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Short Communication

Genotype–phenotype correlation in dihydropteridine reductase deficiency

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Inherited deficiency of dihydropteridine reductase (DHPR, EC 1.66.99.7) impairs the regeneration of tetrahydrobiopterin (BH_4), the essential cofactor of phenylalanine (Phe) (PAH, EC 1.14.16.1), tyrosine (Tyr) (TYH, EC 1.14.16.2) and tryptophan (Trp) (TRH, EC 1.14.16.4) hydroxylases, which is oxidized to qBH_2 during a coupled reaction with these enzymes. The main metabolic derangements caused by DHPR deficiency (McKusick 261630) are hyperphenylalaninaemia and impaired production of monoamine neurotransmitters derived from Tyr and Trp — dopamine, noradrenaline and serotonin. Untreated patients can early develop a severe and progressive neurological picture (Blau et al 1996a). The control of hyperphenylalaninaemia and biogenic amine deficiency is necessary to improve their prognosis, together with folinic acid supplementation to avoid folate depletion (Spada et al 1996). However, in some DHPR patients a milder phenotype has been described characterized by absent neurological signs. These patients respond to a BH_4 monotherapy or do not require any treatment (Blau et al 1992).

The DHPR gene (QDPR), on 4p15.3, includes seven exons (Dianzani et al 1998). It encodes for a protein of 244 amino acids, active as a homodimer. So far, 21 mutations have been described in QDPR uniformly scattered throughout the coding region (Dianzani et al 1998; Smooker et al 1999), with different mutations having

different effects on the protein, as determined by *in vitro* studies (Smooker et al 1993; Zhang et al 1996). Most mutations have been found in single chromosomes. So far, only five of them have been identified more than once (de Sanctis et al 1996).

In the present study we evaluated genotype–phenotype correlation in 21 completely characterized DHPR patients. Molecular, biochemical and clinical data on DHPR-deficient patients are stored in the BIOMDB and BIODEF database ⟨http://www.unizh.ch/%7eblau/biomdb1.html⟩ (Blau et al 1996b).

PHENOTYPE AND MOLECULAR CHARACTERIZATION

In the 21 patients included in the present study, hyperphenylalaninaemia was detected at neonatal mass screening or later and a definite diagnosis of DHPR deficiency was obtained by measurement of urinary pterins and enzyme activity on dried blood spots.

In the attempt to distinguish the different phenotypes, the following clinical and biochemical parameters were evaluated, when available: pretreatment blood Phe concentration; neurotransmitter metabolite concentrations in cerebrospinal fluid (CSF); CSF pterin level; urinary pterin excretion; response to oral BH_4 loading test (7.5 or 20 mg BH_4 per kg body weight); response to the combined Phe (100 mg/kg body weight) and BH_4 oral loading (Ponzone et al 1993); DHPR activity in erythrocytes and/or in fibroblasts; response to treatment and type of treatment required (Blau et al 1996a).

The molecular characterization in 18 of the 21 patients considered was reported previously (Dianzani et al 1998; Smooker et al 1999): 15 patients were mutation homozygotes, whereas the remaining three patients were compound heterozygotes for different mutations. Thus, in this study we included the six patients homozygous for the G23D mutation, three for L14P, two for H158Y, two for R221X, two for IVS5G^{+1} > A, one for IVS4G^{-1} > A, one for G151S and one for F212C. The other patients considered were compound heterozygotes for R221X/G18V, R221X/L14P and IVS5G^{+1} > A/G218ins9bp mutations.

Additionally, in three unrelated patients from Malta, genomic DNA was extracted from peripheral blood leukocytes, using the phenol–chloroform method. Because the G23D mutation had been found in another patient from Malta belonging to this series, the G23D mutation has been searched for. Exon 1 PCR amplification was performed by using the appropriate primers. The amplified products were incubated at 37°C with the specific endonuclease *Hin*fI. When a G-to-A change occurs at codon 23, a *Hin*fI restriction site is created in the 106 bp amplified product, yielding two fragments of 68 and 38 bp (de Sanctis 1996).

RESULTS AND DISCUSSION

The clinical aspects of each patient are correlated with the responsible mutations in Table 1. Lack of standardization in the procedures employed for screening, diagnosis and treatment, however, hampered a full definition of phenotype and prompted us to use the need for and the type of treatment as the main phenotypic

Table 1 Genotype–phenotype correlation in patients with DHPR deficiency

Mutations	*BIODEF no.*	*Phenotype*[a]	*DHPR activity in RBC; fibroblasts* (%)	*Functional defect*
G23D*/G23D* ($n = 6$)	132–150–202	Severe	0; 0	Binding site for NADH
L14P/L14P ($n = 3$)	—	Severe	0; 0	Protein rapid degradation
H158Y*/H158Y* ($n = 2$)	153–165	Severe	0; 0	Increased susceptibility to proteases
R221X/R221X ($n = 2$)	255–300	Severe	0; ND	Truncated protein
IVS5G^{+1} > A/IVS5G^{+1} > A ($n = 2$)	128	Severe	ND; 0	Probable truncated protein
IVS4G^{-1} > C/IVS4G^{-1} > C ($n = 1$)	131	Severe	0; ND	Probable truncated protein
F212C/F212C ($n = 1$)	175	Mild	0; 10	?
G151S*/G151S* ($n = 1$)	198	Mild	0; 4	Mildly reduced activity
R221X/G18V ($n =$)	151	Severe	0; ND	Truncated protein/ binding site for NADH
R221X/L14P ($n = 1$)	—	Severe	0; ND	Truncated protein/ protein rapid degradation
G23D*/Y150C* ($n = 1$)	174	Intermediate	0; ND	Binding site for NADH/ mildly reduced activity
IVS5G^{+1} > A/ G218ins9bp ($n = 1$)	289	Mild or intermediate	0; ND	Probable truncated protein/?

RBC, red blood cells; ND, not determined; *, mutants expressed *in vitro*; ?, not identified or not studied
[a] Clinical phenotype was assessed on the basis of the need for partial, complete or no treatment (see text)

discriminants. Thus, patients were classified into three phenotypes — severe, intermediate and mild — on the basis of the need, respectively, for complete or partial treatment or no treatment at all.

Eighteen patients with a severe phenotype (needing a complete therapy with neurotransmitter precursors and BH_4, besides folinic acid), with no DHPR activity either in red blood cells or in fibroblasts, harboured mutations that grossly alter the enzyme structure, as observed by *in vitro* expression of mutant enzymes (Smooker et al 1993) and inferred by the molecular abnormalities (Dianzani et al 1998). In particular, a severe phenotype has been identified in the six patients homozygous for G23D, in three for L14P, in two for H158Y, in two for R221X, in two for IVS5G^{+1} > A, in one for IVS4G^{-1} > A, and in a compound heterozygote for R221X/G18V mutations (Table 1).

The G23D mutation affects the binding site for NADH, whereas H158Y causes a disruption of the overall protein structure. Both mutations deeply inhibit the enzymatic activity in *in vitro* expression studies (Smooker et al 1993). The nonsense mutation R221X results in a presumably inactive truncated protein.

L14P does not affect the maturation of the mRNA, as full-length RNA is detected by RT-PCR. However, no protein is detectable by immunoprecipitation; therefore either the mRNA cannot be translated or the protein is unstable and subject to rapid degradation, as observed in other nonconservative mutations (Dianzani et al 1998; Smooker et al 1993).

IVS5G^{+1} > A and IVS4G^{-1} > A are splice site mutations that result in the skipping of exon 5 with subsequent frameshift, addition of six nonsense amino acids after residue 146 and premature termination. The mutations would presumably lead to an inactive protein product if translated (Dianzani et al 1998).

Finally, G18V is a missense mutation located in the binding site for NADH (Varughese et al 1992); this mutation may have the same functional effect as G23D.

The four patients with a milder phenotype (needing BH_4 monotherapy only or no treatment at all) are homozygotes for F212C or G151S mutations or compound heterozygotes for G23D/Y150C or IVS5G^{+1} > A/G218ins9bp. The first two patients did not show enzymatic activity in red blood cells but had residual activity in fibroblasts of 4% and 10%, respectively. G151S mutation involves a conservative substitution (Gly to Ser). The mutant enzyme has been expressed *in vitro*, showing that, although the mutation has affected the kinetic parameters, it has not seriously impaired the enzyme activity (Dianzani et al 1998). The other mutant enzyme has not yet been expressed. However, F212C is a substitution that may not grossly alter the protein function.

Y150C affects a Tyr considered to be involved in the pterin binding site and thus essential for the reduction process. Moreover, this Tyr is part of a consensus TyrXXXLys. This sequence is highly conserved among the class of short-chain dehydrogenases, which includes DHPR (Varughese et al 1992, 1994). The mutant enzyme for Y150C was expressed in an *E. coli* system. The kinetic parameters of the Y150C mutant DHPR showed that it is not as effective as the wild-type enzyme but is better than the G23D mutant (Zhang et al 1996).

Finally, the G218ins9bp, which does not disrupt the frame, may not grossly alter the enzyme activity. Expression of this mutant enzyme in an *in vitro* system may provide the only way to confirm this hypothesis (Dianzani et al 1998).

The milder phenotype in the compound heterozygotes studied is most probably due to the mild Y150C and G218ins9bp mutations. Thus, patients who harbour a severe mutation that grossly alters the protein structure/function, in compound heterozygosity with a mild mutation that does not grossly alter the protein structure/function, have a milder phenotype, showing that the mild mutation has a dominant effect, as observed in PKU (Guldberg et al 1998).

In conclusion, these data further confirm the possibility of drawing genotype–phenotype correlations in DHPR deficiency.

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