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Two Patients with Clinically Distinct Manifestation of Pyruvate Dehydrogenase Deficiency Due to Mutations in *PDHA1* Gene

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Abstract: The most common cause of pyruvate dehydrogenase complex (PDHc) deficiency is the deficit of the E1α-subunit. The aim of this study was to describe distinct course of the disease in two boys with mutations in *PDHA1* gene and illustrate the possible obstacles in measurement of PDHc activity. Clinical data and metabolic profiles were collected and evaluated. PDHc and E1α-subunit activities were measured using radiometric assay. Subunits of PDHc were detected by Western blot. *PDHA1* gene was analysed by direct sequencing. In patient 1, the initial hypotonia with psychomotor retardation was observed since early infancy. The child gradually showed symptoms of spasticity and arrest of psychomotor development. In patient 2, the disease manifested by seizures and hyporeflexia in the toddler age. The diagnosis was confirmed at the age of seven years after attacks of dystonia and clinical manifestation of myopathy with normal mental development.

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Brain MRI of both patients revealed lesions typical of Leigh syndrome. Enzymatic analyses revealed PDHc deficiency in isolated lymphocytes in the first but not in the second patient. The direct measurement of PDH E1-subunit revealed deficiency in this individual. In patient 1, a novel hemizigous mutation c.857C>T (Pro250Leu) was detected in the X-linked *PDHA1* gene. Mutation c.367C>T (Arg88Cys) was found in patient 2. We present first two patients with PDHc deficit due to mutations in *PDHA1* gene in the Czech Republic. We document the broad variability of clinical symptoms of this disease. We proved that normal PDHc activity may not exclude the disease.

Introduction

Pyruvate dehydrogenase complex (PDHc) is a crucial multienzyme system linking glycolysis to the tricarboxylic acid cycle by catalysing the decarboxylation of pyruvate to acetyl coenzyme A. PDHc defects as a cause of primary lactic acidosis in children are rare in Slavonic populations (Cerna et al., 2001). The PDHc is composed of multiple copies of 3 enzymes: E1 (subunit α *PDHA1*, subunit β *PDHB*); dihydrolipoyl transacetylase (*DLAT*) (E2; EC 2.3.1.12); and dihydrolipoyl dehydrogenase (*DLD*) (E3; EC 1.8.1.4). The E1 enzyme is a heterotetramer of 2 α and 2 β subunits. The E1α-subunit contains the E1 active site and plays a key role in the function of the PDH complex (Brown et al., 1994). Dysfunction of the pyruvate dehydrogenase complex is most commonly due to mutations in the E1α-subunit gene (*PDHA1*) (Cameron et al., 2004). Although the gene is located on the X chromosome (Xp22), numbers of affected males and females are similar (Quintana et al., 2010).

There is a wide range of clinical presentations from severe neonatal lactic acidosis to mild ataxia or neuropathy, including Leigh syndrome and chronic encephalopathy (Robinson et al., 1987; Brown et al., 1994). The clinical course in females depends on the mutation and the random pattern of X-inactivation (Dahl et al., 1992). However, both males and females may manifest either severe or milder forms of the disease (Dahl et al., 1992; Bachmann-Gagescu et al., 2009; Quintana et al., 2010).

A low lactate/pyruvate ratio in blood is a consistent diagnostic indicator of PDHc deficiency in the case of lactic acidosis (Barnerias et al., 2010). Magnetic resonance imaging (MRI) may document the diffuse reduction of white matter with abnormal gyrification, agenesis of corpus callosum, cerebellar atrophy and symmetric abnormal hyperintensities in basal ganglia and brain stem (Quintana et al., 2010). PDHc activity or PDH E1-subunit activity measurement in lymphocytes, fibroblasts or muscle biopsy may confirm the diagnosis on biochemical level. Subunits of PDHc can be detected by Western blot. The definitive diagnosis is finally certified by molecular-genetic analysis.

In our study, we describe a clinical course of the disease and the results of biochemical and molecular analyses in two patients with distinct presentation

of Leigh syndrome due to PDH deficiency caused by one known and one novel hemizygous mutations in *PDHA1* gene coding E1α-subunit of PDH complex.

Methods

Enzymatic analyses

PDH complex activity and E1-subunit activity were assayed as the release of $^{14}CO_2$ from [1-^{14}C]pyruvate. The activity of PDH complex in the lymphocytes was measured according to Sheu et al. (1981) and in isolated muscle mitochondria according to Constantin-Teodosiu et al. (1991). The activity of the E1-subunit of the PDH complexes was measured according to van Laack et al. (1988). The activity of control enzyme citrate synthase was determined spectrophotometrically (Srere, 1969). Protein content was determined by the method of Lowry et al. (1951).

Western blot

Proteins from muscle mitochondria separated in 10% polyacrylamide gel were transferred onto nitrocellulose membrane (Hybond-C extra, Amersham) by electroblotting. The blots were blocked in phosphate buffer saline (PBS) containing 0.03% Tween 20 and 5% non-fat milk for 60 min. Incubation with the primary antibody (patient 1: chicken anti-bovine PDH complex IgG, 1:1000, Hena Ltd.; patient 2: PDH antibody cocktail 6 μg/ml, Mitosciences, USA) was performed for 60 min in the same buffer. Blots were washed in PBS containing 0.03% Tween 20 (4×10 min/wash) and incubated for 1 h with the appropriate secondary antibody in PBS containing 0.03% Tween 20 and 5% non-fat milk. Blots were washed in PBS containing 0.03% Tween 20 (4×10 min/wash) and the proteins were visualized by SuperSignal West Femto maximum sensitivity substrate (Pierce, USA) and detected by VersaDoc 4000 Imaging System (Bio-Rad Laboratories, Hercules, USA).

Molecular-genetic analyses

Total genomic DNA was isolated from blood lymphocytes by phenol-chloroform extraction. All 11 exons and adjacent intronic regions of the *PDHA1* were amplified by PCR and analysed by direct sequencing at genetic analyzer ABI3100 Avant (Applied Biosystems, USA). PCR primers were partly used according to Okijama et al. (2006) and partly designed with use of Primer3 software (Rozen and Skaletsky, 2000). The presence of both mutation was confirmed by PCR-RFLP with use of specific endonucleases (*Bst*NI for mutation c.857C>T, *Hin*FI for mutation c.367C>T).

Case report

Patient 1

The boy was born from the 6th pregnancy at the 38th week of gestation with birth weight 2,700 g. Parents, grandparents and two older sisters are healthy.

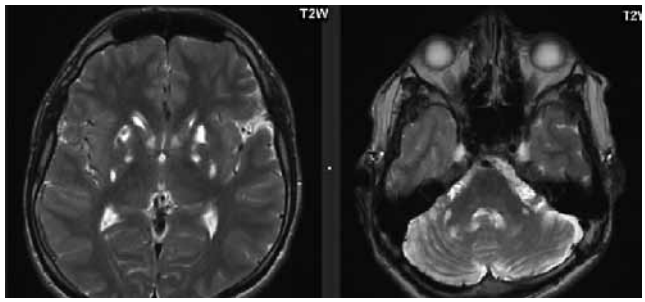

Figure 1 – Brain MRI of the first patient with Leigh syndrome. Axial T2 weighted images. Bilateral, symmetric abnormal hyperintensity in the putamina, caudate and globi pallidi in the first image; bilateral hyperintensity in the cerebellar dentate nuclei, middle cerebellar peduncule and cerebellum atrophy in the second image.

The early postnatal adaptation was uneventful, but progressive central hypotonic syndrome was observed since the 2nd month when repeated episodes of acute listlessness and the eye-bulbs movement discoordination were observed. A right-sided hemisyndrome was documented at the age of 2 years. Speech consisted of un-articulated sounds. He had severe development delay. Antiepileptic therapy was introduced after repeated seizures. CT investigation in infancy revealed frontal lobes atrophy. Brain MRI at the age of 6 years showed bilateral necrotic lesions in the basal ganglia, compatible with the diagnosis of Leigh syndrome.

At present, the boy is 15 years old, with failure to thrive and borderline head circumference. He has craniofacial dysmorphy, kyphoscoliosis and pectus carinatum, severe spastic quadruparesis and large joints stiffness. The boy is wheelchair bound, he uses only few words and his communication is mainly non-verbal. He is very anxious, social communication is a major problem. He does not control his bladder. The epilepsy is compensated on monotherapy with clonazepam. For recent MRI see Figure 1.

Patient 2

Eight-year old boy was born at term with a birth weight of 3,050 g and a length of 50 cm. The early postnatal adaptation was uneventful. Episode of seizures occurred at the age of 14 months. Brain MRI, EEG, and metabolic screenings showed normal findings. The antiepileptic therapy with valproic acid was administered. The hyporeflexia developed gradually since 17 months of age. The severe deterioration with weakness and quadriplegia was observed during acute gastroenteritis seven months later. EMG showed only slightly decreased nerve conduction velocities, spinal MRI was normal. The motor abilities improved to the level before the infection during following two months. His further psychomotor development was fully normal and the boy did not show any symptoms of the disease until the age of 7 years, when the painful dystonia of lower extremities developed. The attacks of dystonia with duration of 5–10 minutes mostly followed the physical but also psychical stress, and occurred approximately once a week. The neurological examination was summarized as myopatic phenotype: calf hypertrophy, areflexia, mild hyperlordosis, waddling gait, the boy was not able to perform a knee-bend.

The school attendance was one year delayed. An indicated novel MRI revealed the lesions consistent with Leigh syndrome.

Results

Biochemistry

The biochemical analyses showed increased levels of aminotransferases (AST 5.9 μkat/l; controls <0.6; ALT 2.3 μkat/l, controls <0.6), hyperuricaemia 490 μmol/l (ref. range 120–340 μmol/l) in patient 1. No laboratory signs of hepatopathy but borderline elevated creatine kinase (3.05 μkat/l, controls <2.27) were present in patient 2.

Both patients manifested lactic acidosis with elevated lactate in blood (2.4–4.3 mmol/l and 2.2–4.6 mmol/l; controls <2.3 mmol/l), cerebrospinal fluid (5.7 mmol/l and 2.8–4.8 mmol/l; controls <2.1 mmol/l), and hyperalaninaemia (1,248 μmol/l and 461–963 μmol/l; controls <500 μmol/l). Lactate to pyruvate ratio was border low in patient 1 and low in patient 2 (11.6 and 7.2; controls 10.0–20.0).

Enzymatic analyses

Activity of PDH complex was analysed in isolated lymphocytes from both patients, in muscle and liver homogenates of patient 1, and in isolated muscle mitochondria from patient 2 (Table 1). Activity of E1-subunit was analysed only in lymphocytes of patient 2 and his maternal relatives (Table 1). In patient 1, the decrease in PDHc activity was observed in all available samples with the most pronounced reduction in muscle (6.4% of lower reference range). In patient 2, the PDHc activity was in reference range in isolated lymphocytes, it was slightly decreased in muscle mitochondria (94% of lower reference range). Nevertheless, a marked deficiency of the E1-subunit activity was found in lymphoctes (7.4% of lower reference range). In lymphocytes from maternal relatives of patient 2, the E1-subunit activity was in reference range.

Table 1 – Activity of the PDH complex and E1-subunit in several tissues of the patients and lymphocytes from maternal relatives of patient 2

		PDHc activity ($nmol^{14}CO_2$/min/mg of protein)		PDH E1 activity (pmol/min/mg of protein)	
		activities	ref. range	activities	ref. range
Patient 1	*Lymphocytes*	**0.14–0.26**	0.25–2.2		
	Muscle	**0.128**	2–6.21		
	Liver	**0.064**	0.2–4.6		
Patient 2	*Lymphocytes*	0.410	0.25–2.2	**0.015**	0.202–0.454
	Muscle mitochondria	**9.410**	10–44.4		
Sister of patient 2 – *lymphocytes*				0.357	0.202–0.454
Grandmother of patient 2 – *lymphocytes*				0.224	0.202–0.454
Aunt of patient 2 – *lymphocytes*				0.210	0.202–0.454
Mother of patient 2 – *lymphocytes*				not done	

Protein analyses

The steady-state levels of the PDH subunits were analysed in isolated muscle mitochondria. In both patients, significantly decreased amount of E1α and E1β was observed. The amount of E2 subunit and E3 binding protein (E3BP) was unchanged in comparison with controls (Figure 2).

Mutation analysis

Sequencing of all exons of *PDHA1* gene revealed the novel hemizygous mutation c.857C>T in exon 7 in patient 1. The mutation results in substitution of highly conserved proline for leucine in position 250 of the protein. The mutation was not found in his mother's DNA isolated from blood as well as it was not present in 200 control DNA samples. Thus, mutation is either *de novo* in the patient or it results from germinal mosaicism in mother.

In patient 2, mutation analysis of the *PDHA1* exons revealed hemizygous mutation c.367C>T in exon 3. The mutation changes arginine for cystein in position 88 of the protein. Patient's mother and sister were heterozygotes for the mutation. The mutation was not found in his grandmother and maternal aunt.

Discussion

Clinical course

The clinical manifestation of PDHc deficit is highly variable. Three classical phenotypes of *PDHA1* deficit were characterized by Robinson et al. (1987): (1) neonatal neurological distress, lactic acidosis, facial dysmorphia, and prenatal brain lesions; (2) mental retardation and Leigh syndrome; and (3) relapsing ataxia with prolonged survival. In the group of 22 patients with PDHc deficit published by Barnerias et al. (2010), the main presenting symptoms were hypotonia (n=11), epilepsy (n=9), paroxysmal ataxia (n=6), paroxysmal dystonia (n=6) and signs of brainstem involvement (n=5). Only 3 of 22 patients manifested neonatal lactic acidosis in this group (Barnerias et al., 2010). Quintana et al. (2010) described

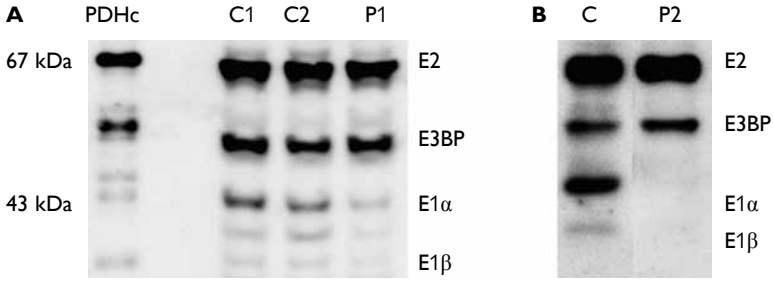

Figure 2 – Immunoblot analysis of PDHc subunits in isolated muscle mitochondria of patient 1 (P1, A) and patient 2 (P2, B) revealed markedly decreased levels of E1α and E1β subunits in comparison with controls (C1, C2, C).

the clinical features in 20 of 40 patients diagnosed with PDHc deficiency. The male-female ratio was balanced (9:11). Fatal lactic acidosis occurred in two males. Other patients manifested psychomotor delay (n=16), epilepsy (n=10), cerebral malformations or cerebellar atrophy (n=11), and microcephaly (n=9). Other symptoms included facial dysmorphy, hypotonia, visual or hearing defects, hypospadia, failure to thrive, and dystonic hemiplegia. Leigh syndrome was the clinical presentation in four of the males, but curiously, none of the 11 females showed this presentation. An increased lactate level in blood and/or cerebrospinal fluid was found in all patients.

In the presented study, MRI examination revealed Leigh syndrome in both our patients. Curiously, patient 1 is severely cognitively affected and quadruspastic, while the mental development is surprisingly normal in patient 2. The disease manifestation in patient 1 corresponds with classical clinical phenotype of PDH deficiency with hypotonia, cerebellar symptoms, seizures, dystonia, microcephaly, and craniofacial dysmorphy (Robinson et al., 1987). The clinical course of the disease was less severe in our second patient with isolated dystonia of lower extremities. Indeed, this may be the only symptom of PDH deficiency (Head et al., 2004). In the beginning of the disease hypotonia was present in both patients, with the progressive development in the patient 1. In the second patient, hypotonia showed episodic character with spontaneous resolutions.

The neurological state of patient 2 remained stable like in many of less severe affected patients with normal mental development, although the progressive neurological deterioration may occur in such patients after several years (Debray et al., 2006). On the other hand, mental development may be normal even in adulthood as in cognitively normal 18 years old patient (Bachmann-Gagescu et al., 2009).

Biochemical findings

The PDH deficiency is an important cause of primary lactic acidosis in children (Lissens et al., 2000). The increased blood and cerebrospinal fluid lactate levels were present in both our patients. In concordance with other studies, biochemical investigations did not help to predict the phenotype of patients, as the correlation between biochemical findings and clinical manifestation is poor and even impossible in the PDH deficiency (Robinson et al., 1987). The patients with various severity of clinical impairment may show mild elevation of lactate and alanine levels in blood or very severe lactic acidosis. Decreased lactate/pyruvate ratio in case of lactic acidosis (normal range 10–20) supports the clinical suspicion on PDHc deficit. Some authors consider already values under 15 significant (Barnerias et al., 2010). Lactate/pyruvate ratios indicate a suspicion on PDHc deficit in both our patients using Barnerias's criteria.

Analysis of the PDHc activity in available samples is crucial for the diagnosis. However, the obtained data may significantly differ among tissues and residual

PDHc activity may reach normal values as observed in patient 2 lymphocytes. The diagnostics then relies on the Western blot analysis of the PDHc subunits steady-state levels and on *PDHA1* mutation analysis. Based on our results as well as observations of others (Quintana et al., 2010), the measurement of the E1-subunit specific activity seems to be more sensitive enzymatic assay for PDH E1-subunit deficiency than the enzymatic analyses of the whole PDHc activity. Furthermore, it can be run in parallel with PDHc activity measurement and it may confirm the diagnosis of PDHA1 deficiency in lymphocytes without a need of muscle or skin biopsy.

The question raises to which extent (i) the PDHc and E1 activities and (ii) the PDHc activity and E1α/β-subunit protein level can be directly correlated. Unfortunately, literary evidence is heterogenous with no thoroughgoing simultaneous comparison of the PDHc activity, E1 activity and E1α/β protein level in patients with the same type of mutation in *PDHA1* gene. In males, residual PDHc activity can vary in dependence on the specific mutation (amino acid change) involved, subsequent post-translational modifications, and/or the site of mutation, with oddly different phenotypes in the cases of e.g. regulatory phosphorylation site mutation (Morten et al., 1998) or mutation in site of E1α/β-subunits interaction region (Marsac et al., 1997). It was demonstrated previously (Robinson, 2001) that E1α/β-subunits amounts decrease proportionately. This is caused by degradation of E1β-subunit elicited by E1 heterotetramer instability caused by decreased amounts of defect E1α-subunit, which in turn can lead to lower PDHc activity. In females, non-random X-inactivation results in disproportional expression of normal and mutated alleles, thus resulting in normal or affected phenotype (Dahl et al., 1992). The complexity of correlations is illustrated for instance in the study of Ostergaard et al. (2009), in which 2 patients with missense mutation (E206K and M153V) showed dramatically distinct PDHc activity values and E1α/β Western blot profile. Interestingly, a female patient (E206K) exhibiting nearly normal PDHc activity values and normal level of E1α/β protein was far severely affected. In male patient (M153V) with PDHc activity about twice reduced in comparison with previous patient and with decreased amounts of E1α/β-subunits, disease presentation was milder. We may conclude that no clear correlation among the PDHc activity, E1α/β protein level and clinical phenotype exists in PDHc deficit.

Genetics

Patient 1 harbours novel mutation (P250L) in E1α-subunit. Nevertheless, other pathogenic mutation affecting the listed codon has been already described in patient with (slightly) different clinical phenotype. Cameron et al. (2004) described 1-year-old boy with cerebral atrophy, acute peripheral neuropathy and osteopenia caused by P250T substitution in E1α-subunit. Mutation R88C in E1α-subunit, found in patient 2, has been previously described by Debray et al. (2006) in 7-year-old girl with Guillain-Barré syndrome (acute peripheral motor neuropathy). Furthermore,

Marsac et al. (1997) described a child suffering from motor neuropathy and lactic acidosis during infectious episodes caused by mutation R88S. On contrary to our patient 2, the residual PDH activity in lymphocytes of their patient was very low. Phenotype variability in patients with mutations in the same codons suggests the possible influence of epigenetic factors in the PDH deficiency caused by *PDHA1* mutations. This was illustrated also in the case report of 18 years old mentally normal patient (Bachmann-Gagescu et al., 2009), who harboured the common mutation in *PDHA1* gene (R263G). Previous patients with this mutation had presented with mental retardation and/or Leigh syndrome, while this patient's clinical outcome was excellent and his brain MRI was also normal (Bachmann-Gagescu et al., 2009).

Therapy

The high-fat, low sugar ketogenic diet has reportedly been therapeutically useful in some PDH-deficient patients. However, it has the disadvantages of unpalatability and of possible nutritional deficiencies (Weber et al., 2001). Even an avoidance of carbohydrate-rich foods may reduce the frequency of minor episodes of weakness in some patients (Debray et al., 2006). Initial treatment with thiamine (50 mg/kg/day) is recommended (Barnerias et al., 2010), although only one from twenty two patients exhibited dramatic improvement after thiamine administration. This corresponds to our experience, as no significant effect has been observed in our patients yet.

Conclusion

PDH deficiency is a mitochondrial disorder with various clinical presentations including Leigh syndrome. Different natural courses of the disease in described patients prove the great clinical variability even within Leigh syndrome. This fact emphasizes the necessity of taking PDH deficiency into differential diagnosis of each patient with not clearly explained nonspecific neurological involvement of variable severity, including particularly development delay, ataxia hypotonia and dystonia. These symptoms usually go along with lactic acidosis. We show in our study that the sufficient PDHc activity measured in isolated lymphocytes does not exclude the diagnosis of PDHc deficit. Then, the direct measuring of PDH E1-subunit should be performed in suspected cases. Described patients are the first ones in the Czech Republic harbouring mutations in the *PDHA1* gene coding the E1α-subunit. Mutation observed in patient 1 is novel and not reported previously.

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