The Clinical and Molecular Cytogenetic Analyses of Six Patients with Pelizaeus-Merzbacher Disease From Four Families

Dört Aileden Pelizaeus-Merzbacher Sendromlu Altı Hastanın Klinik ve Moleküler Sitogenetik Analizleri

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ABSTRACT

Objective: Pelizaeus-Merzbacher Disease is a rare X-linked recessive leukodystrophy caused by a mutation in the proteolipid protein (PLP) gene on chromosome Xq22. PMD is an early-onset neurological disorder characterized by nystagmus, spastic quadriplegia, ataxia, and developmental delay. Genetic analysis has identified Xq22 microduplications (60-70%), point mutations (10–25%), and deletions (5-10%) within the coding region of the PLP genes in Pelizaeus-Merzbacher Disease. This study evaluated six patients with *PLP1* deletion and duplication in four Turkish families.

Material and Methods: To detect the duplication and deletion of *PLP1*, chromosomal microarray analysis, and multiplex ligation-related probe amplification assays were performed.

Results: In these four families, two brothers had a hemizygous deletion in the *PLP1* gene, their carrier mother had a deletion in the *PLP1* gene, and another two unrelated boys and one girl had duplication of the *PLP1*. Also, we identified the rare case of two brother patients who were found to have a hemizygous deletion in the *PLP1* gene. Their carrier mother had unexplained dementia.

Conclusion: Genotype-phenotype correlations of the *PLP1* mutation in these families were identified in this study while trying to elucidate the genetic etiology of six individuals from four different families.

Key Words: Dysmyelinating Disorders,Pelizaeus-Merzbacher Disease, *PLP1* Gene

ÖZ

Amaç: Pelizaeus-Merzbacher Hastalığı, Xq22 kromozomu üzerindeki proteolipid protein (PLP) genindeki bir mutasyonun neden olduğu X'e bağlı resesif nadir görülen bir lökodistrofidir. PMD, nistagmus, spastik kuadripleji, ataksi ve gelişimsel gecikme ile karakterize erken başlangıçlı bir nörolojik bozukluktur. Genetik analiz, Pelizaeus-Merzbacher Hastalığında PLP genlerinin kodlama bölgesinde Xq22 mikroduplikasyonlarını (%60-70), nokta mutasyonlarını (%10-25) ve delesyonları (%5-10) tanımlamıştır. Bu çalışma, dört Türk ailede *PLP1* delesyonu ve duplikasyonu olan altı hastayı değerlendirdi.

Gereç ve Yöntemler: *PLP1*'in duplikasyonu ve delesyonunu saptamak için kromozomal mikroarray analizi ve multipleks ligasyona bağlı prob amplifikasyon deneyleri yapıldı.

Bulgular: Bu dört ailede, iki erkek kardeşte *PLP1* geninde hemizigot delesyonu, taşıyıcı annelerinde *PLP1* geninde delesyon ve akraba olmayan diğer iki erkek ve bir kızda *PLP1* duplikasyonu vardı. Ayrıca, *PLP1* geninde hemizigot delesyona sahip olduğu tespit edilen iki erkek kardeş hastanın nadir vakasını belirledik. Taşıyıcı annelerinde açıklanamayan bunama vardı.

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Ethics Committee Approval / *Etik Kurul Onayı*: This study was conducted in accordance with the Helsinki Declaration Principles. The study was approved by Kocaeli Derince Training and Research Hospital, Clinical Research Ethics Committee (Document Number: 2020-121/10.09.2020).

Contribution of the Authors / Yazarların katkısı*: AKKUŞ N:** Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results, Taking responsibility in necessary literature review for the study, Taking responsibility in the writing of the whole or important parts of the study, Reviewing the article before submission scientifically besides spelling and grammar. ***ÖZYAVUZ ÇUBUK P: Constructing the hypothesis or idea of research and/or article, Planning methodology to reach the Conclusions, Organizing, supervising the course of progress and taking the responsibility of the research/study, Taking responsibility in patient follow-up, collection of relevant biological materials, data management and reporting, execution of the experiments, Taking responsibility in logical interpretation and conclusion of the results.

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Sonuç: Bu çalışmada, dört farklı aileden altı bireyin genetik etiyolojisi aydınlatılmaya çalışılırken, bu ailelerdeki *PLP1* mutasyonunun genotip-fenotip korelasyonları belirlendi.

Anahtar Sözcükler: Dismiyelinizan Hastalıklar, Pelizaeus-Merzbacher Hastalığı, *PLP1* Geni

INTRODUCTION

Pelizaeus-Merzbacher's disease (PMD, MIM 312080) is a rare disease due to X-linked recessive features and mutations in the *PLP1* gene on the Xq22 chromosome. It causes dysmyelination by affecting the Central Nervous System (CNS) (1). Identified as the chronic form of pediatric leukoencephalopathy, PMD is a failure of myelin metabolism and axonal myelination in oligodendrocytes (2). Several studies have reported point mutation, duplications, insertions, and deletions in the genetic material of patients with PMD. Approximately 60-70% of PMD duplication involving *PLP1* has been reported as the most common mutations in this disease (3). Harmful mutations are rare in this disease, and point mutations like splicing, missense, and nonsense have been detected in only 10-25% of patients. *PLP1* gene duplication is the most common reason for the impaired myelin construction of the CNS by producing a structural protein (4-6). *PLP1* is formed of seven exons encoding a major myelin protein in the CNS myelin. *PLP1* gene encodes two proteins *PLP1* and its isoform DM20. Both proteins are much more expressed by oligodendrocytes (2,4).

Delayed motor functions, with muscular hypotonia and nystagmus, are disorders often seen in PMD patients. Cognitive defects are determined in patients with PMD. Speech-related language development is affected, and most patients may receive language training if they have delays or significant language problems (7,8). Magnetic resonance imaging (MRI) of patients with PMD reveals a diffuse pattern of the CNS, including cerebral hemispheres, cerebellum, and brainstem(9). In this study, we performed chromosomal microarray analysis (CMA) and multiplex ligation-related probe amplification (MLPA) to examine a cohort of 6 patients with PMD and elucidate the relationship between their genotypes and phenotypes.

Clinical and genetic features of patients for the definitive diagnosis of this rare hereditary PMD disease that can contribute to genetic counseling and prenatal diagnosis in Türkiye were analyzed. We further delineate and expand the PLP-related genotype-phenotype correlations and phenotypic spectrum.

MATERIAL and METHODS

No pathological finding was determined in karyotype for all patients included in the study. Blood samples were obtained from the patients and parents, and genomic DNA was isolated from peripheral blood using the salting-out method.

PLP1 gene MLPA or chromosomal microarray analysis was performed. Furthermore, mothers were examined for genetic carriers of the *PLP1* gene.

Affymetryc Cytoscan Optima (312K) array was performed on patients 1, 4, and 6.

Multiplex Ligation-dependent Probe Amplification(MLPA) using the Pelizaeus Merzbacher Disease region of chromosome X confirmed the deletions and duplications within the *PLP1* gene for all patients.

The study was approved by Kocaeli Derince Training and Research Hospital, Clinical Research Ethics Committee (Document Number: 2020-121/10.09.2020).

MLPA analysis

The SALSA MLPA Probemix P022 *PLP1* kit (MRC Holland, Amsterdam, The Netherlands) was used to detect deletions or duplications in the *PLP1* gene and Xq22 region. MLPA was performed according to the manufacturer's recommendations. The SALSA MLPA Probemix P022-B2 *PLP1* contains 37 MLPA probes, seven for the *PLP1* gene, 20 for the Xq22.2 region, and ten reference probes. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com). MLPA data were analyzed with the Coffalyser software.

Chromosomal Microarray

All microarray procedures were performed using CytoScan Optima Array Kit (Thermo Fisher Scientific, MA, United States). Microarray data were analyzed with Chromosome Analysis Suite (ChAS) 4.3 from Affymetrix, using GRCh37/hg19 libraries.

Interpretation of copy number variants (CNVs)

Variants were classified as variants of unknown significance according to the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen) recommendation. CNVs were compared to variants reported in the Database of Genomic Variants (DGV, http://projects.tcag.ca/variation), Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER, https://decipher.sanger.ac.uk), ClinVar and the in-house database at the Department of Medical Genetics, Haseki Education and Research Hospital.

RESULTS

Diagnosis of PMD was determined in 6 patients from 4 families. All individuals had nonconsanguineous parents.

PLP1 gene duplications were identified in 3 patients (patients 4,5 and 6), and *PLP1* gene deletions were observed in 3 patients (family 1).

In the first family, two affected siblings with *PLP1* hemizygous deletion, and their mother had a deletion of the *PLP1* gene of exons 2 through 8 (Figure 1). Patient 1, a two years old boy with

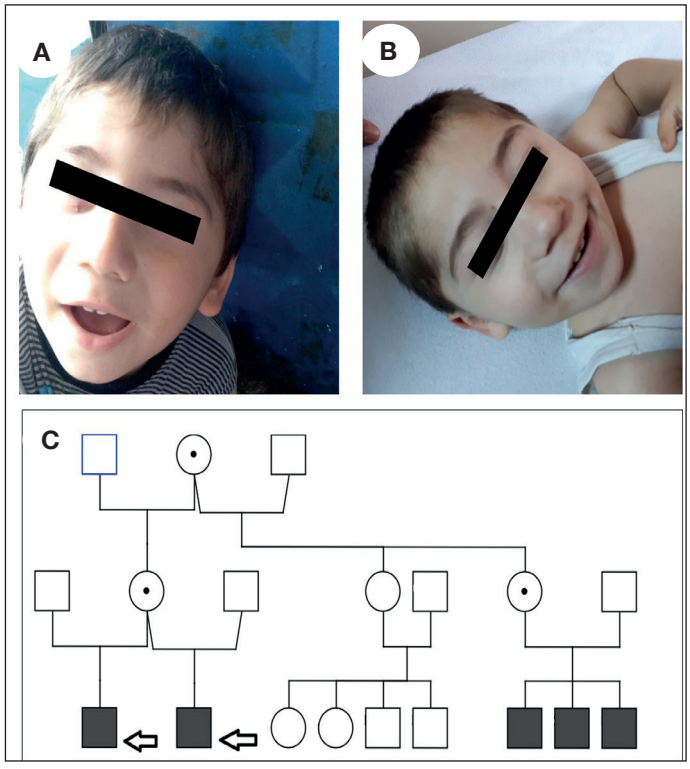

Figure 1: Photographs of patients and pedigree of the first family **a.** Photograph of the patient **b.** Photograph of the patient **c.** Pedigree of the first family.

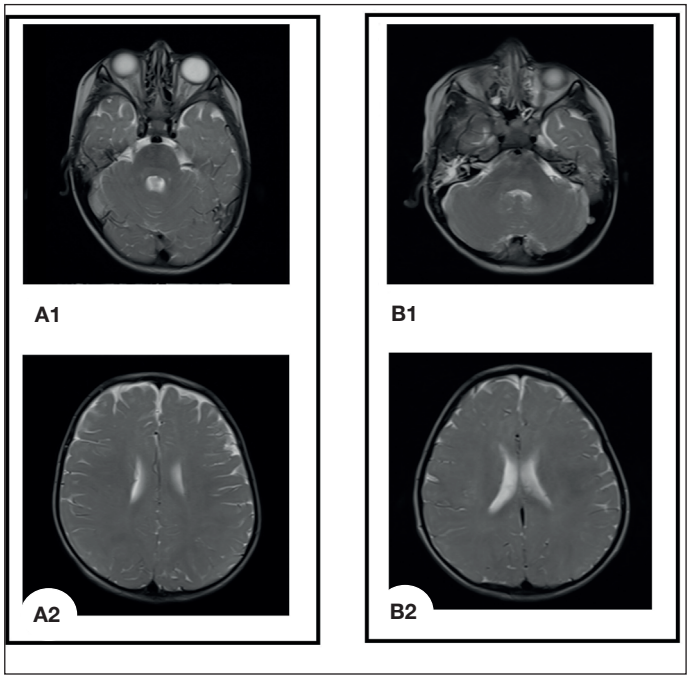

Figure 2: a.1 and b.1) Hyperintense, consistent with hypomyelination in the dorsal brain stem in axial T2W sequence

a.2 and b.2) Hyperintensity consistent with diffuse hypomyelination in the white matter in the centrum semiovale plane in the axial T2W image

hypotonia, developmental delay, and nystagmus, was referred for evaluation. The index patient was the first child of the mother. He was born at term by cesarean section, and his birth weight was 3400 gr. Height and occipitofrontal circumference

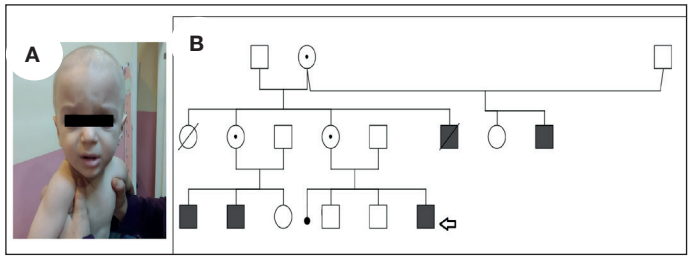

Figure 3: Photograph of the patient and pedigree of the second family **a)** Photograph of the patient **b)** Pedigree of the second family.

(OFC) was not recorded. Child-related measurements were as follows: weight was 10 kg (<3 percentile), and length was 80 cm (<3 percentile). He was unable to walk or talk and lacks head control.

Patient 2, the big brother of our index patient, was a four-year old boy with a severe developmental delay. He was unable to sit, walk or talk. His weight was 12 kg (10 percentile), his length is 85 cm (<3 percentile), and his OFC is 46 cm(<3 percentile) (Figure 1).

Consequently, they showed severe psychomotor developmental delay and hypotonia.

Patient 3 is the mother of patients 1 and 2. The mother, who was 30 years old, had mild intellectual disability. Brain MRI could not be carried out. Physical examination was normal, and no nystagmus (Figure 2).

In the second family, patient 4 demonstrated a duplication of the *PLP1* gene of exons 2 through 8. The mother of patient 4 had carriers of *PLP1* duplication and had a resting tremor (Figure 3). Patient 4, a one-year-old boy, was evaluated for nystagmus, vomiting, and severe developmental delay. He was born as the third child of nonconsanguineous parents at the 38th gestational week at a birth weight of 3200 gr. Immediately after birth, because of Meconium Aspiration Syndrome (MAS), he was referred to the neonatal intensive care unit. Birth height and OFC were not recorded. At the time of examination, his weight was 6700 gr (<3p), his body length was 72 cm (3-10p), and OFC was 46.5 cm (25-50p). The patient had hypotonia, and at 8 months of age, he couldn't manage head control. Brain MRI findings were normal.

In the third family, duplication of the *PLP1* gene was detected by MLPA analysis of patient 5. He weighted 8.5 kg at 25 months of age (<3p) and was 80 cm (<3p) in height, and head circumference was not recorded. Physical examination revealed spastic quadriplegia, bilateral nystagmus, and cachectic findings, and the patient was unable to talk, walk or even hold his neck. Hypotonia and severe developmental delay were observed. Brain MRI could not be carried out. The patient's older brother had *PLP1* duplication. Additionally, his mother was heterozygous for the same duplication and showed no PMD symptoms.

The patient's uncle and older brother were reported to have the same clinical findings. However, we could not carry out their examination and neuroimaging (Figure 4).

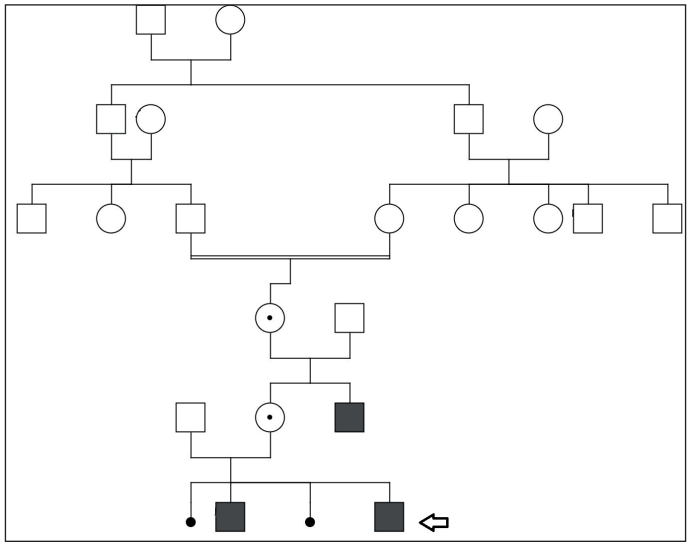

Figure 4: Pedigree of the third family.

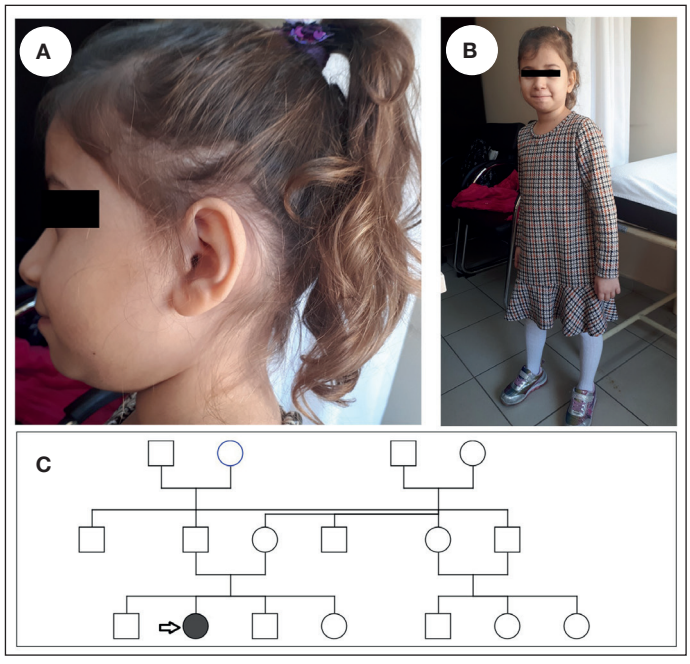

Figure 5: Photograph of the patient and pedigree of the fourth family **a and b)** Photograph of the patient **c)** Pedigree of the fourth family.

In the fourth family, patient 6 showed duplication of the *PLP1* gene of exons 2 through 8. Her mother was negative for the PLP duplication by MLPA analyses, suggesting that the duplication occurred as a de novo event (Figure 5). However, all other patients' mothers were carriers (Table I).

We detected *PLP1* gene deletions in half of the patients. The other half of the patients had *PLP1* gene duplication. Additionally, while 83% of the patients had a maternal inheritance, one patient had de novo duplication.

The most common finding determined in all patients was intellectual disability or global developmental retardation and developmental delay. While male patients had severe mental motor retardation and developmental delay, female patients had mild intellectual disability or developmental delay (Table II).

The female patients mentioned in the article had learning difficulties and comprehension difficulties. Patient 6 has had fine motor development disorders and did not learn to read and write. Along with these findings, patient 3 had dementia findings. However, in other male patients, hypotonia and severe developmental delay were observed.

DISCUSSION

We here reported two brothers with a hemizygous deletion in the *PLP1* gene, their carrier mother with a deletion in the *PLP1* gene, and another two boys and one girl with a duplication of the *PLP1* from four families in total. The clinical symptoms of the four male patients in this study included a lack of stable head control and severe mental motor retardation, and the other two female patients had mild mental retardation. Male patients of the *PLP1* with deletions and duplications had more severe mental motor retardation than female patients. Consistent with the literature, we did not find any significant difference in clinical signs between *PLP1* with deletions and duplications of the male patients.

An uncommon X-linked recessive central nervous system disease with neonatal neurological deficits, including hypomyelination features, is called PMD (MIM 312080). Significant pendular nystagmus, tremors, spasticity, and generalized hypotonia, which develop into a motor developmental delay in early infancy, are the clinical manifestations of this syndrome (10). Developmental and psychomotor delay, ataxia, microcephaly, hearing disorders, the rotary motion of the head, dysmyelination of the CNS, and spasticity are the common features in patients with PMD (11,12). We described six patients with PMD from four different families. The male patients of *PLP1* deletion were severe intellectual disability or global developmental delays and lacked head control. These patients had severe developmental delay, generalized hypotonia, spasticity, bilateral nystagmus, dysarthria, dysphagia, spastic quadriplegia, and were cachectic.

Mutations in the *PLP1* (proteolipid protein 1) gene encoding the isoform DM20, which is attached to the proteolipid protein and oligodendrocytes, the two major myelin proteins in the CNS, are the main cause of the PMD disease that belongs to the series of HLDs (hypomyelination leukodystrophy) (13).

The process of abnormal CNS myelination occurs due to point mutations and proliferation in the *PLP1* gene, causing Pelizaeus-Merzbacher's disease (PMD; MIM 312080). It can progress to spastic paraplegia (SPG2; MIM 312920) which is a type of X-linked HLD. *PLP1* deletions are much less common than duplications. Consistent with the literature, a complete genotype-phenotype correlation cannot be established in our patients either.

Table I: Genomic finding in six patients with Pelizaeus Merzbacher Disease.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
MLPA	*PLP1* exon2-8 hemizygous deletion	*PLP1* exon2-8 hemizygous deletion	*PLP1* exon2-8 heterozygous deletion	*PLP1* exon2-8 hemizygous duplication	*PLP1* exon2-8 hemizygous duplication	*PLP1* exon2-8 duplication
Microarray analysis	arr[hg19] Xq22.2(102995019-103162012)x0	NA	NA	arr[hg19] Xq22.2(102643610-103305273) x2	NA	arr[hg19] Xq22.1q23(100,213,231-109,412,333)x3
Deletion/ duplication	167 kb deletion	NA	NA	662 kb duplication	NA	9,199 kb duplication
Origin	Maternal	Maternal	Maternal	Maternal	Maternal	De novo

Table II: Clinical findings in six patients with Pelizaeus Merzbacher Disease.

Family Patient	Family 1			Family 2	Family 3	Family 4
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age at examination/ Gender	2y/M	4y/M	30y/F	1y/M	2y/M	11y/F
Consanguinity	-	-	-	-	-	-
Psychomotor development						
Develepmontel delay	Severe develepmontel delay	Severe develepmontel delay	NA	+	Severe develepmontel delay	Mild mental retardation
Mental motor retardation	Severe mental motor retardation	Severe mental motor retardation	Mild mental retardation	Severe mental motor retardation	Severe mental motor retardation	Mild mental retardation
Head control, Sitting and Walking	-	-	+	-	-	+
Growth retardation	+	+	-	-	+	+
Hypotonia/ Bedridden	+	+	-	+	+	-
Seizures	-	-	-	-	-	-
Nystagmus	+	+	-	+	+	-
Other symptoms	Spastic quadriplegia, and cachectic	Spastic quadriplegia, and cachectic	Dementia	Meconium Aspiration Syndrome (MAS)	Spastic quadriplegia, and cachectic	Frequent falls
MRI findings	Diffuse hypomyelination	Diffuse hypomyelination	NA	Normal	NA	At one year of age, thin corpus callosum. 10 years were normal
Clinical findings in the mother	Dementia	Dementia	Dementia	-	-	-

The *PLP1*-null syndrome is a relatively mild neurology syndrome that is also graduated as a mild form of PMD, caused by other *PLP1* null mutations and complete deletion of *PLP1* (4,11,15-17).

HLDs, which are PLP-related disorders, can affect males, while the phenotypes may cause diseases ranging from mild hereditary spastic paraplegia to severe forms of type 2 PMD (SPG2) (15). *PLP1* missense mutations constituting the most severe form of PMD (connatal form) are *PLP1*-related disorders, and the most common types of PMD duplications are SPG2 and classical PMD (1,14).

Considering the affected siblings, it was apparent that the absence of hyperreflexia and subtle eye-movement abnormalities of the surrogate carrier mother indicates a familial form of PMD. However, progressive leukodystrophy with dementia may develop in the later life of carrier females with point mutation or deletion that may cause late-onset spastic paraplegia phenotype of variable severity. The mother of our patients 1 and 2 had unexplained dementia, and she was the carrier of *PLP1* deletion. Patient 6 had *PLP1* duplication. She exhibited a delay in all motor developmental milestones and had a history of frequent falls and an awkward gait. She had been going to primary school with personal assistance help. There was no nystagmus and no history of seizures. However, their short and long-term memory was impaired.

Sixty to seventy percent of PMD patients have complete replication of the *PLP1* gene on Xq22. PLP duplications prevent regular myelination resulting in an increased dose of *PLP1*. An increased dose of *PLP1* is related to the classic form of the disease, but patients may have phenotypes ranging from severe connatal to mild PMD. The disease is usually asymptomatic even if *PLP1* duplication exists in carrier females (18). Similarly, the mother of patient 5 had *PLP1* duplication and was asymptomatic. On the other hand, patient 3's carrier mother who had a deletion in the *PLP1* had unexplained dementia. In this case, genetic tests play an important role in the diagnosis of the disease. Also, patient 6 had duplication in the *PLP1* with mild mental motor retardation, developmental delay, and frequent fall.

CMA is the first-line test for individuals with developmental delays (19-21). Microarray analysis, the increased detection rate of chromosomal imbalances in the human genome, has allowed the diagnosis of syndromic phenotypes with previously unknown etiologies. CMA detects microdeletion and microduplication syndrome in this group with a diagnostic yield (22). Microarray-based screening analysis results of patients with undiagnosed neurologic disease revealed the potential use of this method in providing a diagnosis for these patients.

CONCLUSION

Although PMD is a neurological disorder, it has no specific pathognomonic clinical features. In these cases, the importance of genetic evaluation to achieve a final diagnosis is emphasized since there are no specific clinical findings. Diagnosis and recognition of these neurological diseases are essential for appropriate genetic counseling and disease prognosis. Further research is needed to explain the pathophysiological mechanism of PMD. In this way, treatment methods can be developed.

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