showed approximately the same *PLP/PRNP* ratio with corresponding human genomic DNA (single to male and double to female). In the control analyses of DRFLP, no male controls showed a heterozygous pattern, and all female controls revealed the U/C ratio identical to the theoretical calculation. Together with absence of reported pseudogenes of *PLP* and *PRNP*, there may be no other genes that are amplified by our primers and may affect the detected signals.

DRFLP analysis of exon 4 also was effective for detecting *PLP* gene duplication, although application of this method is limited to heterozygous mothers. We could confirm that three of four families carried the *PLP* duplications and one did not. Using DRFLP together with CM-PCR, duplication of the *PLP* gene may be rigorously detected in families with PMD. It is of note that Hodes et al. (1993) mentioned a case who was heterozygous at this site; meanwhile, all of our cases were homozygous.

In the clinical investigations, these duplicated cases showed typical manifestations as observed in PMD due to exonic mutations. Thus, overexpression of the *PLP* gene product may affect myelination as severely as exonic mutations, resulting in the same phenotype of a CNS dysmyelinating disorder. Similar finding have been reported in autosomal dominant CMT due to gene duplication or point mutation (Roa et al. 1993*b*). It is interesting to note that autosomal recessive CMT has also been proposed, in a rare case, to be due to an exonic mutation on one chromosome and a deletion on the other chromosome (Roa et al. 1993*a*). This correspondence may be a key point in explaining the pathogenesis of gene duplication–associated neurological disease.

The mechanism by which an increased *PLP* dosage causes disease is unclear. In CMT1A patients who have a duplicated *PMP22* gene, the overexpressed gene products impair peripheral nervous system myelination (Lupski et al. 1992). Transgenic mice overexpressing PLP show both CNS hypomyelination and dysmyelination, features also found in PMD patients, which suggests that not only functional gene defects but also increased amounts of gene products can disrupt CNS myelination. Therefore, overexpression of PLP is the most likely cause of PMD in our four patients.

Although, in our study, all patients' mothers transmitted the duplication to the probands, they are all asymptomatic carriers. This is the most striking difference from CMT1A with dominant inheritance. It is not clear how X inactivation occurs on their duplicated allele and how much the duplicated allele is transcribed. In transgenic mice expressing PLP, the degree of protein overexpression is closely related to the severity of dysmyelination (Kagawa et al. 1994). If there is a threshold level of expression for developing clinical manifestations, and the mothers produce lower levels of PLP than affected patients due to the X inactivation, it is reasonable that they would have no signs of dysmyelination or hypomyelination, despite having an additional *PLP* gene. Other hand, random inactivation may produce mosaicism of expression at the cellular level, and there may be some full overexpressing cells, which may lead to abnormal development in myelination. The answer to this question will require further study.

PLP is known to have two functions in the CNS (Schneider et al. 1992). In early development, before

oligodendrocytes are differentiated from CNS stem cells, the *PLP* gene is transcribed as an alternatively spliced mRNA, encoding a protein termed "DM-20," which is essential for oligodendrocyte differentiation and survival. After CNS development is completed, PLP functions in myelin assembly as a structural transmembrane protein. Thus, *PLP* gene defects impair not only myelin structure but also oligodendrocyte differentiation. It is unlikely that an increased-dose PLP is the only reason for the severe dysmyelination seen in PMD. Developmental defects in DM-20 synthesis may play a major role in the dysmyelination of this disease. Some cases of X-linked spastic paraplegia, with milder clinical features than PMD, have *PLP* gene point mutations in a region that is spliced out in DM-20. This results in normal synthesis of DM20 but abnormal PLP production (Saugier-Verber et al. 1994; Osaka et al. 1995). Therefore, the serious clinical manifestations of PMD may be due to impairment of DM20 production rather than that of PLP. The severe dysmyelination observed in the families analyzed in this study may be attributable mainly to overexpression of DM-20 during development rather than excessive production of PLP.

M6 proteins, which are expressed in neurons, are highly homologous with DM-20 and constitute a gene family (Yan et al. 1993). Although the detailed function of these proteins in CNS development is still unclear, their structural homology with ion channel, nicotinic acetylcholine, and glutamic receptors (Kitagawa et al. 1993) suggests a receptor-like function for this family. Therefore, if DM-20 plays a role in communicating with other cells, such as neurons, via M6 or other developmental genes, the expression dosage of this protein may be crucial to the differentiation and maintenance of oligodendrocytes. Further studies on the function of the DM-20 gene family in CNS development may reveal the pathogenetic role of abnormal *PLP* gene expression in PMD.

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References

Boespflug-Tanguy O, Mimault C, Melki J, Cavagna A, Giraud G, Pham Dinh D, Dastugue B, et al (1994) Genetic homogeneity of Peliazeus-Merzbacher disease: tight linkage to the proteolipoprotein locus in 16 affected families. Am J Hum Genet 55:461–467

Boison D, Stoffel W (1989) Myelin-deficient rat: a point mutation in exon III (A→C, Thr 75→Pro) of myelin proteolipid protein causes dysmyelination and oligodendrocyte death. EMBO J 8:3295–3302

Boulloche J. Aicardi J (1986) Pelizaeus-Merzbacher disease: clinical and nosological study. J Child Neurol 1:233–239

Carango P, Funanage VL, Quiros RE, Debruyn CS, Marks HG (1995) Overexpression of DM20 messenger RNA in two brothers with Pelizaeus-Merzbacher disease. Ann Neurol 38:610–617

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

Doll R, Natowicz MR, Schiffmann R, Smith FI (1992) Molecular diagnostics for myelin proteolipid protein gene mutations in Pelizaeus-Merzbacher disease. Am J Hum Genet 51: 161–169

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

Gencic S, Abuelo D, Ambler M, Hudson LD (1989) Pelizaeus-Merzbacher disease: an X-linked neurologic disorder of myelin metabolism with a novel mutation in the gene encoding proteolipid protein. Am J Hum Genet 45:435–442

Gencic S, Hudson LD (1990) Conservative amino acid substitution in the myelin proteolipid protein of jimpy^{msd} mice. J Neurosci 10:117–124

Hodes ME, Pratt VM, Dlouhy SR (1993) 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 86:8128–8131

Iwaki A, Muramoto T, Iwaki I, Furumi H, deLeon MD, Tateishi J, Fukumaki Y (1993) A missense mutation in the proteolipid protein gene responsible for Pelizaeus-Merzbacher disease in a Japanese family. Hum Mol Genet 2:19–22

Kagawa T, Ikenaka K, Inoue Y, Kuriyama S, Tsujii T, Nakao J, Nakajima K, et al (1994) Glial cell degeneration and hypomyelination caused by overexpression of myelin proteolipid protein gene. Neuron 13:427–442

Kitagawa K, Sinoway MP, Yang C, Gould RM, Colman DR (1993) A proteolipid protein gene family: expression in sharks and rays and possible evolution from an ancestral gene encoding a pore-forming polypeptide. Neuron 11:433–448

Kobayashi H, Hoffman EP (1994) The *rumpshaker* mutation in spastic paraplegia. Nat Genet 7:351–352

Kurosawa K, Iwaki A, Miyake S, Imaizumi K, Kuroki Y, Fukumaki Y (1993) A novel insertional mutation at exon VII of the myelin proteolipid protein gene in Pelizaeus-Merzbacher disease. Hum Mol Genet 2:2187–2189

Lupski JR, Oca RM, Slaugenhaupt S, Pentao L, Guzzetta V, Trask BJ, Saucedo OC (1991) DNA duplication associated with Charcot-Marie-Tooth disease type 1A. Cell 66:219–232

Lupski, JR, Wise CA, Kuwano A, Pentao L, Parke JT, Glaze

DG, Ledbetter DH, et al (1992) Gene dosage is a mechanism for Charcot-Marie-Tooth disease type 1A. Nat Genet 1:29–33

Merzbacher L (1910) Eine eigenartige familiar-hereditäre Erkrankungsform (Aplasia axialis extracorticalis congenita). Z Ges Neurol Psychiatr 3:1–138

Nadon NL, Duncan ID, Hudson LD (1990) A point mutation in the proteolipid protein gene of the shaking pup interrupts oligodendrocyte development. Development 110:529–537

Nave KA, Lai C, Bloom F, Milner RJ (1989) Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in mRNA splicing. Proc Natl Acad Sci USA 83:9264–9268

Nezu A (1995) Neurophysiological study in Pelizaeus-Melzbacher disease. Brain Dev 17:175–181

Osaka H, Kawanishi C, Inoue K, Uesugi H, Kurisaki H, Nishiyama K, Yamada Y, et al (1995) Novel nonsense proteolipid protein gene mutation as a cause of X-linked spastic paraplegia in twin males. Biophys Biochem Res Commun 215:835–841

Pelizaeus F (1885) Über eine eigentümliche Form spastischer Lähmung mit Zerebralerscheinungen auf hereditärer Grundlage (multiple Sklerose). Arch Psychiatr Nervenkr 16: 698–710

Pham Dinh D, Boespflug-Tanguy O, Mimault C, Cavagna A, Giraud G, Leberre G, Lemarec B, et al (1993) Pelizaeus-Merzbacher disease: a frame shift deletion/insertion event in the myelin proteolipid gene. Hum Mol Genet 2:465–467

Pham Dinh D, Popot JL, Boespflug-Tanguy O, Landrieu P, Deleuze JF, Boue J, Jolles P, et al (1991) Pelizaeus-Merzbacher disease: a valine to phenylalanine point mutation in a putative extracellular loop of myelin proteolipid. Proc Natl Acad Sci USA 88:7562–7566

Pratt VM, Kiefer JR, Lähdetie J, Schleutker J, Hodes ME, Dlouhy SR (1993) Linkage of a new mutation in the proteolipid protein (PLP) gene to Pelizaeus-Merzbacher disease (PMD) in a large Finnish kindred. Am J Hum Genet 52: 1053–1056

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

Readhead C, Schneider A, Griffiths I, Nave KA (1994) Premature arrest of myelin formation in transgenic mice with increased proteolipid protein gene dosage. Neuron 12:583–595

Roa BB, Garcia CA, Pentao L, Killian JM, Trask BJ, Suter U, Snipes GJ, et al (1993*a*) Evidence for a recessive PMP22 point mutation in Charcot-Marie-Tooth disease type 1A. Nat Genet 5:189–194

Roa BB, Garcia CA, Suter U, Kulpa DA, Wise CA, Mueller J, Welcher AA, et al (1993*b*) Charcot-Marie-Tooth disease type 1A: association with a spontaneous point mutation in the *PMP22* gene. N Engl J Med 329:96–101

Saugier-Verber P, Munnich A, Bonneau D, Rozet JM, Le Merer M, Gil R, Boespflug-Tanguy O (1994) X-linked spastic paraplegia and Pelizaeus-Merzbacher disease are allelic disorders at the proteolipid protein locus. Nat Genet 6:257–262

Schneider A, Montague P, Griffiths I, Fanarraga M, Kennedy P, Brophy P, Nave KA (1992) Uncoupling of hypomyelination and glial cell death by a mutation in the proteolipid protein gene. Nature 358:758–761

Seitelberger F (1970) Pelizaeus-Merzbacher disease. In: Vinken PJ, Bruyn GW (eds) Handbook of clinical neurology. North Holland, Amsterdam, pp 150–202

Suter U, Welcher AA, Snipes GJ (1993) Progress in the molecular understanding of hereditary peripheral neuropathies reveals new insights into the biology of the peripheral nerve system. Trends Neurosci 16:50–56

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

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

Yan Y, Lagenaur C, Narayanan V (1993) Molecular cloning of M6: identification of a PLP/DM20 gene family. Neuron 11:423–431