Cerebral palsy and pyruvate dehydrogenase deficiency: identification of two new mutations in the E1α gene

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Abstract Pyruvate dehydrogenase (PDH) complex deficiency, a common cause of congenital lactic acidosis, is mostly due to mutations in the X-linked gene coding for the E1α subunit of the complex. We have studied two unrelated girls presenting a static encephalopathy with spastic quadriplegia, microcephaly and seizures and in one girl, hypocalcaemia, a new finding in PDH complex deficiency. PDH deficiency was diagnosed in adolescence and both girls had low PDH complex activity in muscle but normal amounts of all subunits on Western blotting, and a normal lactate/pyruvate ratio in blood and CSF. Mutation analysis of the E1α gene at the cDNA or DNA level revealed an arginine to histidine substitution at amino acid position 288 (R288H) in the girl with hypocalcaemia and a 12 bp insertion, predicting a four amino acid duplication at the C-terminal end of the protein in the second girl. They both carried a normal and a mutated E1α gene and X-inactivation studies showed skewed patterns.

Conclusion Mutation identification in pyruvate dehydrogenase complex deficiency remains important especially for the determination of the recurrence risk and for reliable genetic counselling in couples with an affected child.

Key words Pyruvate dehydrogenase E1α · Clinical presentation · Mutation analysis

Abbreviation PDH pyruvate dehydrogenase · SSCP single strand conformational polymorphism

Introduction

Lactic acidosis is a frequent manifestation of metabolic disease in newborns and young children. An important cause of primary lactic acidosis is a defect in the pyruvate dehydrogenase (PDH) complex [3, 25]. This multi-enzyme complex plays an important role in the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA and consists of multiple subunits of a thiamine pyrophosphate-dependent pyruvate decarboxylase, E1 which is a heterotetramer of 2α and 2β subunits, dihydrolipoamide acetyltransferase (E2), dihydrolipoamide dehydrogenase (E3), dihydrolipoamide dehydrogenase-binding protein (E3BP, formerly known as...
protein X) [1, 14, 17, 21], E1 kinase and phospho-E1 phosphatase [26]. The first three components (E1, E2 and E3) are the major catalytic subunits of the complex, while the E1 kinase and the phospho-E1 phosphatase regulate the activity of the PDH complex by phosphorylation (inactivation)/dephosphorylation (activation) of the α component of E1.

A wide spectrum of clinical presentations has been associated with PDH complex deficiency. Symptoms range from an intermittent ataxia over a progressive disease with mental retardation and neurological complications to an early neonatal presentation with severe lactic acidosis and early death [3, 7, 18, 26]. At the molecular and biochemical level, defects in the PDH complex are most often attributed to deficiencies in the E1 component, and more specifically to the X chromosome-linked E1α subunit [9]. To date, more than 50 different mutations in E1α have been described [20]. In our sample of unrelated patients we have identified 37 mutations in E1α [2, 11–13, 22–24; unpublished data]; however, mutations are only found in about 50% of the patients with biochemical and clinical indication of PDH complex deficiency. Therefore, mutation analysis in suspected PDH complex deficiency remains important for the molecular confirmation, for the determination of the recurrence risk and for genetic counselling in couples with an affected child.

Materials and methods

Biochemical studies

PDH complex activity was assayed in mitochondrial fractions prepared from fresh muscle tissue and immunochemical study of the PDH complex subunits with anti-PDH complex antibodies was performed as previously described [29].

Molecular analysis of the PDH-E1α gene

Mutation analysis of the PDH-E1α gene was performed on either cDNA prepared from total RNA isolated from fibroblasts (case 1) or on DNA from white blood cells (case 2). Isolation of total RNA, cDNA synthesis, PCR amplification and SSCP analysis of six fragments covering the whole coding region of the E1α gene has been described [23]. For genomic analysis of the 11 exons of the gene, 10 primer pairs were used (primer sequences available on request; 19). All exons are amplified individually from sequences within flanking introns, except for exons 7 and 8 which are amplified as one fragment. Except for this fragment, all others could be used for single strand conformational polymorphism (SSCP), as described for cDNA fragments, or could be directly sequenced using the amplification primers for sequencing. The fragment containing exons 7 and 8 was directly sequenced using the amplification primers and an additional intron 7 (reverse) primer 8i5bis 5′-CTCAATGTGTTCTAGATCG-3′.

Case reports

Case 1

This female patient (born in 1982) was the sixth child of healthy non-consanguineous parents. Pregnancy and delivery were without complications. She was first investigated at the age of 10 months because of psychomotor retardation and microcephaly. Metabolic studies revealed increased urinary concentrations of alanine and lactate. EEG was abnormal with general slowing and epileptic discharges. CT scan of the brain showed dilated ventricles and cerebral atrophy. Further studies were not performed at that time.

At the age of 11 years she was admitted to hospital because of intestinal obstruction. A volvulus of the transverse colon was diagnosed for which she underwent surgery. Pre-operative screening showed severe hypocalcaemia, necessitating vigorous supplementation. The electrolyte disturbance persisted after the successful abdominal surgery and she was discharged with oral supplementation.

She was again admitted to hospital 2 years later with intestinal obstruction caused by a volvulus of the transverse colon. She was operated and the transverse colon was derotated and decompressed. After surgery persistent hypocalcaemia was noted. Additional studies demonstrated high urinary loss of calcium (increased calcium/creatinine ratio of 3; normal <0.7), with a normal parathyroid hormone level suggesting hypoparathyroidism. There were no signs of other renal tubular dysfunction and creatinine clearance was normal. Ultrasound studies of the kidneys were also normal.

Case 2

The female patient (born in 1984) was born at term; birth weight 3020 g. Her parents were healthy and unrelated. At the age of 5 months infantile spasms were observed. Head growth dropped from 0 SD before 6 months of age to −1.5 SD thereafter. She...