PLP1 gene duplication as a cause of the classic form of Pelizaeus-Merzbacher disease – case report

Duplikacja genu PLP1 *jako przyczyna klasycznej postaci choroby Pelizaeusa-Merzbachera – opis przypadku*

Jacek Mądry[1], Dorota Hoffman-Zacharska[2], Leszek Królicki[3], Maciej Jakuciński[3], Andrzej Friedman[1]

[1]Department of Neurology, Bródno Voivodship Hospital, Medical University of Warsaw
[2]Department of Medical Genetics, Institute of Mother and Child, Warsaw
[3]Department of Nuclear Medicine and Magnetic Resonance, Bródno Voivodship Hospital, Warsaw

Neurologia i Neurochirurgia Polska 2010; 44, 5: 511–515

Abstract

Pelizaeus-Merzbacher disease (PMD) is a rare X-linked dysmyelination disorder of the central nervous system (CNS). PMD is caused by mutations in the *PLP1* gene located at Xq22 and encoding the major myelin component in CNS, proteolipid protein 1 (PLP1). The disease is clinically heterogeneous. Phenotypes are generally categorized into classic and connatal forms. Connatal PMD has more rapid progression with early death, while patients with classic PMD generally survive to adulthood. Both forms of the disease are caused by point mutations as well as rearrangements – multiplication (mainly duplication) and deletion of the *PLP1* gene.

We present a case of a male patient affected by the classic form of PMD with benign course, except severe dysarthria with the characteristic laryngeal stridor, which is more typical for connatal form of the disease. The diagnosis has been confirmed at the molecular level. The patient has duplication of all 7 exons of the *PLP1* gene. This duplication was inherited from the patient's mother, who is an unaffected carrier of the mutation. The patient's family pedigree analysis revealed the

Streszczenie

Choroba Pelizaeusa-Merzbachera (*Pelizaeus-Merzbacher disease* – PMD) to rzadka, sprzężona z chromosomem X choroba dysmielinizacyjna ośrodkowego układu nerwowego (OUN). Spowodowana jest mutacjami znajdującego się w locus Xq22 genu *PLP1*, kodującego główny składnik otoczki mielinowej OUN, białko proteolipidowe 1 (PLP1). Charakteryzuje się heterogennością obrazu klinicznego. Wyróżniamy dwie jej postacie – wrodzoną i klasyczną. W obu formach pierwsze objawy występują już we wczesnym dzieciństwie. W postaci wrodzonej progresja choroby jest jednak zdecydowanie szybsza, w postaci klasycznej pacjenci osiągają zazwyczaj średni wiek życia. Podłoże molekularne obu postaci choroby stanowią zarówno mutacje punktowe, jak i rearanżacje genu – multiplikacje (głównie duplikacje) oraz delecje genu *PLP1*.

W pracy przedstawiono opis przypadku mężczyzny z klasyczną postacią PMD o łagodnym przebiegu, u którego występowały objawy charakterystyczne dla postaci wrodzonej – nasilone zaburzenia artykulacji mowy o typie stridoru krtaniowego. Rozpoznanie choroby zostało potwierdzone

Correspondence address: Andrzej Friedman, Klinika Neurologii Wojewódzkiego Szpitala Bródnowskiego, ul. Kondratowicza 8, 03-242 Warszawa, tel. +48 22 326 58 15, fax +48 22 326 58 15, e-mail: friedman@amwaw.edu.pl
Received: 18.01.2010; accepted: 25.06.2010

interfamilial variability of the phenotype among affected male relatives.

Key words: Pelizaeus-Merzbacher disease, dysmyelination disorder, leukodystrophy, *PLP1*, gene duplication, MLPA.

badaniami genetycznymi. U pacjenta stwierdzono duplikację wszystkich eksonów genu *PLP1*. Mutacja została odziedziczona od matki, bezobjawowej nosicielki. Analiza rodowodowa wykazuje u chorego rodzinną postać choroby o zmiennym obrazie fenotypowym u spokrewnionych chorych płci męskiej.

Słowa kluczowe: choroba Pelizaeusa-Merzbachera, choroba dysmielinizacyjna, leukodystrofia, *PLP1*, duplikacja genu, MLPA.

Introduction

Pelizaeus-Merzbacher disease (PMD; OMIM 312080) is a rare X-linked recessive leukodystrophy [1]. It is an allelic form of spastic paraplegia type 2 (SPG2; OMIM 312920) [1]. Both disorders are caused by mutations of the *PLP1* gene (OMIM 300401), located at locus Xq22 and coding proteolipid protein 1 (PLP1) – the main component of myelin in the central nervous system (CNS) [1,2]. *PLP1*-related disorders represent a spectrum of the CNS white matter diseases caused by the gene dosage effect and point mutations of the gene. Increased dosage of the PLP1 protein is the major cause of PMD. About 60-70% of patients have a submicroscopic duplication on chromosome X, including the entire *PLP1* gene. In rare cases, patients having three or even five *PLP1* copies have been described [2,3]. Point mutations in the *PLP1* gene are found in 15-20% of cases [2,3]. A few known patients have deletion or null mutation of the *PLP1* gene which result in protein loss [4]. As an X-linked recessive disorder, PMD affects hemizygous males, while female carriers are generally asymptomatic. They can show, however, some neurological symptoms, especially if they carry *PLP1* point mutations [5].

PLP1-related phenotypes show considerable variation, but the common features include nystagmus, stridor, ataxia, psychomotor developmental delay and spasticity. The onset of disease is usually in the first year of life. Disease severity ranges from the most severe, connatal PMD through the classic form to mild PMD and SPG2. The molecular background of such phenotypic variability is not completely understood but there is some general association between the disease severity and the type of causative mutation [6]. Connatal form is mainly associated with missense mutations in the most conserved *PLP1* regions. Gene duplications are most often found in patients with classic form of the disease. The less severe forms of the disease result from loss of the *PLP1* gene products [4]. Intrafamilial variability in the disease course suggests the influence of additional modifier genes on the final phenotype [7].

PMD is divided into classic and connatal types. Although these two types differ in severity, their features can overlap. The connatal form is the most severe form of the disease. It appears from birth and involves delayed mental and physical development and severe neurological symptoms. Affected boys usually die at an early age due to quick progression of the illness. In rare cases they can live until the third decade of life but are completely speechless [2,8]. The classic form has slower progress of symptoms including muscle weakness, nystagmus and delayed motor development in the first year of life followed by ataxia, movement disorders of the trunk and upper limbs characteristic for dystonia, and sometimes athetosis and choreatic movements. Pyramidal symptoms with spastic paraparesis appear and develop slowly. Boys are able to articulate single words and most of them start to walk independently. In adolescence, a regression in patients' development and walking skills can be observed. They often achieve an average level of intellectual development. Patients suffering from classic PMD can usually survive to the third, fourth or even seventh decade of life [2].

In this report we present a case of a patient with classical form of PMD, confirmed by genetic analysis and caused by the duplication of all exons of the *PLP1* gene, inherited from his mother, an asymptomatic carrier of this mutation on one X chromosome.

Case report

A 20-year-old man was admitted to the Department of Neurology in Bródno Hospital. According to his mother, the first symptoms of his illness appeared just

before his first birthday. They included head tremor at rest, nystagmus and truncal ataxia, which at first made him unable to sit, then to stand unsupported. The symptoms were followed by ataxia of limbs, and speech articulation disorder. At that time, the diagnosis of cerebral palsy was made. When he was 10 years old, weakness of the lower limbs was noticed and head tremor at rest became more intensive. Despite these symptoms the patient was able to walk with help.

Magnetic resonance imaging (MRI) at the age of 13 showed incomplete white matter myelination in the brain with a band-like, tiger-striped image of white matter with areas of highly myelinated white matter located around blood vessels surrounded by spheres of lower myelination. Magnetic resonance spectroscopy (MRS) revealed decreased choline/creatine ratio (with a stable level of creatine) and reduced NAA/tCr in the regions of dysmyelination. Visual evoked potentials showed abnormally delayed latency of the P_{max}. Other tests revealed normal cerebrospinal fluid, lack of oligoclonal protein and protein IgG antibodies and normal level of copper, ceruloplasmin, protein, albumin, immunoglobulin and very long chain fatty acids (VLCFA) measured in serum. Peripheral motor and sensory nerves' conduction velocity was normal.

Neurological examination at the present admission demonstrated mild nystagmus with the fast phase towards the right, laryngeal stridor, muscle weakness and decreased muscle tone in upper limbs, hyperextension of elbow joints, kinetic tremor of head and upper limbs, truncal and upper limb ataxia, dystonia of neck, trunk and upper limbs, spastic paraparesis of lower limbs with joint contractures of ankles and knees, increased deep tendon reflexes, bilateral Babinski sign and foot clonus. Symptoms of dystonia had typical features of action dystonia and were absent in the lying position. Brain MRI revealed that the myelination process had stopped at the child level (Fig. 1).

Psychological examination confirmed average level of intellectual development – 99 points according to WAIS-R.

As the clinical picture and the family data showing X-linked inheritance of the disorder (Fig. 2) suggested the diagnosis of PMD, molecular analysis of the *PLP1* gene was performed. *PLP1* copy number was determined by multiple ligation-dependent probe amplification (MLPA) reaction using the commercially available test reagent set SALSA P022 (MRC-Holland) containing probes covering all *PLP1* exons as well as control probes for autosomal, chromosome X and Y regions (www.mrc-holland.com). The Ethical Committee of the Institute of Mother and Child approved the genetic study. The DNA test was performed for the patient and his mother, from whom informed consent was obtained. In the case of the patient, analysis revealed whole gene duplication (Fig. 3). The patient's mother, who according to pedigree data was suspected of being a mutation carrier, was confirmed as an asymptomatic *PLP1* gene duplication carrier.

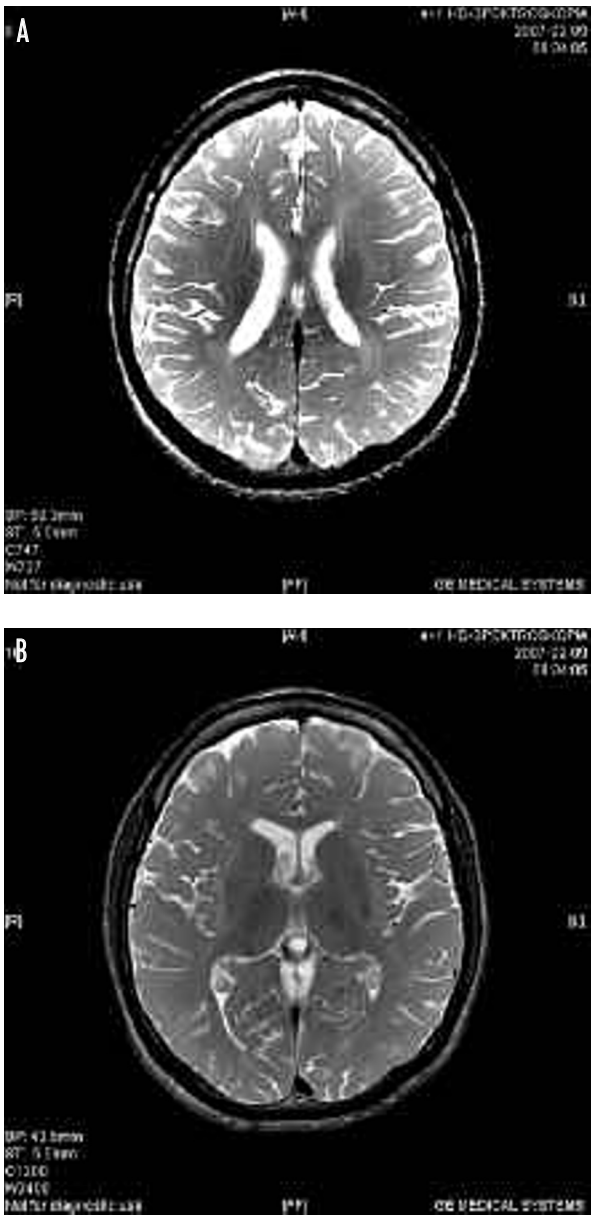

Fig. 1. Magnetic resonance images of the proband showing a typical picture of leukodystrophy: hyperintense areas located in subcortical white matter of frontal lobes (A) and band-like hyperintensity in subcortical white matter in the region of both insulas (B)

Discussion

PMD is a rare disorder of unknown prevalence in Poland. Data for the United States population indicate a prevalence of 1 per 200 000-500 000 [2]. PMD accounts for 6.5% of all leukodystrophies in Germany [9]. Most PMD patients have mutations in the *PLP1* gene, but there is a group of 5-10% of patients with no detectable defect in this gene. They may have mutations in *PLP1* remote from routinely examined regions (deep in introns or the promoter region) or develop Pelizaeus-Merzbacher-like disease 1 (PMLD1, OMIM 608804), caused by mutations affecting the *GJA12* gene (OMIM 608803). The disorder is virtually identical to PMD but of a different type of inheritance – autosomal recessive. The variability of the clinical picture of the disease may cause additional problems with correct diagnosis. Individuals with PMD/SP2 are often initially diagnosed with cerebral palsy or static encephalopathy. All these diagnostic problems may cause that PMD may be more prevalent than is recognized.

Analysis of the patient's family pedigree suggested the familial form of the disease with the patient's mother being an obligatory carrier of the mutation. PMD diagnosis was confirmed at the molecular level. The patient has duplication of all exons of the *PLP1* gene and his mother is a mutation carrier. Three male members of the family also suffered from the disease. Based on available data, we can classify their illness as connatal and classic form of PMD. Our patient probably has the mildest form of the disease, but because of the lack of full clinical data from other affected members of the family, a detailed comparison is not possible.

From the clinical point of view, our patient showed the typical classic form of PMD, with slow progression and average intellectual development. The only deviation from the classic form was the progressive dysarthria with laryngeal stridor, which allowed him to speak using only simple words, slowly and with much effort. This severe disorder of speech articulation is more characteristic for the connatal form than for the mild form of PMD [2,8].

Analysis of the patient's history shows that in the case of such a rare disease as PMD, additionally characterized by a heterogeneous clinical picture, genetic molecular tests are necessary to establish the final diagnosis. Confirmation of presence of the causative mutation is also very important for the whole family, making it possi-

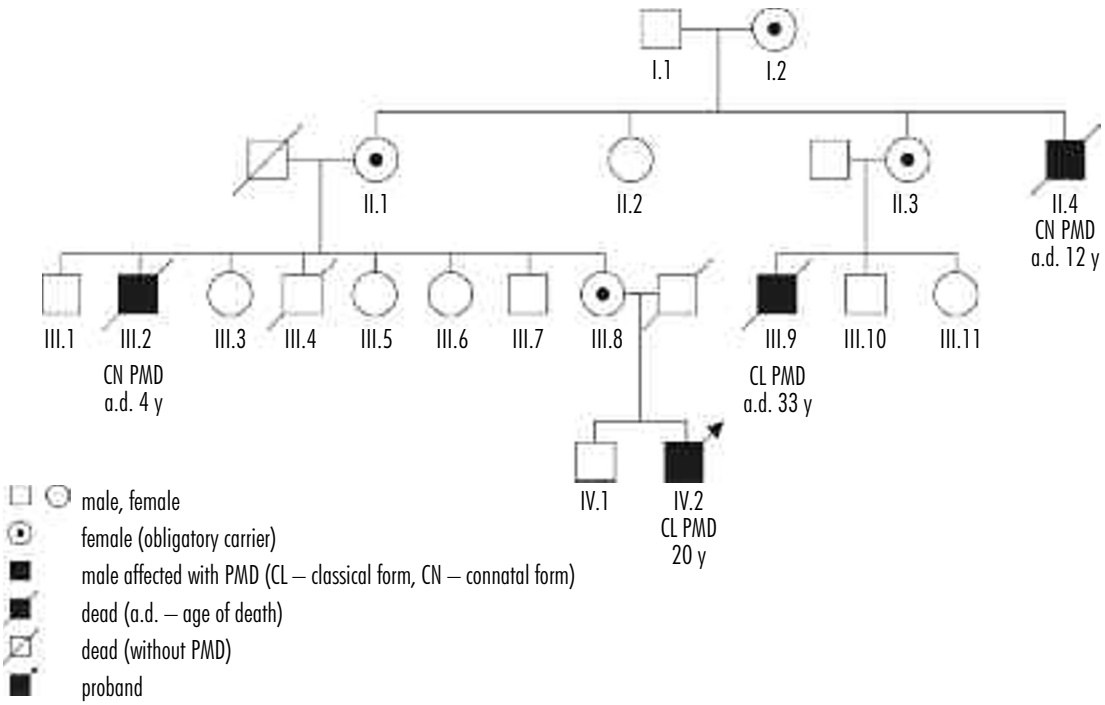

Fig. 2. The pedigree of the proband's family (CP, IV-2). Pelizaeus-Merzbacher disease is presented in the family in three generations showing the intrafamilial clinical picture variability (CL PMD III-9, IV-2; CN PMD II-4, III-2). Molecular diagnosis was performed only for the proband and his mother.

Other females who are obligatory carriers are marked on the basis of pedigree analysis

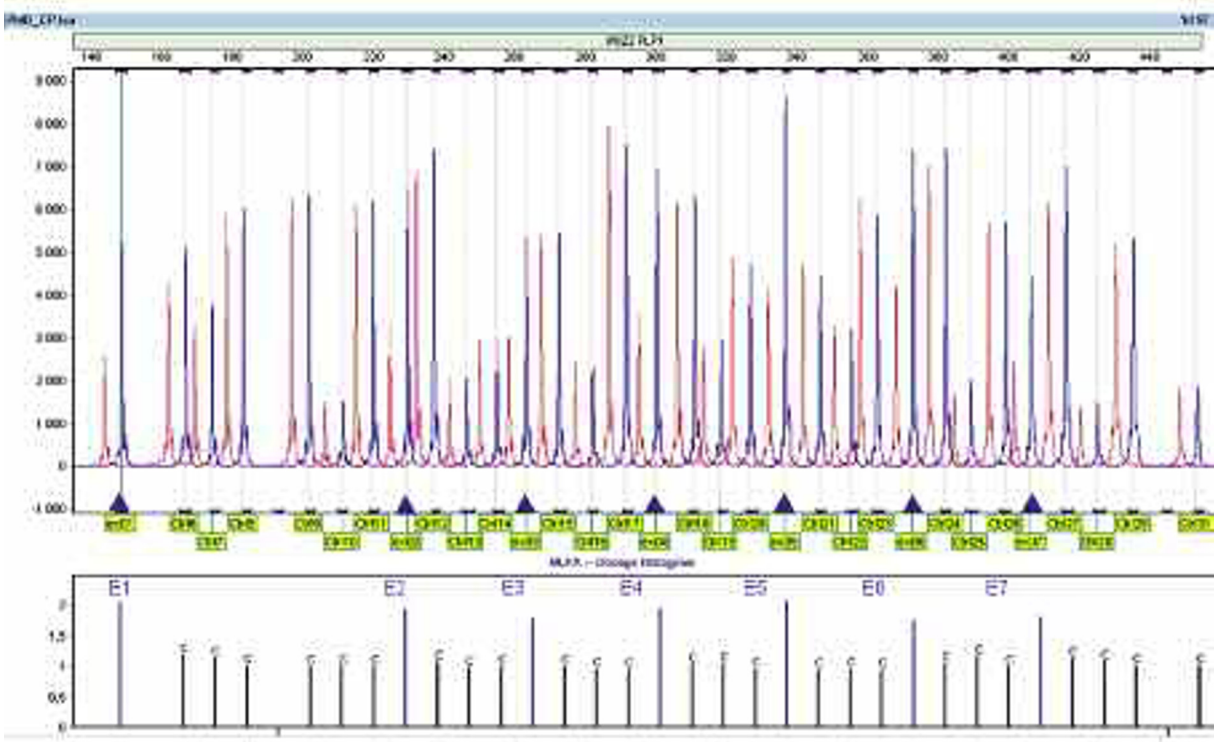

Fig. 3. Graphical results of detection of the *PLP1* exons 1-7 duplication for our patient. Samples containing 100 ng of DNA were analysed by using MLPA P022 probe mix. (A) Peak profile of the MLPA analysis for proband C.P. (black) compared to control profile (grey). The difference in *PLP1* exons 1-7 peak heights (indicated by arrows) indicates the copy number change – duplication in proband's gene. Data are summarized in dosage histogram (Ex1-7 – exon probes, C – control probes).

(B) Normalization MLPA ratio plot for proband PMD-CP and analysed controls PMD-K+M (non-PMD male), PMD-K+F (female), PMD-K-, no DNA control. Analysis was performed by using the population normalization method. Exonic duplication is apparent from the presence of the outlying data points for *PLP1* probes 'above' the threshold line (> 1.25) on the proband's plot. On the female control plot the *PLP1* and chromosome X derived probes are also located in this area

ble to test the potential carriers of the gene, and provide the proper genetic counselling.

Acknowledgements

This work was supported by grant No. N N401 130436 from the Polish Ministry of Science and Higher Education.

Disclosure

Authors report no conflict of interest.

References

1. OMIM; Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD), available at: http://www.ncbi.nlm.nih.gov/omim/ (accessed October 2009).
2. Garbern J.Y., Krajewski K., Hobson G. PLP1-related disorders. GeneReviews 2006; available at: http://www.genereviews.org (accessed October 2009).
3. Wolf N.I., Sisterman E.A., Cundall M., et al. Three or more copies of the proteolipid protein gene PLP1 cause severe Pelizaeus-Merzbacher disease. *Brain* 2005; 128: 743-751.
4. Garbern J.Y. Pelizaeus-Merzbacher disease: genetic and cellular pathogenesis. *Cell Mol Life Sci* 2007; 264: 50-65.
5. Woodward K., Kirtland K., Dlouhy S., et al. X inactivation phenotype in carriers of Pelizaeus-Merzbacher disease: skewed in carriers of duplication and random in carriers of point mutations. *Eur Jour of Hum Genet* 2000; 8: 449-454.
6. Cailloux F., Gauthier-Barichard F., Mimault C., et al. Genotype-phenotype correlation in inherited brain myelination brain myelination defects due to proteolipid protein gene mutations. *Eur J Hum Genet* 2000; 8: 837-845.
7. Fattal-Valevski A., DiMaio M.S., Hisam F.M., et al. Variable expression of a novel PLP1 mutation in members of a family with Pelizaeus-Merzbacher disease. *J Child Neurol* 2009; 24: 618-624.
8. Hanefeld F.A., Brockman K., Pouwels P.J. Quantitative proton MRS of Pelizaeus-Merzbacher disease. Evidence of dys- and hypomyelination. *Neurology* 2005; 65: 701-706.
9. Heim P., Claussen M., Hoffman B., et al. Leukodystrophy incidence in Germany. *Am J Med Genet* 1997; 71: 475-478.