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Short Communication

Prenatal Diagnosis of Dihydropteridine Reductase Deficiency in a Twin Pregnancy

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Introduction

Three inborn errors of metabolism are known to cause deficiency of tetrahydrobiopterin (BH_4), the natural cofactor of aromatic amino acid hydroxylases (1). Guanosine triphosphate cyclohydrolase I deficiency (Mc Kusick 23391) and 6-pyruvoyl tetrahydropterin synthase deficiency (Mc Kusick 26164) lead to impaired BH_4 biosynthesis, dihydropteridine reductase deficiency (DHPR; Mc Kusick 26263) prevents BH_4 from being regenerated after oxidation in the coupled reaction with hydroxylases. All these traits are inherited recessively. Patients develop, in addition to hyperphenylalaninemia, severe neurological symptoms due to biogenic amine deficiency, and they are not responsive to a phenylalanine restricted diet. Since the prognosis may be poor even if patients were treated early with diet and substitutive therapy, prenatal diagnosis was recently made possible following different procedures (2).

Problems inherent in prenatal diagnosis of a twin pregnancy at risk for DHPR deficiency are presented.

Case Report and Methods

Two second cousin parents, originally from Italy, known to be heterozygotes for DHPR deficiency since they had an affected girl (3) and an heterozygous boy, requested a prenatal diagnosis in the course of a third pregnancy.

An ultrasound scan was performed at 15 weeks and a twin gestation was visualized: a single posterior placenta with two amniotic sacs separated by a thin membrane were typical of a monozygotic pregnancy, and consistent with the male sex of both fetuses. In order to avoid the increased risk of repeated needle insertions, amniocentesis was performed after selecting a site in the maternal abdomen suitable to sample both the sacs without crossing the placenta. Under real-time ultrasound control, a 20-Gauge spinal needle was inserted transabdominally into the first amniotic sac (fetus A), 20 ml of amniotic fluid (AF) were removed, and 2 ml of indigo carmine were injected. The stylet was inserted and the amniotic membrane was crossed. A few drops of colorless AF confirmed that the second sac (fetus B) had been entered, but not a sufficient sample for pterin analysis and amniocyte culturing could be aspirated, probably because the fetus was moving. As the mother had already had a vaginal hemorrage, and the ultrasound picture was predictive for monozygotic gestation, a second insertion of the needle was not performed. However, the parents being informed of a residual 17% risk of a dizygotic pregnancy (4) chose to avoid any risk of fetus B being affected.

Cordocentesis was performed at 19 weeks gestation under ultrasound real-time visualization by the same technique employed for amniocentesis. Samples of fetal blood were obtained by transabdominal punc-

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Table 1. Pterins, neurotransmitter metabolites and amino acids in amniotic fluid, and DHPR activity in amniocytes and in fetal erythrocytes.

Fetus at risk	Weeks of gestation	amniotic fluid				amniotic fluid		DHPR activity	
		N (nmol/l)	B	5-HIAA	HVA	Phe (μmol/l)	Tyr	amnio-cytes*	erythro-cytes**
Fetus A	15	37	23	125	168	61	57	13.4	
	19	51	19	146	188	50	46		1.8
Fetus B	19	49	18	142	174	54	50		1.4
Normal values	15 / 23	29 / 62	12 / 29	92 / 135	50 / 144	<120	<109	22 / 49	2 / 5

* nmol NADH/min mg prot
** mU/mg Hb
N = neopterin; B = biopterin; 5-HIAA = 5-hydroxyindoleacetic acid; HVA = homovanillic acid

ture of the umbilical vein at the site of placental insertion for each fetus by two different needle insertions, and the purity of fetal blood was immediately controlled by Coulter Counter Channelyzer. Contextually, AF samples were taken from both sacs.

Pterins in AF were measured, after oxidation with manganese dioxide and subsequent deproteinization with trichloroacetic acid, by HPLC (5). 5-Hydroxyindolacetic acid (5-HIAA) and homovanillic acid (HVA) in AF were measured by HPLC with an ESA Coulochem 5100 A electrochemical detector (2). Amino acids were measured using a Biotronic ion-exchange amino acid analyser. DHPR activity was measured in amniocyte extracts obtained after 21 days of culturing and in fetal erythrocytes, as described by (6).

Spontaneous premature delivery of two phenotypically normal males occurred at 34 weeks gestation.

Results and Discussion

Biochemical analyses of AF were not informative for antenatal diagnosis, as the concentrations of amino acids, pterins and neurotransmitter metabolites were in the normal range (Table 1). The reduced DHPR activity detected in cultured amniocytes from fetus A, as well as in fetal erythrocytes from both fetuses, was consistent with their heterozygosity, as demonstrated after birth by the assay of enzyme activity in peripheral erythrocytes.

These findings confirm that in pregnancies at risk for DHPR deficiency the measurement of AF neopterin and biopterin allows the detection of only the affected fetus, whereas the enzyme assay on amniocytes or fetal erythrocytes is necessary to distinguish the heterozygous from the normal fetus. Neurotransmitter metabolite concentrations were normal in these cases as well as in the case of a fetus homozygous for DHPR deficiency (2). These findings might explain the absence in such patients of neonatal symptoms of biogenic amine deficiency, possibly because of dopamine and serotonin supply from the mother to the fetus throughout gestation.

The few prenatal diagnoses of DHPR deficiency till now have been performed in the second trimester of pregnancy. Since DHPR activity is expressed in all fetal tissues (7), either early amniocentesis or chorionic villus sampling will allow the first-trimester diagnosis. However, in twin gestations every procedure will be complicated by the need of repeated obstetric manoeuvres to exclude dizygosity even in front of an ultrasound appearance of monozygotic pregnancy, so still allowing all possible options.

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