Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency in Dutch neonates


Departments of 1 Biomedical Genetics and 2 Molecular Genetics, Stichting Klinische Genetica Zuid-Oost Nederland; 3 Department of Pediatrics, Academic Hospital Maastricht; 4 University Medical Center Utrecht; 5 Department of Pediatrics, Academic Hospital Groningen, The Netherlands; 6 Division of Clinical Chemistry and Biochemistry, University Children’s Hospital, Zurich, Switzerland

*Correspondence: Department of Biomedical Genetics, Stichting Klinische Genetica Zuid-Oost Nederland, Joseph Bechlaan 113, 6229 GR Maastricht, The Netherlands. E-mail: leo.spaapen@gen.unimaas.nl

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Summary  Four neonates with a positive phenylalanine screening test (Phe concentrations between 258 and 1250 μmol/L) were investigated further to differentiate between phenylalanine hydroxylase (PAH) deficiency and variant hyperphenylalaninaemia (HPA) forms. In patients 1 and 2 a tetrahydrobiopterin (BH4) load caused a significant decrease of the plasma Phe levels. A combined phenylalanine/BH4 loading test was performed in patients 2, 3 and 4. In the latter two patients, plasma Phe concentrations completely normalized within 8 h after the BH4 load (20 mg/kg). Basal urinary pterins were normal in all four patients. The activity of dihydropteridine reductase (DHPR) was normal in patients 1, 2 and 3 and 50% of control values in patient 4 (not in the range of DHPR-deficient patients). In patient 3 a subsequent phenylalanine loading test with concomitant analysis of plasma biopterins revealed a normal increase of biopterin, excluding a BH4 biosynthesis defect. Pterins and neurotransmitter metabolites in CSF of patients 1, 3 and 4 were normal. DNA mutations detected in the PAH gene of patients 1–4 were A313T, and L367fsinsC; V190A and R243X; A300S and A403V; R241C and A403V. The results are suggestive for mutant PAH enzymes with decreased affinity for the cofactor BH4.

Hyperphenylalaninaemia (HPA) is a disorder caused by a deficient or a decreased activity of phenylalanine-4-hydroxylase (PAH, EC 1.14.16.1) due either to a
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mutated enzyme protein or to a deficiency of its obligatory cofactor tetrahydrobiopterin (BH₄). The latter group comprises defects in the biosynthesis and in the regeneration of BH₄ (Scriven et al 1995). Detection of HPA is included in the newborn mass screening programme. Differential diagnostic investigations are necessary, however to detect BH₄ deficiencies even if phenylalanine (Phe) concentrations are only slightly elevated (Ponzone et al 1993). Screening for BH₄ deficiency is performed by analysis of pterins in urine and measurement of dihydropyridine reductase (DHPR) activity in erythrocytes or skin fibroblasts (Blau and Blaszkovics 1996). In the Netherlands as well as in several other countries, a BH₄ loading test is included in the screening protocol of HPA. However, if the initial Phe concentration is below 400 μmol/L, a combined Phe/BH₄ loading is performed (Ponzone et al 1993). Recently, Kure and colleagues (1999) reported that serum Phe concentrations in four patients with mild HPA decreased after a BH₄ challenge. Urinary pterins in their patients and DHPR activities in blood appeared to be normal. In addition, mutations were detected in the PAH genes of those patients.

In the follow-up of a positive newborn PKU-screening test we found four children to be responsive after either a BH₄ challenge or a combined Phe/BH₄ loading test. DHPR deficiency was excluded and urinary pterins appeared to be normal. Mutations were found in the PAH genes of all these patients; thus this group of patients belongs to a new variant of BH₄-responsive PAH deficiency.

PATIENTS AND METHODS

Patient 1, a girl, was born prematurely after a pregnancy of 28 weeks because of maternal complications. Her birth weight was 750 g, she was dysmature and suffered from severe neonatal complications. Patients 2, 3 and 4 were born at term after uncomplicated pregnancies. They were admitted to the academic hospital for evaluation of a positive newborn PKU screening test. Combined Phe/BH₄ loading tests were performed according to Ponzone and colleagues (1993).

Amino acids were analysed by means of automated ion-exchange chromatography with postcolumn ninhydrin derivatization (Biochrom 20, Amersham Pharmacia Biotech). DHPR activity in erythrocytes was measured as described previously (Surplice et al 1990) or in cultured skin fibroblasts according to Bonafe and colleagues (2000). Urinary pterins were analysed by a HPLC procedure adapted from Fukushima and Nixon (1980) and Nixon and colleagues (1980). Neurotransmitter metabolites were analysed in CSF of patients 1, 3 and 4 as described (Blau et al 1999). Mutations in the PAH gene were detected by means of single-strand conformational analysis and subsequent sequence analysis (van der Sijs-Bos et al 1996).

RESULTS AND DISCUSSION

The positive PKU screening test in patient 1 was followed by a BH₄ load of 20 mg/kg body weight. Figure 1 shows the response of plasma Phe to the BH₄ load. Because of the rapid decrease of the plasma Phe concentration, treatment with BH₄ (5 mg/kg per day) was continued during the next 8 days. From day 9 the treatment was stopped.

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