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Mutations and Phenotypes in Dihydropteridine Reductase Deficiency in Italy

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Introduction

Dihydropteridine reductase (DHPR: E.C. 1.6.99.7) is an essential enzyme in the hydroxylating system of the aromatic amino acids and catalyses the regeneration of tetrahydrobiopterin (BH_4), the natural cofactor of phenylalanine, tyrosine and tryptophan hydroxylases.

DHPR deficiency has been the first defect described (1) and represents the second most common defect among BH_4 deficiencies, covering about one third of all forms (2).

The cDNA encoding DHPR has been cloned in 1987 (3,4) and is about 1.2 Kb long. The gene has been assigned to the short arm of chromosome 4, in the region 15.3. It encodes for a protein of 244 amino acid, active as a homodimer.

So far, sixteen mutations have been described: fourteen mutations are uniformly scattered throughout the coding region (5-9); two further mutations have been identified in the donor splice sites of introns 3 and 4, respectively (8). The majority of mutations are missense mutations, with only two resulting in premature termination of the peptide chain. A single insertion of one amino acid (5) and a frameshift due to one base deletion, which also affects splicing (9), have also been described. Most mutations have been found in single chromosomes. Only three of them have been identified more than once. IVS 4ntl has been found in two patients from United States (8, Dianzani I et al., manuscript in preparation). The others, G23D and H158Y, have been identified in Italian patients (6,7).

We report here the distribution of the DHPR mutations in the Italian population. Moreover, since DHPR deficiency is a heterogeneous disease at clinical level, we attempted to evaluate genotype/phenotype correlation in a subset of completely characterized patients.

Materials and Methods

Patients

Nine unrelated Italian patients with DHPR deficiency were included in this study. Moreover, we considered a patient from Malta, whose clinical status and genotype were reported in previous studies (10,6).

Hyperphenylalaninemia 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 spot.

Phenotype determination

In the attempt to distinguish the different pheno-

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types, some clinical and biochemical parameters were evaluated, when available: pretreatment blood phenylalanine concentration, neurotransmitter metabolites concentrations in cerebrospinal fluid (CSF), CSF pterin levels, urinary pterin excretion, response to oral BH_4 loading test (7.5 or 20 mg/kg body weight BH_4), response to the combined phenylalanine (100 mg/kg body weight) and BH_4 (20 mg/kg body weight) oral loading (11), DHPR activity in erythrocytes and/or in fibroblasts, response to treatment (12).

DNA analysis

Genomic DNA was extracted from peripheral blood leucocytes, using the phenol-chloroform method. G23D and H158Y mutations were searched for in all patients. PCR amplifications of genomic DNA were performed by using appropriate primers. In particular, to detect G23D PCR was performed with primers 5'-CCAGTTGCGGGCCCGAAAAG-3' and 5'-GCGTGGATCCGCGGCGGCTGCA-3'. The amplified product was then incubated at 37°C with the specific endonuclease Hinf I. When a G->A change occurs at codon 23, a Hinf I restriction site is created in the 106 bp amplified product, yielding two fragments of 68 and 38 bp. To detect H158Y, a PCR was performed with primers 5'-AGTGGTCACTGAGCCATCT-3' and 5'-ACGGGAACCCCAAGCACTT-3'. The amplified product was then incubated at 37°C with the specific endonuclease Hph I. When a C->T change occurs at codon 158, a Hph I restriction site is abolished in the 166 bp amplified product, yielding a fragment of 166 bp, instead of two fragments of 104 and 62 bp.

Results

Molecular characterization

The molecular characterization of four patients was reported previously (6,7): two patients were homozygous for G23D, one was homozygous for H158Y and the last patient was a compound heterozygous for G23D and a new identified mutation, Y150C. This mutation was identified in a larger analysis of the DHPR coding sequence (Dianzani I. et al, manuscript in preparation). By enzyme digestion a further patient was found homozygote for G23D and another homozygote for H158Y.

Three remaining patients, who did not carry either G23D or H158Y, will be subjected to an extensive exon-screening study to identify the causal mutations. Geographic origin of the characterized chromosomes could be ascertained: G23D was found in one patient from Sicily, two from Apulia, and in one from Malta (in total 7 chromosomes); H158Y was found in two patients from Sicily (4 chromosomes in total). We could not establish the Italian origin of the new identified mutation, Y150C, because paternity in this patient has not been confirmed.

Phenotype classification

In Table 1 the clinical aspects of each patients are correlated to the responsible mutations. Since DHPR deficiency is very heterogeneous clinically, we evaluated all available clinical and biochemical parameters to attempt a phenotype classification. A fully definition of phenotype, however, is hampered by differences in procedures employed for screening and diagnosis, as well as for treatment. Moreover, the response to the BH_4 oral load and to the combined phenylalanine + BH_4 oral load could not be compared, because of lack standardization of these tests.

Thus, patients were classified into three phenotypes, severe, intermediate, and mild, on the basis of the need for complete or partial or none treatment, respectively. Five patients with a severe phenotype (needing a complete therapy with neurotrasmitter precursors and BH_4) lacked DHPR activity either in red blood cells or in fibroblasts and harboured mutations G23D and H158Y in

Table 1. Genotype/phenotype correlation in DHPR deficiency in Italy.

Mutation	Phenotype	DHPR activity (RBC-Fibroblasts)	Functional defect	BH_4 oral load (mg/kg)
G23D/G23D (n=3)	Severe	0%-0%	Binding site for NADH	Response only to 20
H158Y/H158Y (n=2)	Severe	0%-0%	Increased susceptibility to proteases	Response only to 20
G23D/Y150C (n=1)	Intermediate	0%-ND	G23D: Binding site for NADH/Y150C:?	Respones only to 20

n=number of patients; ND=Not Determined; RBC=Red Blood Cells

homozygosity.

The single patient with an intermediate phenotype (needing BH_4 monotherapy only) is a compound heterozygote for G23D and Y150C.

Discussion

In this study we attempted to identify the molecular basis of DHPR deficiency in Italy. So far three mutations have been identified in Italian patients: two of them, G23D and H158Y, are associated to a severe phenotype. G23D affects the binding site for NADH, whereas H158Y causes disruption of the overall protein structure. Both mutations deeply inhibit the enzymatic activity in in vitro expression studies (6,13). It is noteworthy that the single patient with an intermediate phenotype is a compound heterozygote for G23D and Y150C. Thus, the milder phenotype showed by this patient is probably due to a less severe functional damage induced by the concurrent Y150C mutation. Y150C affects a tyrosine considered to be involved in the pterin binding site, thus essential for the reduction process (14). Moreover, this tyrosine is part of a consensus TyrXXXLys. This sequence is highly conserved among the class of short chain dehydrogenases, which includes DHPR (15).

Two patients homozygous for G151S and F212C mutations, previously described by Blau (16), could be considered to have a mild form, by our classification. They did not require treatment at all or needed only BH_4 treatment. They did not show enzymatic activity in red blood cells, but had a residual activity in fibroblasts, of 4 and 10%, respectively. These two mutant enzymes have not been expressed in in vitro system. G151S mutation involves a conservative substitution (Gly to Ser), that might explain the mild disease state in this patient. In conclusion, two mutations, G23D and H158Y, account together for 55% of DHPR deficient patients in Italy. It is interesting to note that a cluster of DHPR deficient patients had been identified in Southern Italy and the same distribution had been found also for PTPS deficiency (17). Either heterozygote advantage or genetic drift could be hypothesized to explain the clustering of BH_4 defects in Italy, but neither of these mechanisms has been demonstrated so far.

Our data suggest that a small number of mutations accounts for most cases of DHPR deficiency in Italy. Accordingly, clinical phenotype results more homogeneous as compared to other populations.

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