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Short clinical report

Pyruvate dehydrogenase deficiency caused by a new mutation of *PDHX* gene in two Moroccan patients

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ABSTRACT

Pyruvate dehydrogenase deficiency is one of the genetic defects of mitochondrial energy metabolism. Clinical features are heterogeneous, ranging from fatal lactic acidosis in the newborn period to chronic neurodegenerative abnormalities. Most cases have mutations in the gene for the E1alpha subunit of the pyruvate dehydrogenase complex. Primary defects of the E3 binding protein component of the pyruvate dehydrogenase complex are rarier. We describe two unrelated Moroccan patients with the same new mutation c.1182 + 2T > C in the E3 binding protein gene *PDHX* and different clinical forms.

1. Introduction

Pyruvate dehydrogenase complex (PDHc) deficiency is an important cause of primary lactic acidosis and neurodegenerative disease of varying severity in infancy and childhood [1,2]. PDHc is a large mitochondrial multienzyme complex consisting of three catalytic enzymes: Pyruvate dehydrogenase (El), dihydrolipoamide acetyltransferase (E2), and dihydrolipoamide dehydrogenase (E3), two specific regulatory enzymes (pyruvate dehydrogenase phosphatase and kinase) and a binding protein linking the E3 subunit to the E2 core called protein X or E3BP. PDHc activity is involved in the conversion of pyruvate to acetyl CoA, linking the glycolytic pathway and the citric acid cycle; therefore it plays a central role in energy metabolism. The majority of cases of PDHc deficiency are caused by mutations in the X-linked E1α subunit gene (*PDHA1* gene) [2]. Primary defects of the other subunits appear to be rarer, and only a few cases of E3BP deficiency (*PDHX* gene; 11p13) have been reported [3–16]. Clinical presentation of patients with PDH deficiency is heterogeneous, ranging from fatal lactic acidosis in the newborn period to chronic neurodegenerative abnormalities [1].

We report two unrelated Moroccan patients presenting with PDHc deficiency due to E3BP deficiency. Molecular studies revealed that the two patients beared the same new mutation in the E3BP gene. A founder effect of this new mutation was searched.

2. Patients and methods

2.1. Case reports

2.1.1. Patient 1

The first patient was a 8 years-old Moroccan male, the third child of healthy consanguineous parents. Pregnancy and delivery had been uneventful and the child was born at term. His siblings were healthy. The parents didn't report any problem until day 30. At this age, the parents noticed that their child had little interaction with his environment and reacted badly to sounds. He had moderate global developmental delay with particularly poor coordination. No diagnosis had been established until the age of 8 years when the patient was seen at our outpatient clinic. His height and weight were normal. He presented instability in walking with frequent falls until age 5 years, currently less obvious, marked during prolonged exercise or running. He has no dysmorphic features. MRI of the brain showed thinning of the corpus callosum, vermian atrophy and discrete atrophy of the left ventricle. Biochemical studies revealed elevated blood alanine and lactate (3–4 mmol/l) concentrations with normal lactate-to-

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pyruvate ratios (L/P range: 13–17; $N < 17$). Cerebrospinal fluid lactate (6.8 mmol/l; $N < 1.7$ mmol/l) and pyruvate (0.45 mmol/l; $N < 0.17$ mmol/l) concentrations were also elevated with a normal lactate to pyruvate ratio ($L/P = 15$; $N < 19$). PDH deficiency was investigated in cultured fibroblasts: the PDH activity was reduced to 27% of controls. Enzymatic activities of respiratory chain complexes in muscle mitochondria were normal. A high fat and low carbohydrate diet was started with thiamine supplementation.

2.1.2. Patient 2

The second patient was a female infant, two months old, the first child of healthy consanguineous parents of Moroccan origin living in France. Pregnancy and delivery had been uneventful and the child was born at term. At birth, she presented with hypoxia (initial pH at 6.96 and lactatemia at 13.6 mmol/l). Blood lactate levels decreased very slowly and remained abnormal from 21.6 mmol/l at 14 h to 4–5 mmol/l after day 10, with persistently normal lactate to pyruvate ratios (8–12). Cerebrospinal fluid lactate was elevated at 4.9 mmol/l. Conventional MRI of the brain showed signal abnormalities with bilateral and symmetrical decreased diffusion coefficient in cortico-subcortical parieto-occipital area, a thinning of the corpus callosum and normal basal ganglia. PDH activity in lymphocytes was reduced to 33% of controls. Dysmorphic features were not found. Neurological examination showed hypotonia and a minor developmental delay. The patient was treated by a ketogenic diet and thiamine supplementation. She made developmental progress, her psychomotor development was slightly delayed (evaluated at 12 months when she was 15 months-old) and at the age of 2.5 years, blood lactate concentrations remained in the range 2.4–4 mM, with pyruvate levels between 0.24 and 0.40 mM.

2.2. Molecular studies

Informed consent was obtained from the probands' parents prior to implementation of the genetic studies reported here. Mutation analysis was carried out by sequencing *PDHA1* and *PDHX* genes on genomic DNA prepared from cultured fibroblasts and blood samples of patients. *PDHX* cDNA was also analyzed to look for the consequences of the splice donor site mutation at the RNA level. The *PDHX* mutation status of parents was investigated from blood DNA analysis. Haplotype analysis was performed using six microsatellite polymorphisms flanking the *PDHX* gene and single nucleotide polymorphisms (SNPs) within the *PDHX* gene.

3. Results

Mutation analysis revealed a normal *PDHA1* gene sequence. Subsequent genetic studies showed that both patients were homozygous for a single base pair substitution (c.1182 + 2T > C) in the splice donor site of intron 9 of the *PDHX* gene. cDNA studies revealed that the lost of the natural donor splice site of intron 9 led to the activation of a cryptic site in exon 9 and deletion of 29 bp in the mRNA. This deletion creates a frameshift with appearance of a premature stop codon (p.Ile386SerfsX13).

Parents were found heterozygous for the *PDHX* c.1182 + 2T > C mutation in both families.

To investigate whether the two unrelated Moroccan patients could have a common ancestor, haplotypes were established by analysis of microsatellites flanking the *PDHX* gene and intragenic SNPs. A shared homozygous region of at least 0.120 Mb was found covering the gene and a flanking region suggesting a founder effect (Table 1).

Table 1
Microsatellites and single nucleotides polymorphisms surrounding the homozygous c.1182 + 2T > C mutation of *PDHX* in the two patients.

Genetic markers	Patient 1	Patient 2	Position (bp) on chromosome 11
D11S1322	228–230	224–224	31555676–31555899
D11S1751	296–296	296–296	33826245–33826543
D11S1392	204–204	200–200	34640077–34640281
D11S4200	110–110	110–110	34852544–34852675
rs3818401	*wt–wt*	*wt–wt*	*34894775*
rs2243948	*wt–wt*	*wt–wt*	*34935468*
rs2767035	*wt–wt*	*wt–wt*	*34938645*
rs10768108	*C > G-C > G*	*C > G-C > G*	*34956321*
rs111818080	*wt–wt*	*wt–wt*	*34962655*
PDHX c.1182 + 2 T > C	*T > C-T > C*	*T > C-T > C*	*34962853*
rs74524673	*wt–wt*	*wt–wt*	*34973322*
D11S4203	258–258	244–244	35813457–35813694
D11S1911	294–294	300–300	37209496–37209789

The homozygous region is boxed for each family and the common haplotype is shaded; the position of *PDHX* gene on chromosome 11 is 34894253–34974251 (Reference Sequence NM_003477.2, NCBI36).

4. Discussion

Mutations in *PDHX* gene that encodes E3BP appear to be a rare cause of PDHc deficiency. The first paper describing a case of E3BP deficiency has been published by Robinson and colleagues in 1990 [3]. With the patients described here, there are now 26 defined cases from 21 unrelated families. Key features of these patients are summarized in Table 2. The clinical presentation of E3BP deficiency is variable and undistinguishable from PDH E1α deficiency. Age at onset varies from the neonatal period (13 cases < 1 month) to infancy (8 cases < 13 months) or childhood (5 cases). The main clinical features are delayed psychomotor development with mental retardation, hypotonia and a variable degree of lactic acidosis. Some patients develop ataxia or dystonia, particularly during episodes of metabolic decompensation associated with intercurrent illness. Seizures have been observed in 6 patients and visual impairment in five patients. The main neuropathological findings are the absence or thinning of the corpus callosum (11 patients), some degree of cortical atrophy, predominantly affecting white matter (10 patients), ventricle dilatation (3 patients), periventricular and subependymal cysts (3 patients) or necrotic lesions of the basal ganglia (6 patients). Biochemically, measurement of blood and cerebrospinal fluid lactate and pyruvate are the most important tests for recognition of PDHc deficiency. Marked lactate increase with a lactate/pyruvate ratio <20 is a clue to the diagnosis. Almost all patients with the neonatal form develop lactic acidosis (blood lactate in the range 5–23 mM) whereas patients presenting later in infancy usually had blood lactate concentrations in the range 3–5 mM. Skin fibroblasts are commonly used for PDH assay but fresh lymphocytes are also convenient. The residual PDH complex activity varies from 6 to 33% of mean normal activity. Causative mutations have been defined in almost all the known patients with E3BP deficiency. A striking finding is that all of the reported mutations are expected to result in complete deficiency of E3PB protein. Half of the patients have splicing mutations; others have frameshift or nonsense mutations. Mutation analysis in our two patients revealed the same splicing mutation of the *PDHX* gene. This is a new mutation that has never been described. This mutation at the 5′ donor splice site (c.1182 + 2T > C) leads to a deletion of 29 bp of exon 9 and a frameshift (p.Ile386SerfsX13). As expected when a homozygous mutation is delineated for an autosomal recessive disorder, both families are consanguineous. There is a high incidence of parental consanguinity in Morocco, it was found to be 15.25% [17]. The patients presented here have different forms