

PRACE ORYGINALNE/ORIGINAL ARTICLES

Dorota Hoffman-Zacharska[1,2], Hanna Mierzewska[3], Elżbieta Szczepanik[3], Jarosław Poznański[4], Tomasz Mazurczak[3], Anna Jakubiuk-Tomaszuk[5], Jacek Mądry[6], Anatol Kierdaszuk[7,] Jerzy Bal[1]

THE SPECTRUM OF *PLP1* GENE MUTATIONS IN PATIENTS WITH THE CLASSICAL FORM OF THE PELIZAEUS-MERZBACHER DISEASE*

SPEKTRUM MUTACJI GENU *PLP1* U PACJENTÓW Z KLASYCZNĄ POSTACIĄ CHOROBY PELIZAEUSA-MERZBACHERA

[1]Department of Medical Genetics; Institute of Mother and Child, Warsaw, Poland
[2]Institute of Genetics and Biotechnology; Warsaw University, Poland
[3]Clinic of Neurology for Children and Adolescents; Institute of Mother and Child, Warsaw,
[4]Department of Biophysics; Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw,
[5]Department of Clinical Genetics, Medical University of Bialystok, Poland
[6]Department of Neurology, Faculty of Health Science, Warsaw Medical University
[7]Clinic of Neurology for Children and Adolescents, Provincial Hospital in Biala Podlaska

Abstract

The Pelizaeus-Merzbacher disease (PMD) is a rare X-linked recessive hypomyelination disorder caused by mutations of the proteolipid protein1 gene (PLP1). There is a spectrum of PLP1-related disorders from very severe connatal PMD, through classical PMD to mild spastic paraplegia type 2 (SPG2), with some correlation between the type of mutation and the phenotype. In general, missense mutations give rise to more severe forms of the disease, deletions and null mutations to mild PMD and SPG2. The most common variations, duplications, result in the classical-intermediate form of PMD.

The aim: *To report the analysis of mutations in the PLP1 gene and phenotype differences in ten male patients diagnosed with PMD. Although they had different types of PLP1 mutations (duplications, missense and nonsense mutations), all of them were clinically classified with the classical form of PMD (clPMD).*

Material and methods: *The subjects of analysis were ten male patients aged 1.5 to 21 years who were diagnosed with PMD. All patients developed the first clinical symptoms between 1 an 8 months of age and showed developmental delay, mainly in motor skills. All were classified with the classical form of the disease, according to international clinical criteria and the electrophysiological and brain MRI criteria of hypomyelination. The molecular analysis of the PLP1 gene involved dosage analysis and direct sequencing of all exons and promotor region of the gene.*

Results: *The clinical diagnosis of PMD was confirmed for all subjects by molecular analysis of the PLP1 gene. Although all had the classical form of PMD, it was caused by mutations of different types: duplications of the entire gene, missense and nonsense mutations.*

Conclusions: *Our clinical and molecular findings showed that the phenotypic spectrum resulting from PLP1 mutations seems to be broader in patients with the PLP1 gene duplication compared to patients with both nonsense and missense mutation. Nevertheless, apart from the type of mutation, all our patients' clinical manifestation falls into the category of the classical form of PMD according to international criteria. Obviously the type of mutations, but also other unidentified factors may affect the clinical course of PMD.*

Key words: gene duplication, hypomyelination disorder, leukodystrophy, Pelizaeus-Merzbacher disease, *PLP1*, point mutations

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Streszczenie

Choroba Pelizaeusa-Merzbachera (ang. Pelizaus-Merzbacher Disease, PMD) jest rzadką , dziedziczoną jako cecha recesywna, sprzężona z płcią, chorobą hipomielinizacyjną, której podłoże molekularne stanowią mutacje genu PLP1. Choroby powodowane mutacjami tego genu określane są jako tzw. choroby PLP1-zależne i stanowią spektrum jednostek od ciężkiej, wrodzonej postaci PMD przez klasyczną PMD do spastycznej paraplegii typu 2 (ang. Spastic Paraparesis type 2, SPG2) o łagodniejszym przebiegu, charakteryzujących się częściową korelacją pomiędzy typem mutacji w genie a fenotypem. W większości przypadków mutacje missens stanowią podłoże najcięższych postaci choroby, delecje i mutacje typu „null" lżejszych postaci PMD i SPG2. Najczęstsze mutacje, duplikacje genu powodują wystąpienie klasycznej postaci PMD.

Cel pracy: *Przedstawienie wyników analizy zmienności fenotypowej w kontekście podłoża molekularnego-mutacji genu PLP1 u dziecięciu pacjentów płci męskiej ze zdiagnozowaną PMD. Wszyscy zostali zakwalifikowani jako pacjenci z klasyczną postacią choroby, pomimo, że w genie PLP1 zidentyfikowano u nich mutacje różnego typu (duplikacje, mutacje punktowe typu missens i nonsens).*

Materiał i metody: *Dziesięciu pacjentów płci męskiej w wieku od 1,5 do 21 lat z rozpoznaną klasyczna postacią PMD zdiagnozowaną zgodnie z międzynarodowymi kryteriami klinicznymi oraz elektrofizjologicznymi i neuroobrazowymi kryteriami hipomielinizacji. Analiza molekularna genu PLP1 objęła badania dawki genu, jak również bezpośrednie sekwencjonowanie wszystkich eksonów i obszaru promotorowego genu.*

Wyniki: *Rozpoznanie kliniczne PMD, zostało u pacjentów potwierdzone badaniem molekularnym genu PLP1. Pomimo, że u wszystkich badanych stwierdzono klasyczną postać PMD to jej przyczyną były mutacje różnego typu; duplikacje całego genu, zmiany typu missens i nonsens.*

Wnioski: *Zmienność fenotypowa wydaje się być większa u pacjentów z duplikacją genu PLP1 w porównaniu z pacjentami, u których zidentyfikowano mutacje typu missens i nonsens. Pomimo to, niezależnie od typu mutacji, obraz kliniczny wszystkich badanych odpowiadał cechom klasycznej postaci PMD.*

Słowa kluczowe: choroba Pelizaeusa-Merzbachera, choroby hipomielinizacyjne, duplikacja genu, leukodystrofia, mutacje punktowe, *PLP1*

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INTRODUCTION

The Pelizaeus-Merzbacher disease (PMD; MIM 312080) is a rare form of inherited leukodystrophies – diseases affecting the white matter of the central nervous system (CNS). PMD is classified as a hypomyelinating disorder (1), inherited as an X-linked recessive trait and is caused by mutations in the *PLP1* gene (MIM 300401) localized in *locus* Xq22. The gene encodes the proteolipid protein 1 (PLP1) and its smaller, differently spliced form DM20 (2). The PLP1/DM20 is the major component of the oligodendrocytes' myelin in CNS, where it may stabilize and maintain the myelin sheath (2, 3). DM20 expression can be detected in the early stages of CNS development, before the onset of myelination, and this isoform is probably involved in oligodendrocyte differentiation (4). The DM20 form lacks 35 PLP-specific amino acids and is not able to compensate for the loss of proteolipid protein in myelin (5,6).

Mutations in the *PLP1* gene cause two allelic disorders, PMD and SPG2 (MIM 312920), characterized by a wide clinical spectrum, from severe connatal and classic PMD to "uncomplicated" and "complicated" forms of SPG. Generally the PMD/SPG2 phenotype variability is not completely understood. However, because of the intrafamilial heterogeneity of the disease's severity, not only the type of mutation and its localization in the *PLP1* gene, but also the influence of modifier genes may play a role in the final clinical phenotype. PLP1/DM20 expression not only in oligodendrocytes, but also in Schwann cells and neurons (motor, cortical, brainstem) may have relevance for the phenotypic variability of *PLP1*-related disorders.

The aim is to report the analysis of mutations in the *PLP1* gene and phenotype differences in ten boys diagnosed with PMD. Although they had different types of *PLP1* mutations (duplications, missense and nonsense mutations), all of them were classified clinically with the classical form of PMD (clPMD).

MATERIAL AND METHODS

Subjects

10 male patients aged from 1.5 to 21 y. with suspicion of PMD (7 unrelated probands, 2 affected brothers and 1 cousin) were referred for *PLP1* gene analysis. The patients presented the classical form of PMD according both to the international clinical criteria (onset within the first 5 years of life with the nystagmus occurring within the first 2 months of age, initial hypotonia followed by spastic quadriparesis, ataxia, titubation, dystonia, athetosis, and

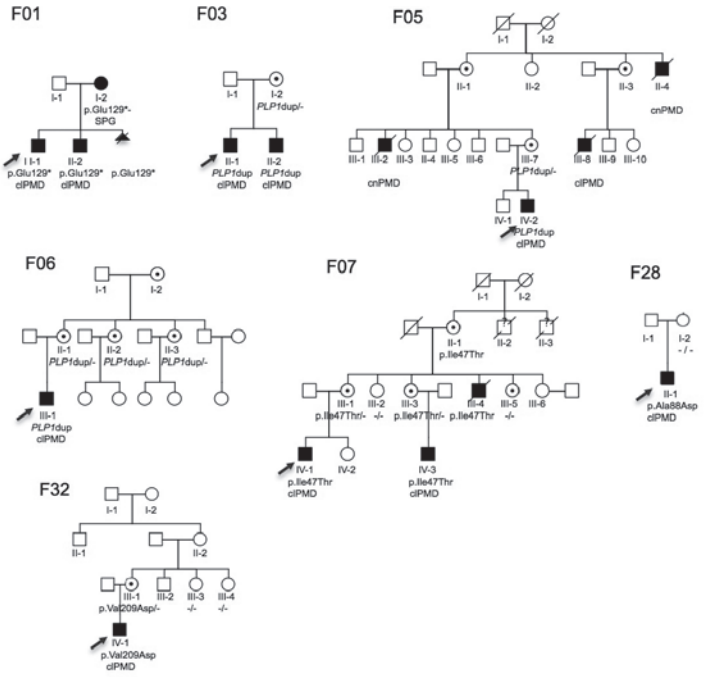

Fig. 1. Pedigrees of the PMD families presented.
cnPMD – connatal PMD, clPMD – classicPMD, SPG – spastic paraparesis

Ryc. 1. Rodowody prezentowanych rodzin obciązonych PMD.
cnPMD – wrodzona postać PMD, clPMD – klasyczna postać PMD, SPG – spastyczna parapareza.

cognitive impairment) and to the electrophysiological and brain MRI criteria of hypomyelination (mild hyperintensity on T2, combined with normal signal on T1 and highly abnormal, hyperintensive signal on FLAIR sequences) (7, 8, 9,10). The severity of the clinical picture was assessed according to a 0-4 degree scale based on the best motor function the patients were able to achieve up to 10 years: from 0 – patients with no motor achievement; 1 – patients who achieved head control between 2-4 yrs; 2 – patients who were able to achieve the sitting position between 3-5 yrs, and might achieve dysarthric but comprehensible speech; 3 – patients who achieved the sitting position between 1-2 yrs of age and walked with support at 2-6 yrs, but lost this capacity between 14-20 yrs of age, achieved dysarthric but comprehensible speech; 4 – patients who were walking independently (8).

DNA samples

DNA analysis was performed for 20 subjects: 7 PMD probands and their healthy and affected relatives. In two cases molecular diagnosis was performed in secondary laboratories (Institute fur Human Genetics in Hamburg, Klinisch Genetisch Centrum Nijmegen). To confirm the nature of the identified mutation, inherited vs. *de novo*, we performed the carrier test for all the patients' mothers and 1 grandmother. The carrier status was also tested in 8 women at risk. In 1 case a prenatal diagnosis was performed (Fig. 1).

The healthy control group consisted of 100 unrelated males 20-73 years old; mean 29.9 [SD± 9.7]. All DNA samples were obtained with written informed consent. The Institute of Mother and Child Ethics Committee approved the study.

PLP1 mutation analysis

The *PLP1* dosage analysis was performed for all probands by Multiple Ligation-dependent Probe Amplification reaction (MLPA) using the tests SALSA P022 A1 or B1 and P071 (MRC-Holland), containing probes covering all *PLP1* exons and 20 different regions for the Xq22.2 (11). Reactions were performed according to the manufacturer's recommendations (100 ng of DNA/ reaction, hybridization time 16 h and 35 PCR cycles). Reaction products were separated with 3130 Genetic Analyzer (Applied Biosystems). We used Gene Marker v.1.51 software (SoftGenetics LLC) to dosage ratio analysis (standard parameters, dosage ratio boundaries <0.75 and >1.25 for deletion and duplication respectively).

For the patients without *PLP1* duplications, the presence of the point mutations was analyzed. The direct sequencing of all 7 exons and the promoter region of the *PLP1* gene, according to Osaka et al. 1999 (12), were performed. Sequencing was performed using the BigDye v.3.2 terminator sequencing kit (Applied Biosystems), reaction products run on an ABI 3130 Genetic Analyzer, data analyzed with MutationSurveyor V3.24 software (SoftGenetics LLC). For the two new missense mutations, 100 healthy males were examined to determine the frequency of this mutation in the control population.

The identified variants were labelled according to the *PLP1* reference sequence NM_000533.3 (NCBI RefSeq; http://www.ncbi.nlm.nih.gov/nuccore). The impact of missense mutations on protein structure and function were analysed by PolyPhen-2 v.2.1 software, HumVar model (13) [http://genetics.bwh.harvard.edu/pph2] and MutPred tool (14) [http://mutpred.mutdb.org].

RESULTS

Clinical aspects

The first clinical signs and symptoms were observed between 1 and 8 months of age in all 10 patients. Developmental delay, mainly in motor skills was noted in all cases. Nystagmus was also a constant finding as well as mainly axial muscular hypotonia presented during the first 1-2 years of age. In patients who achieved head control, nystagmus was accompanied by head nodding (spasmus nutans). The symptoms showed a tendency to alleviate with age. Mild stridor was noted at infancy in 5 out of 10 patients, in two of them only during crying. Spastic progressive paraplegia was visible after the age of 2-4 years in all patients but one (F05_IV-2). He presented cerebellar ataxia regarded as cerebral palsy up to the age of 8 years. By the age of 2-4 years, six patients also revealed symptoms of cerebellar ataxia and dyskinesias, mainly of choreoatetotic type. In older patients choreoathetosis was dismissed, while focal or multifocal dystonia developed, frequently induced by movements and overlapping with spasticity. The intellectual level ranged from severe mental retardation to normal IQ. Only one patient (F05_IV-2) achieved temporarily autonomous walking as a teenager, but after the age of 20 his neurological state deteriorated markedly. He also achieved comprehensive but mildly

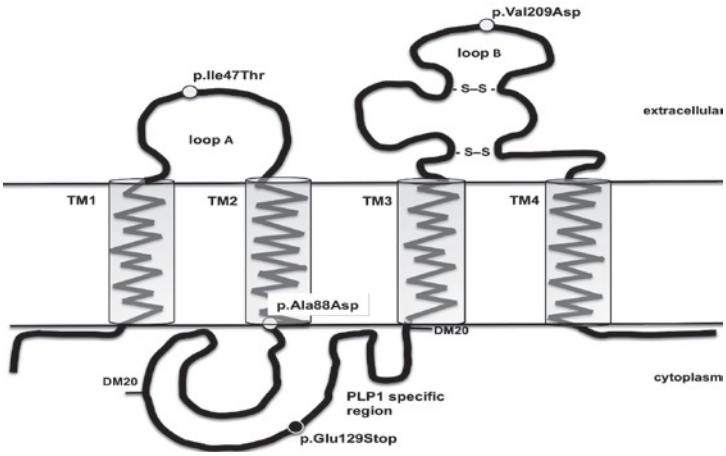

Fig. 2. Characterization of the *PLP1* point mutations identified in PMD patients. Localization of the *PLP1* gene mutations in relation to putative PLP1 topology including four transmembrane domains (TM1-4), two extracellular (LA and LB) and one cytoplasmic (LC) loops. The PLP1-specific 35 amino acids region (absent in DM20) is part of the cytoplasmic loop; in loop B two disulphate bridges are localized (based on topology by Weims and Stoffel, 1992). Identified mutations are indicated as white circles – missense substitutions, black circle – nonsense.

Ryc. 2. Charakterystyka mutacji punktowych genu PLP1 zidentyfikowanych u prezentowanych pacjentów z PMD. Lokalizacja mutacji białka PLP1 w odniesieniu do założonej toplogii obejmującej cztery domeny przezbłonowe (TM 1-4), dwie pętle zewnątrzkomórkowe (LA i LB) oraz jedna cytoplazmatyczną (LC). Specyficzny dla białka PLP1 35-cio aminokwasowy fragment (którego brak w formie DM20) położony jest w cytoplazmatycznej pętli LC; w pętli B zlokalizowane są dwa mostki dwusiarczkowe (na podstawie topologii zaproponowanej przez Weims and Stoffel).

dysarthric speech and attended a mainstream secondary school and started to study at university.

All patients underwent brain MRI examination that revealed diffuse symmetrical central white matter hypomyelination. Brainstem evoked potentials revealed severely prolonged central latencies in all the patients.

One of the patients' mothers (F01_I-2), being a carrier of the point terminating mutation p.Gln129*, was symptomatic. This 26-year old woman presented cognitive dysfunction, nystagmoid eye movements, slowly progressive dysartria, spastic paraparesis and cerebellar signs. Her MRI examination of the brain revealed abnormal diffuse hypo/dysmyelination of cerebral white matter.

Molecular analysis

The clinical diagnosis of PMD was confirmed for all subjects by molecular analysis of the *PLP1* gene. The probands, representing 7 families, have changes of different types: duplications of the entire gene (43%) missense (43%) and nonsense (14%) mutations. Duplications of the X chromosome segments, encompassing the *PLP1* gene, were identified in 3 families (4 patients – 2 unrelated subjects, 2 brothers, Fig. 1 F03, F05 and F06). All duplications were inherited from asymptomatic carrier mothers.

The nonsense mutation p.Gln129* (c.385C>T), already described as causing PMD (p.Gln128Term) (15), was found in one family in two affected boys and their symptomatic mother, diagnosed with SPG (Fig.1 F01). Different missense mutations were identified in three families (Fig. 2 F07, F28 and F32), one already known - p.Val209Asp (c.626T>A) (16) and two not reported previously – p.Ile47Thr (c.140 T>C) and p.Ala88Asp (c.263C>A). *In silico* analysis using the PolyPhen-2 tool confirmed the potential pathogenicity of both of them. Both were predicted as probably damaging with the p.Ile47Thr score of 0.882 and p.Ala88Asp 0.956. The MutPred tool, giving the general scores of deleterious effect probabilities, 0.832 and 0.852 respectively, confirmed these data. The substitutions c.140 T>C and c.263C>A were not identified in the examined healthy male cohort (100 alleles). According to the analysis done with the aid of the GPS server (GPS 2.0), Thr49, located in the extracellular loop A (Fig.2) is a putative target for CAMK/CAMKL/LKB human kinases (17). The replacement Ile47Thr visibly reduces the score for the Thr49 phosphorylation (8,59 down to 5.82). Moreover, Thr47 itself becomes a putative target for STE/STE7/MAP2K1 and STE/STE7/MAP2K2 kinases. In view of the above data, the Ile47Thr replacement may change the phosphorylation pattern of loop A, and in consequence may modify the PLP1 functionality.

According to the proposed topology of PLP1 (Fig. 2), the Ala88 residue is located in the proximity of the internal side of the cellular membrane. The Ala88Asp replacement creates unfavourable interactions between side chains of Asp88 and Glu89, resulting in the reorientation of transmembrane helix TM2 and/or in a distortion of the proximal region of the cellular membrane. Both should affect the organisation of the core framework of four transmembrane helices (TM1-TM4) of PLP1, and thus interfere with its biological function.

The clinical, neurological, MRI and molecular characterization of the patients is presented in Table I.

Family studies

The PMD carrier status causing mutations was analysed in the mothers of all the probands and revealed that *de novo* mutation only occurred in the family F32. In the family F01 the prenatal test was proposed, revealing that the male foetus was a carrier of *PLP1* mutation identified in the family. In three families (F06, F07 and F32), all the sisters of the probands' mothers decided to define their mutation carrier status, so appropriate analysis was performed depending on the type of mutation in the families (F06 MLPA analysis, F07 and F032 direct sequencing of exons 2 and 6 respectively) (Fig. 1).

DISCUSSION

Mutations of the *PLP1* causing PMD/SPG2 fall into two categories, the gene rearrangements (mainly duplications) and sequence variations, which account for 50-60% and 15-25% of the cases, respectively (7). Genotype-phenotype correlations are in some degree associated: the missense mutations, if localised in highly conserved regions, cause the most severe form of PMD, deletions and null mutations lead to a mild form of PMD and SPG2 presenting with peripheral neuropathy. The duplications result in the classical-

Table I. Clinical, neuroradiological and molecular data of the PMD patients

Tabela I. Dane kliniczne, neuroobrazowe i molekularne przezentowanych pacjentów z PMD.

ID	Age of onset	First symptoms	Age at present	Clinical symptoms	Intelectual level	Hypomyelination	PMD form/ score*	PLP1 molecular findings				
								Exon	Mutation	Prediction	Inheritance	Reference
F01_II-1	6 m.	Hypotonia Nystagmus Developmental delay	15 y	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR moderate	+	Classical/2	3	nonsens p.Glu129* (c.385C>T)	protein loss	Inherited	Hubner; 2005
F01_II-2	3 m.	Hypotonia Nystagmus Developmental delay	13 y	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR moderate	+	Classical/2					
F03_II-1	3 m.	Hypotonia Nystagmus Stridor Developmental delay	15 y	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR severe	+	Classical/ 1/2	1_7p	gene duplication Ex1_7 du	overex pression	Inherited	HGMD#
F03_II-2	birth	Hypotonia Nystagmus Dyskinesia Developmental delay	15 y	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR mild	+	Classical/2					
F05_IV-2	8 m.	Hypotonia Nystagmus Head tremor (spasmus nutans) Cerebellar ataxia Motor delay	24 y	Pyramido-extrapyramidal syndrome (spastic paraparesis), dystonia Head tremor Dysartria	IQ 99	+	Classical/4	1_7	gene duplication Ex1_7 dup	overex pression	Inherited	HGMD#
F06_III-1	3 m.	Hypotonia Nystagmus Head tremor (spasmus nutans) Developmental delay Cerebellar ataxia Dyskinesia	5 y	Spastic tertraparesis Dyskinesia Cerebellar ataxia Mild nystagmus	IQ 85	+	Classical/3	1_7	gene duplication Ex1_7 dup	overex pression	Inherited	HGMD#
F07_IV-1	7 m.	Hypotonia Nystagmus Developmental delay	18 y	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR moderate	+	Classical/2	2	missense p.Ile47Thr (c.140 T>C)	probably damaging	Inherited	this study
F07_IV-3	4 m.	Hypotonia Nystagmus Stridor Head tremor	14 y	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR moderate	+	Classical/2					
F28_II-1	2 m.	Hypotonia Nystagmus Stridor Head tremor (spasmus nutans) dyskinesia	12 y.	Spastic tertraparesis Dyskinesia Amblyopia (optic atrophy)	MR mild	+	Classical/2	3	missense p.Ala88Asp (c.263C>A)	probably damaging	de novo	this study
F32_IV-1	6 m.	Hypotonia Developmental delay Nystagmus	7 y	Spastic tertraparesis Dyskinesia Axial hypotonia Mild nystagmus	MR mild	+	Classical/ 2	4	missense p.Val209Asp (c.626T>A)	possibly damaging	Inherited	Inoue; 1997

*Clinical severity score according to Cailloux F et al, 2000

The PLP1 reference sequence NM_000533.3 (GenBank) was used to describe identified variants; variations are labelled according to HGVS recommendations version 2.0 (A in ATG initiation codon being +1) [http://www.hgvs.org/rec.html]. Functional prediction for missense mutations is based on PolPhen-2 HumVar model [http://genetics.bwh.harvard.edu/pph2]. Mutations are predicted as probably damaging (more confident prediction) or possibly damaging (less confident prediction) based on pairs of false positive rate (FPR) – 10%/20% FPR for HumVar [Adzhubei et al. 2010]. #HDMD Professional 2011.3 (Relase 30.09.2011) reports 20 cases of duplications of different range encompassing PLP1 gene [detailed references http://www.hgmd.cf.ac

intermediate form of PMD, triplication in a much more severe form (18). These genotype-phenotype correlations may be explained by distinct mutational mechanisms at the cellular level (19, 20, 3).

In this study we describe a group of patients with the classical PMD, despite different types of the *PLP1* gene mutations. There were no differences among the groups with respect to the age of the patients at the time of the disease's onset, and the types of the first signs and symptoms of the neurological status in the course of the diseases. There was, however, a difference between the groups regarding the range of PMD severity score and their cognitive level. We observed intra-familial heterogeneity of phenotype (Fig.1). Such data may confirm the hypothesis of the involvement of modifying genes in the final phenotype, especially in patients with *PLP1* duplications. The X-chromosome submicroscopic duplications in region Xq22.2 including the entire *PLP1* gene, are the most common reason for PMD (7). The range of duplication segments is variable in size, and each duplication event seems to be unique – family specific (21).

In the group of our probands, the frequency of point mutations was even a little higher than of duplications (57 vs 43%), but we have to keep in mind the limitations of interpretation due to the group size. Among patients with point mutations we identified one case of a nonsense change p.Gln129*, in two affected brothers and their symptomatic mother (F01, Fig. 1). This truncating mutation is localized to the PLP-specific cytoplasmatic loop of the protein and alters the PLP1 but retains the DM20 form (Fig. 2). This kind of mutation was described in atypical mild forms of PMD, which suggested the protective role of the DM20 isofom (22). However, it seems not to be the case, as other patients with much more severe forms of PMD, carrying this type of mutation have also been described. Among them there was a family with p.Gln129* where, as presented in F01, all the affected boys had clPMD and all female mutation carriers were symptomatic, with progressive disease of later onset (23). This and our data are contradictory to the others, suggesting that there is a risk of being the symptomatic mutation carrier for women in families only with a mild form of PMD (16).

Missense mutations identified in three families were localized in different parts of the PLP1 (Fig. 2), two of them in the extracellular loops of the protein, p.Ile47Thr in loop A and p.Val209Asp in loop B. Substitution p.Val209Asp was described for clPMD (17), p.Ile47Thr and p.Ala88Asp, located in the transmembrane domain (TM), have not been reported previously. Mutation p.Ile47Thr cosegregates with the PMD phenotype in family (F07, Fig1), p.Ala88Asp arose *de novo* (the only one case among patients, 14%). If we try to fit our data into the scheme of genotype-phenotype correlation in *PLP1*-related disorders proposed by Callioux and co-workers (8) we see that most patients classified with score 2 have point mutations in the gene. The phenotypic presentation of a missense mutation depends on its location. The most severe forms of PMD (scores 0,1) result from mutations in highly conserved, the mild form (scores 2,3) in partially conserved and SPG (score 4) in non-conservative regions of PLP1. New identified substitutions p.Ile47Thr and p.Ala88Asp, giving a very similar phenotype, affect non-conservative aa 47 and highly-conservative aa 88. The Ile47 is localized in the extracellular loop A. Based on data showing that substitutions of neighbouring, also non-conservative, Leu46 (Leu46Pro and Leu46Arg) result in severe PMD (8), we can assume that any changes in this loop may be deleterious. The aa Ala88 is localised in the transmembrane domain. The substitution Ala88Asp may affect its structure/function. The third identified substitution p.Val209Asp lies in the extracellular loop B, where two cysteine-mediated disulfide bridges, playing an important role in correct PLP1 folding, are localized. Mutations in this loop are "over-represented" among PLP1 missense mutations, suggesting its important role in proper protein folding. Generally the pathogenic mechanism of missense mutations in PLP1 is thought to be due to the accumulation of the miss-folded protein in the rough endoplasmatic reticulum (ER) and failure in its transport to the cell membrane. Depending on the localization of the substitution, miss-folded protein can reach the ER or the endosomal/lysosomal compartment, which can be correlated with severe and mild phenotypes, respectively. In both cases the death of oligodendrocytes results from the induction of the unfolded protein response (UPR) followed by the activation of chaperone genes and apoptosis (24). This is one of the postulated mechanisms of PMD/SPG pathogenesis. In the case of *PLP1* duplications, protein overexpression causes its accumulation in endosome/lysosome, aberrant protein transport and myelin assembly (25). Clinical manifestation varied between patients with gene duplication but in general they show the clPMD, which is often milder than in patients with point mutations. However, among our patients with *PLP1* duplication, we can observe nearly the full spectrum of disease severity forms (1/2, 2, 3 and even 4). This indicates that even for best-known mutations, such as *PLP1* duplication, it is very difficult to predict the course of the disease, but on the other hand molecular analysis of *PLP1* may result in the expansion of the disease phenotype to less typical patients. At this moment the *PLP1* diagnostic test (duplication/deletion and sequence analysis) makes it possible to confirm the diagnosis for about 60% of male patients, the remaining 40% do not have identifiable *PLP1* mutations (7). These data suggest, that the mutation may occur in regions of the gene not routinely analysed (upstream/downstream sequences and introns) but also indicate genetic heterogeneity in the group of hypomyelinating leukodystrophies (HMLs). PMD caused by *PLP1* mutations is a prototype of the HMLs describedin OMIM classification as hypomyelinative leukodystrophy type 1 - HLD1. Other forms include: HLD2 (known as Pelizaeus-Merzbacher Like Disease 1; MIM 608804) autosomal recessive form caused by mutations in *GJC2* gene (MIM 608803), HLD3 (MIM 260600) autosomal recessive caused by mutations in *AIMP1* gene (MIM 603605), HLD4 (MIM612233) also known as autosomal recessive mitochondrial Hsp60

chaperonopathy, caused by mutation in *HSPD1* gene (MIM118190), HLD5 (MIM 610532) linked to *FAM126A* gene (MIM 610531), HLD6 (MIM 612438) identified on a clinical basis and linked to TUBB4A gene (MIM 602662), HLD7 (MIM 607692) and HLD8 (OMIM 614381) both autosomal recessive, caused by mutations in *POLR3A* (MIM 614258) and *POLR3B* (MIM614366) genes coding the 2 largest subunit of RNA polymerase III. This group also includes the X-linked Allan-Herndon-Dudley syndrome (MIM 300523) caused by mutation in the *SLC16A2* gene (MIM 300095) [7, 25].

CONCLUSIONS

Our clinical and molecular findings showed that the phenotypic spectrum resulting from *PLP1* mutations seems to be broader in patients with the *PLP1* gene duplication compared to the patients with both nonsense and missense mutation. Nevertheless, apart from the type of mutation, all our patients' clinical manifestation falls into the category of the classical form of PDM according to international criteria. Obviously, some factors discussed above and also unidentified ones affect the clinical course of PMD.

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Address for correspondence:
Dorota Hoffman-Zacharska
Department of Medical Genetics;
Institute of Mother and Child
01-211 Warsaw; Kasprzaka 17
tel. (+48 22) 32-77-313
fax (+48 22) 32-77-200
e-mail: dhoffman@wp.pl