

SHORT COMMUNICATION

Evaluation of a fetus at risk for dihydropteridine reductase deficiency by direct mutation analysis using denaturing gradient gel electrophoresis

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Dihydropteridine reductase (DHPR) is an enzyme involved in the recycling of tetrahydrobiopterin (BH₄), which is an obligate co-factor of the aromatic amino acid hydroxylases. DHPR deficiency is a rare, autosomal recessive disorder caused by mutations in the *QDPR* gene. DHPR-deficient patients are diagnosed by a lack of response to a low phenylalanine diet and by severe neurological symptoms. Final diagnosis is made by measurements of neurotransmitters and pterin metabolites in cerebrospinal fluid (CSF) and urine, in addition to DHPR enzyme activity, which can be assessed in whole red blood cells. Treatment of DHPR deficiency can be difficult and the outcome is not always satisfying, even if all treatment strategies are followed. Therefore prenatal diagnosis is of great importance in affected families. Prenatal diagnosis is possible by measuring DHPR activity in different cell types but this is time consuming. More than 25 different mutations have to date been identified in the *QDPR* gene and direct identification of a mutation in a fetus would be easy and rapid. We have developed a method based on denaturing gradient gel electrophoresis (DGGE) for the analysis of the *QDPR* gene. The method is useful for rapid and simultaneous scanning of all exons and flanking intronic sequences of the *QDPR* gene. We describe the first prenatal diagnosis conducted using this method. Copyright © 2001 John Wiley & Sons, Ltd.

KEY WORDS: dihydropteridine reductase (DHPR) deficiency; denaturing gradient gel electrophoresis (DGGE); prenatal diagnosis

INTRODUCTION

Dihydropteridine reductase (DHPR, E.C.1.6.99.7) is responsible for the regeneration of dihydrobiopterin (BH₂) formed during the hydroxylation of phenylalanine, tyrosine and tryptophan. DHPR deficiency (Phenylketonuria II; McKusick 261630) is an autosomal recessively inherited disease that accounts for approximately one-third of all tetrahydrobiopterin deficiencies (Blau and Dhondt, 2001). DHPR deficiency results in hyperphenylalaninemia (HPA) and deficiency of various neurotransmitters (dopamine, epinephrine and serotonin) in the central nervous system (Blau *et al.*, 2001a). The lack of neurotransmitters causes severe neurological symptoms such as psychomotor retardation, hypotonia of the trunk with limb hypertonia, myoclonic epilepsy and hypersalivation. The biochemical defect should be considered when a BH₄ loading test results in a decrease in phenylalanine blood levels in hyperphenylalaninemia patients with normal or high urinary excretion of biopterin and decreased levels of cerebrospinal fluid (CSF) neurotransmitter metabolites such as homovanillic acid and 5-hydroxyindoleacetic acid. DHPR

deficiency is caused by mutations in the gene coding for quinonoid dihydropteridine reductase (*QDPR*). The ultimate diagnosis relies on DHPR enzyme activity combined with mutation analysis of the *QDPR* gene. To date more than 25 different mutations have been identified and registered in the BIOMDB database (Blau *et al.*, 2001b). Treatment of DHPR deficiency implies replacement with neurotransmitter precursors and a low phenylalanine diet, but the treatment is not always successful and the disease is often fatal. Prenatal diagnosis is therefore an important issue in this disease.

Prenatal diagnosis of DHPR deficiency has previously been carried out by measuring DHPR enzyme activity in cord blood samples, cultured amniotic cells or cells cultured from chorionic villus samples (CVS) (Firgaira *et al.*, 1983). Although these methods are useful, direct mutation detection may provide a faster and more accurate diagnosis (Smooker *et al.*, 1993). We have recently described a rapid, reliable and sensitive screening method for the detection of DHPR mutations based on denaturing gradient gel electrophoresis (DGGE) followed by sequencing (Romstad *et al.*, 2000), and have demonstrated the feasibility of the method by genotyping 16 Turkish families with DHPR deficiency. The present report describes the first prenatal diagnosis of DHPR deficiency based on mutation analysis using DGGE.

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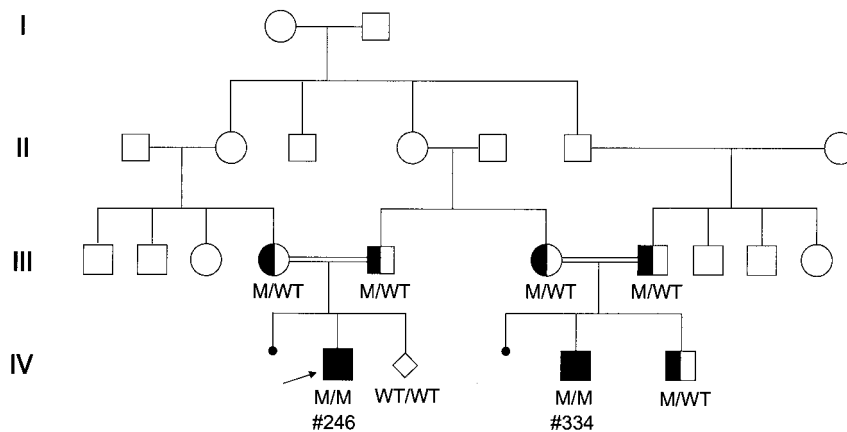


Figure 1—Pedigree of the family examined in the present study. M represents the mutant allele, carrying the mutation W90X, while WT represents the wild-type allele. The numbers #246 and #334 refer to identification numbers from the International Database of Tetrahydrobiopterin Deficiencies (BIODEF) (Blau and Dhondt, 2001)

PATIENTS AND METHODS

A Turkish couple with a child with DHPR deficiency (BIODEF#246) requested prenatal diagnosis. A cousin of the index case is also affected by DHPR deficiency (BIODEF#334) (Figure 1). CVS (20 mg sample) was performed in the 8th week of pregnancy. DNA was extracted directly from the CVS using a commercial kit (DNA Isolation Kit, Puregene, Gentra Inc., Minneapolis, Minnesota, USA). Polymerase chain reaction (PCR) for DGGE analysis of the exons was performed using previously published primers and conditions (Romstad *et al.*, 2000). Samples (25 μ l) were loaded on the denaturing gel consisting of a linear polyacrylamide gradient ranging from 6% to 9%, and a linear denaturing gradient ranging from 0% to 100% (100% denaturant is 7.0 M urea and 400 ml/l formamide). In the analysis, DNA from the father, the mother, the index case (BIODEF#246), the CVS sample and a normal control were included. Based on the DGGE profile, the exon was subjected to direct sequencing. To confirm the maternity and to exclude maternal contamination, analyses of eight polymorphic markers (HUMD21S11, HUMVWA, HUMFES, HUMTH01, HUMF13A1, HUMGABARBI, HUMAPOAI1 and

HUMACTBP2) were also carried out (Imboden *et al.*, 1993; Kimpton *et al.*, 1996).

RESULTS AND DISCUSSION

Using PCR in combination with DGGE, DNA from the father, the mother, the index case and the CVS sample was analysed for the presence of mutations in the *QDPR* gene. As shown in Figure 2A, both heterozygous parents (lanes 1 and 2) showed a four-band DGGE pattern in exon 3, corresponding to the wild-type allele, the mutant allele, and the two heteroduplexes. Following sequencing of exon 3, the mutation was found to be W90X (350G>A, NCBI Accession No. NM_000320). Lane 3 shows the index case, which is homozygous for the mutant allele and forms a single, but shifted band in the gel compared with the normal control (lane 5). The CVS sample (lane 4) formed a single band at the position corresponding to the wild-type allele. Thus, the fetus has inherited both wild-type alleles and is completely healthy. Analysis of polymorphic markers confirmed maternity, and did at the same time exclude a heavy contamination with maternal tissue in the CVS sample. Figure 2B shows the result from analysis of two of the polymorphic microsatellite markers.

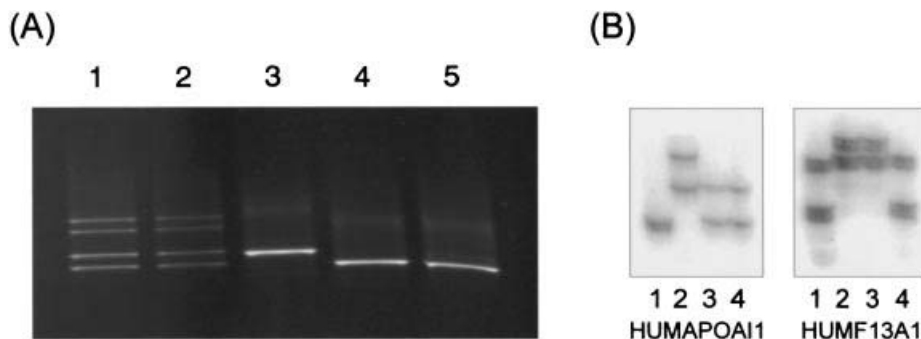


Figure 2—(A) DGGE analysis of *QDPR* exon 3. Lane 1, father heterozygous for W90X; lane 2, mother heterozygous for W90X; lane 3, index case BIODEF#246 homozygous for W90X; lane 4, CVS; and lane 5, normal control sample. (B) Analysis of microsatellite markers HUMAPOI1 and HUMF13A1. Lane 1, father; lane 2, mother; lane 3, index BIODEF#246; and lane 4, CVS

The W90X mutation is a nonsense mutation, causing a stop-codon due to the exchange of G to an A at codon 90 (TGG→TGA). The translated protein is thus shortened by 155 amino acids. The index patient and his cousin have a severe phenotype (determined from the level of treatment required) but have exhibited a good response to therapy.

Direct mutation analysis by DGGE is an accurate and rapid method for detection of carriers and for prenatal diagnosis in DHPR deficiency. Additional sequencing is not necessary, but was performed in the present case as a control due to the homoallelic constellation. Knowing the mutations in the family in advance facilitates a very rapid analysis for carrier status. The present example serves to illustrate the potential of direct gene analysis by DGGE in a case of prenatal diagnosis for a severe metabolic disease.

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