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Pelizaeus-Merzbacher disease in patients with molecularly confirmed diagnosis

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Abstract

Pelizaeus-Merzbacher disease (PMD) is X-linked hypomyelinating leukodystrophy caused by mutations of the PLP1 gene, which codes the proteolipid protein 1. The result of mutations is abnormal myelination – hypomyelination and dysmyelination of cerebral white matter, and in some form of the disease hypomyelinating peripheral neuropathy. DNA samples from 68 patients suspected of PMD due to the clinical course and hypomyelination at magnetic resonance imaging (MRI) were analyzed. Medical history and detailed clinical course of PMD patients were also analyzed. Different mutations of the PLP1 gene were detected in 14 boys from 11 families (~20%). Amongst the molecularly confirmed patients, 13 presented classical PMD forms but clinical phenotypes varied in the severity even amongst siblings. One patient presented a severe connatal form. One mother, obligate carrier, presented complicated SPG2 (spastic paraparesis). There was no phenotype-genotype correlation in our material. In many cases PMD was suspected with a delay of many years, sometimes only after birth of another affected child in the family. Pelizaeus-Merzbacher disease was most frequently misdiagnosed as cerebral palsy.

Key words: *Pelizaeus-Merzbacher disease, hypomyelination, dysmyelination, leukodystrophy, PLP1 gene mutations, MRI.*

Introduction

Pelizaeus-Merzbacher disease (PMD) is X-linked recessive hypomyelinating leukodystrophy resulting from mutations in the *PLP1* gene. The disease is allelic with spastic paraplegia type 2 (SPG2). The gene codes two protein isoforms, PLP1, the major component of myelin in the central nervous system and DM20 present in the peripheral nervous system [2,8]. Until now four phenotypes of PMD/SPG2 have been described. They include the severe inborn, classical, PLP1-null (with peripheral neuropathy) form of PMD, and the uncomplicated or complicated SPG2 [1-3]. Neuropathological findings in PMD include widespread lack or reduction of myelin sheets, sometimes with patchy appearance of perivascular areas (tigroid pattern) and with relatively preserved neurons and their processes [2]. In our material various pathogenic PLP1 gene mutations were detected in 15 patients.

Thirteen cases have been classified as the classical form according to international clinical, electrophysiological and brain magnetic resonance imaging

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(MRI) criteria of hypomyelination. They manifested variable clinical course, which could be observed even in siblings. One patient presented with the severe connatal form. One mother who was the obligate carrier manifested signs and symptoms of a complicated form of SPG2. Diagnosis of PMD was established with a delay of several years, most frequently after delivery of another affected child in the family. Most frequently PMD was misdiagnosed as cerebral palsy (CP). Below, we present a clinical pattern of the disease in patients with molecularly confirmed PMD and analyze causes of the delay in the appropriate diagnosis.

Material and methods

The analyzed material included DNA samples from 68 boys and their 58 mothers, from different centers in Poland from 2007. The inclusion criteria were hypomyelination at MRI according to R. Schiffmann and van der Knaap, including a hyperintensity of the white matter in T2 and Flair [8], characteristic brain auditory evoked potentials (decreased or absent brainstem auditory evoked potentials of waves III-V) as well as the clinical pattern and medical history. Clinical PMD phenotypes were established according to international clinical criteria as classic (onset within the first 5 years of life with the nystagmus occurring within 1-2 months of age, initial hypotonia followed by spastic quadriparesis, ataxia, titubation, dystonia, athetosis, and cognitive decline), connatal (constant nystagmus, general hypotonia, preserved deep tendon reflexes microcephaly, severe developmental delay, marked pre- and postnatal growth retardation) and complicated SPG2 (spastic paraparesis, incontinence, dementia) [1,2,8]. Pedigree analyses of all families were conducted [4,5].

Analysis of DNA samples taken from peripheral blood was performed as described in the earlier paper [4]. At first, investigation for deletion/duplication was performed by MLPA technique and in cases without PLP1 duplications we looked for the presence of the point mutations [4].

Results

Diagnosis of PMD was established in 14 boys from 11 families. Ten mothers proved to be carriers, including one symptomatic carrier with signs of a complicated spastic paraparesis. The familial cases included two sets of siblings, each with two affected brothers, in one family two first cousins were affected. Respective clinical and molecular data are listed in Table I.

Clinical data

The classical form of PMD of different severity has been diagnosed in 13 patients. One patient manifested a severe connatal form. In patients with the classical PMD the first signs and symptoms were noted between the 1st and the 8th month of life. They included nystagmus, axial hypotonia and a delayed psychomotor development, particularly concerning motor skills. Gradually, with maturation of their nervous system they developed tremor of the head and/or oscillating movements of the head (titubation), linked to nystagmus. Permanent stridor was present in some patients; in most severely affected children it was evident only while crying. In the second half-a-year or the second year of life, cerebellar ataxia became obvious within the trunk and extremities, as well as dyskinesias (mainly choreoathetoid movements), overlapping with intentional movements. In one of the patients, cerebellar ataxia persisted till the 8th year of life. Deep tendon reflexes were constantly present but elevated reflexes were noted most frequently after the 12th month of life. Spasticity did not appear until the second up to fourth year of life; in older children it was more pronounced in lower extremities. Older children manifested mainly focal or multifocal dystonia overlapping with the pyramidal signs. All the thirteen patients had psychomotor retardation, but an emotional and mental development was relatively better preserved than motor activities. One of the patients manifested normal intelligence in Leiter International Performance Scale. Ophthalmological examination detected small, pale optic discs in all patients. Nevertheless, vision was relatively well preserved. A clear neurological deterioration and regress in the development were observed after a few years, and in one case, after more than ten years.

The patient with the connatal form was delivered with signs of intrauterine growth retardation. His psychomotor development was profoundly retarded and failure to thrive was evident. His clinical pattern involved a mild, constant nystagmus, extreme muscular axial and limbs hypotonia with preserved deep tendon reflexes and slight involuntary movements of hands and feet [5]. The mother affected of SPG2 had slowly progressing spasticity of lower limbs, urine incontinence and cognitive decline. Her brain MRI revealed abnormal diffuse hypomyelination/dysmyelination.

Table I. Clinical and molecular data of patients affected by Pelizaeus-Merzbacher disease, molecularly confirmed. For detailed information of molecular data see also [4]

Family No.	Patient No.	Age of first signs (In affected children: nystagmus, axial and limbs hypotony, head tremor, developmental delay, stridor, dyskinesias)	Tentative diagnosis	Age of diagnosis (years)	Signs and symptoms when PMD was suspected Pyramido-extrapyramidal syndrome	Brain MRI: hypomyelination (hyperintensity at T2 and FLAIR)	Leiter scale IQ	Type of mutation [4]
I	1 proband	8-9 months	Cerebral palsy	13	+++	+	49	Point nonsense p. Glu129* (c.385C>T)
	2	3 months	Familial progressive encephalopathy	11	+++	+	41	
II	3 proband	3 months	Cerebral palsy	10	+++	+	30	Point missense p. Ile47Thr (c140T>C)
	4	1 month	Familial progressive encephalopathy	8	+++	+	68	
	Mother – symptomatic	21 years paraparesis	SPG2	26	Spastic paraparesis, urine incontinence, cognitive decline (SPG2)	Hypo- and diffuse dysmyelination	71	
III	5 proband	8 months	Cerebral palsy	9 (molecular confirmation at 21 years)	++	+	99	Duplication of 1-7 exons
IV	6 proband	3 months	Cerebral palsy	1.5	+	+	85	Duplication of 1-7 exons
V	7 proband	7 months	Cerebral palsy	14	+++	+	53	Duplication of 1-7 exons
	8	4 months	Familial progressive encephalopathy	10	+++	+	45	
VI	9 proband	2 months	PMD	11	+++	+	75	Point missense p.Ala88Asp (c.263C>G)
VII	10 proband	6 months	Cerebellar malformation?	4	+	+		Point missense p.Val209Asp (c.626T>A)
VIII	11 proband	Neonatal period	Spinal muscular atrophy	1	Severe developmental delay with severe hypotonia, reflexes (+); hypotrophy	+	30	Duplication of 1-7 exons

Table I. Cont.

Family No.	Patient No.	Age of first signs (In affected children: nystagmus, axial and limbs hypotony, head tremor, developmental delay, stridor, dyskinesias)	Tentative diagnosis	Age of diagnosis (years)	Signs and symptoms when PMD was suspected Pyramido-extrapyramidal syndrome	Brain MRI: hypomyelination (hyperintensity at T2 and FLAIR)	Leiter scale IQ	Type of mutation [4]
IX	12 proband	Infancy < 12 months	Cerebral palsy	17	+++	+	67	Duplication of 1-7 exons
X	13 proband	Infancy < 12 months	Cerebral palsy	1.5	++	+	75	Duplication of 1-7 exons
XI	14 proband	Infancy < 12 months	Cerebral palsy	16	+++	+	65	Duplication of 1-7 exons

In 11 out of the 14 boys (family I, II, V – see Table I), PMD was preceded by diagnosis of cerebral palsy, including three patients diagnosed with its cerebellar form and one patient suspected of cerebellar malformation. All of them were probands (the first affected family members). Just only after a few up to more than ten years, when the disorders deteriorated, or after delivery of similarly affected second child, the patients were referred to geneticists as familial progressive encephalopathy. In 4 patients, PMD was diagnosed in infancy: three brothers of an affected patient and an 11-month-old proband consulted by an experienced physician. In all the cases the decision to perform molecular testing was taken following detection of hypomyelination on MRI imaging (Fig. 1).

Molecular finding

The molecular analysis demonstrated duplication of the entire gene but of a slightly different range in seven cases (family III, IV, VII, IX, X, XI), and four point mutations of missense (family I) and nonsense types (family II, V, VI) in exons 2, 3 and 4 (see Table I) [4].

Discussion

Pelizaeus-Merzbacher disease is the best known leukodystrophy with hypomyelination. In the Czech Republic and in Germany its incidence is estimated at 1 : 90,000 to 1 : 100,000 live births [2,8]. Thus, we may conclude that in Poland many cases of PMD/SPG2 remain undiagnosed. The small group of 15 patients presented above, referred from various pediatric Polish centers since 2007 necessitates considering the reasons for such a low diagnostic efficacy. The first contributing factor may be unspecific character of clinical signs and symptoms. A similar clinical pattern can be found in several inborn and acquired diseases, of which the most frequently encountered is cerebral palsy. The "labelled" patient is referred to rehabilitation centers. In the early period of the disease the increasing muscular tonus is thought to reflect positive effects of rehabilitation. So the progress of the disease is falsely taken for improvement. Additionally some other disturbances alleviate or are modified by the maturation of the nervous system. This also makes the physicians blind to the possibility of another diagnosis. Further development of spasticity is thought to be related to evolution of cerebral palsy and the affected children probably may not return to diagnostic centers.

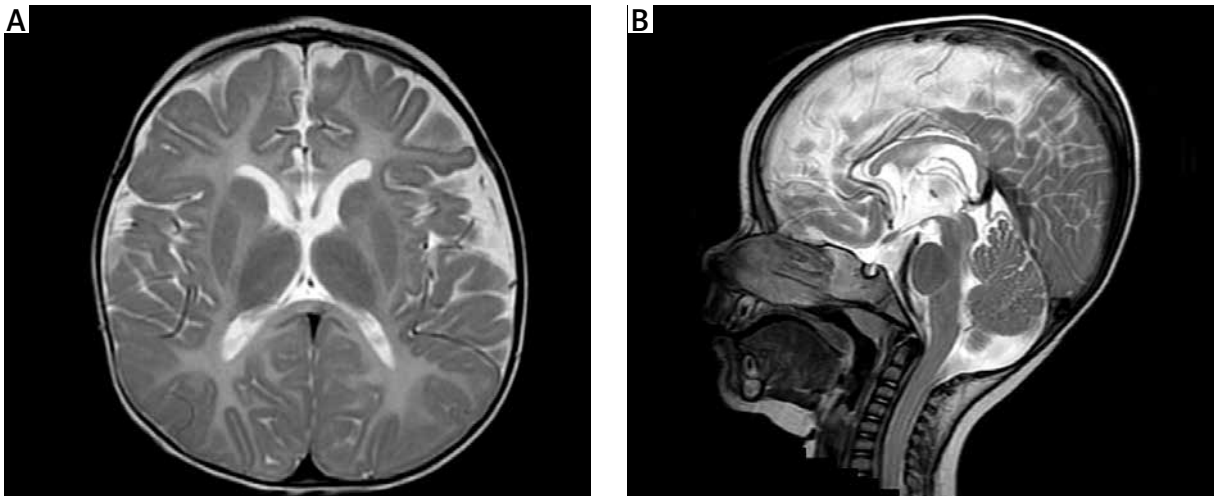

Fig. 1. A) T2-weighted magnetic resonance imaging of the brain of a 1.5-year-old patient affected by Pelizaeus-Merzbacher disease. Transverse section – mild hyperintensity (as compared to cortex) of whole white matter is visible, also in posterior limb of internal capsules. **B)** Sagittal T2-weighted magnetic resonance imaging – see very thin corpus callosum due to the decreased white matter volume.

A "red flag" does not appear until the day when another boy in the patient's family is delivered with similar signs and symptoms. As a result, the former diagnosis is revised and the child is referred to a geneticist.

The delays and errors in diagnosis of PMD are probably caused also by the currently prevalent view that all neurodegenerative diseases always manifest a gradual progressive deterioration. In contrast, the majority of children with PMD, despite neurological disturbances, show progress of various range in their psychomotor, emotional and intellectual development. They frequently acquire a number of abilities due to the enormous developmental potential of the young organism. Most of our patients started to demonstrate evident, gradual deterioration, not before a few, up to ten, years. This is confirmed by literature data [1-3,8].

The vast majority of our patients presented the classical form of PMD but of variable clinical severity even within the same family and the same mutation. This observation together with the small number of patients means that we cannot conclude about any genotype-phenotype correlations. Moreover, the patient with the connatal form of PMD also carried the duplication of the entire gene just as the patients with the classic form, which additionally confirmed the absence of such a correlation.

Differences in the clinical picture were rather related to the time when certain signs appeared as well as their intensity rather than their type. They were neither characteristic nor significant, so they did not result in any new elements in the diagnostic criteria. The variability of the disease course observed in siblings was also seen by other authors [1-3,8]. It can be explained by influence of the remaining genetic material, as well as epigenetic and environmental factors [2,8].

The diagnosis in all probands but one, just as in other rare genetic disorders, was established late, after a few up to over ten years after the appearance of the first symptoms of the disease [2,7]. Due to its X-linked inheritance, the diagnosis of PMD in familial cases may be enhanced by pedigree analysis if males are affected along the feminine line of inheritance. A proportion of PMD cases may develop de novo, which is not always acknowledged by specialists other than geneticists.

The detection of hypomyelination of the central nervous system is an indispensable element to suspect PMD or other hypomyelinating leukodystrophy (HLD). It can be demonstrated exclusively by magnetic resonance imaging (MRI) with analysis of individual time sequences, particularly at T2 and FLAIR [3]. Unfortunately, some of the probands initially had only computed tomography (CT) performed, most frequently for economic reasons. CT does not allow for correct differentiation of the lesion type (hypo-, dys- or demyelination).

Brain auditory evoked potentials (BAEP) are a second medical test which may detect hypomye-

lination but only differentiates this type of abnormality from the other white matter lesions. So the examination is not helpful and additionally available in just a few diagnostic centers.

Fundoscopic examination showed in PMD patients small, hypoplastic optic discs or their pallor, related to hypoplasia or atrophy, but not characteristic of the disease. It can be detected in several inborn or acquired diseases of the white matter [11]. It should be underlined that despite this lesion patients preserve vision for a long time.

The only investigation that can confirm the diagnosis of PMD is molecular analysis, demonstrating mutations in the *PLP1* gene [2,8]. In our material molecular confirmation of PMD was obtained in 15 cases per 68 DNA samples (around 20%) submitted for testing. Duplications encompassing 7 exons (the entire gene) were found in 7 cases, whereas point mutations of missense or nonsense type (see also the Table) were demonstrated in the remaining cases [4,5]. The duplication results in overproduction of PLP and DM20 proteins while the remaining mutations result in their abnormal (missense) or truncated (nonsense) form [2,8].

In the remaining 39 patients mutations have not been detected. It might be due to still insufficient molecular investigative techniques or to the fact that a similar clinical picture may result from other genetic causes. For the last two decades molecular genetics development have allowed to demonstrate heterogeneity of HLD [8,11,12]. Apart from PMD, more than ten HLD were established, manifesting various types of the inheritance pattern [6,8,11]. The clinical course of these diseases may be similar to that of PMD or sometimes additional signs are present e.g. hypodontia, cataract or atrophy of basal ganglia on MRI [9-12]. Moreover, world experts in the white matter diseases state that unclassified leukodystrophies with hypomyelination still exist [9,11]. These new findings may be accessed by available online international internet data bases, such as OMIM or www.genereview.org, that are regularly updated.

Conclusions

The classical PMD form represents a slowly progressing leukodystrophy, with apparent clinical improvement in the first years and deterioration not observed until the later period of life. The MRI is an essential method for detection and differentiation of white matter lesion types. The diagnosis of PMD can be confirmed only with molecular analysis. Genetic confirmation of the diagnosis allows to cover the family with genetic counselling and to suggest an appropriate supportive therapy. It also terminates a long-standing and very expensive diagnostic process, which in addition can be emotionally very painful for all the family members.

It is highly probable that a considerable number of PMD patients remain undiagnosed, so boys with a "label" of cerebral palsy should be watched carefully for alternative diagnosis.

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Disclosure

Authors report no conflict of interest.

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