

MUTATION UPDATE

Mutations in the BH₄-Metabolizing Genes GTP Cyclohydrolase I, 6-Pyruvoyl-Tetrahydropterin Synthase, Sepiapterin Reductase, Carbinolamine-4a-Dehydratase, and Dihydropteridine Reductase

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Tetrahydrobiopterin (BH₄) deficiencies are a highly heterogeneous group of disorders with several hundred patients, and so far a total of 193 different mutant alleles or molecular lesions identified in the GTP cyclohydrolase I (GTPCH), 6-pyruvoyl-tetrahydropterin synthase (PTPS), sepiapterin reductase (SR), carbinolamine-4a-dehydratase (PCD), or dihydropteridine reductase (DHPR) genes. The spectrum of mutations causing a reduction in one of the three biosynthetic (GTPCH, PTPS, and SR) or the two regenerating enzymes (PCD and DHPR) is tabulated and reviewed. Furthermore, current genomic variations or SNPs are also compiled. Mutations in *GCH1* are scattered over the entire gene, and only 5 out of 104 mutant alleles, present in a homozygous state, are reported to cause the autosomal recessive form of inheritable hyperphenylalaninemia (HPA) associated with monoamine neurotransmitter deficiency. Almost all other 99 different mutant alleles in *GCH1* are observed together with a wild-type allele and cause Dopa-responsive dystonia (DRD, Segawa disease) in a dominant fashion with reduced penetrance. Compound heterozygous or homozygous mutations are spread over the entire genes for *PTS* with 44 mutant alleles, for *PCBD* with nine mutant alleles, and for *QDPR* with 29 mutant alleles. These mutations cause an autosomal recessive inherited form of HPA, mostly accompanied by a deficiency of the neurotransmitters dopamine and serotonin. Lack of sepiapterin reductase activity, an autosomal recessive variant of BH₄ deficiency presenting without HPA, was diagnosed in patients with seven different mutant alleles in the *SPR* gene in exons 2 or 3 or in intron 2. Details on all mutations presented here are constantly updated in the BIOMDB database (www.bh4.org). *Hum Mutat* 27(9), 870–878, 2006. © 2006 Wiley-Liss, Inc.

KEY WORDS: tetrahydrobiopterin deficiency; hyperphenylalaninemia; dopa-responsive dystonia; Segawa disease; monoamine neurotransmitter deficiency; *GCH1*; *PTS*; *SPR*; *PCBD*; *QDPR*; *CBR1*; *AKR1B1*; *AKR1C3*

INTRODUCTION

The tetrahydrobiopterin (BH₄) cofactor is essential for various enzyme activities, and is involved in a number of less defined functions on the cellular level (Blau, 2006). The enzymes that depend on BH₄ are the phenylalanine, tyrosine, and tryptophan hydroxylases, all three NO synthase (NOS) isoforms, and the glyceryl-ether monooxygenase [Thöny, 2006; Thöny et al., 2000]. The de novo biosynthesis pathway of BH₄ from GTP involves GTP cyclohydrolase I (GTPCH, gene symbol *GCH1*; MIM#s 233910 and 600225), 6-pyruvoyl-tetrahydropterin synthase (PTPS, gene symbol *PTS*; MIM# 261640), and sepiapterin reductase (SR, gene symbol *SPR*; MIM# 182125). Three additional enzymes can replace SR by catalyzing the last two reduction steps: aldose reductase (AR, gene symbol *AKR1B1*; MIM# 103880), carbonyl reductase (CR, gene symbol *CBR1*; MIM# 114830), and 3 α -hydroxysteroid dehydrogenase type 2 (HSDH2, gene symbol *AKR1C3*; MIM# 603996) [Iino et al., 2003; Park et al., 1991]. Cofactor regeneration requires pterin-4a-carbinolamine dehydratase (PCD, gene symbol *PCBD*, MIM#s 264070 and 126090) and dihydropteridine reductase (DHPR, gene symbol *QDPR*; MIM#

261630). PCD was alternatively termed DCoH, for dimerization cofactor of hepatocyte nuclear factor 1 α (HNF-1 α), as it has been shown to be a protein with dual function [Mendel et al., 1991]. An overview on the BH₄-metabolic enzymes, their corresponding genes, and MIM numbers is given in Table 1.

BH₄ deficiencies, a group of rare inherited neurological diseases with catecholamine and serotonin deficiency, may present

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phenotypically with or without hyperphenylalaninemia (HPA) [Blau et al., 2001b]. They are a heterogenous group of diseases affecting either all organs, including the central nervous system, only the peripheral hepatic phenylalanine hydroxylating system, or only the nervous system. BH₄ deficiency presenting with HPA can be caused by mutations in genes encoding the enzymes involved in its biosynthesis (GTPCH and PTPS) [Thöny and Blau, 1997] or regeneration (PCD/DCoH and DHPR) [Dianzani et al., 1998; Thöny et al., 1998b]. The mutations are all inherited in an autosomal recessive fashion. The autosomal dominant inherited form of GTPCH deficiency (*adGTPCH*; Dopa-responsive dystonia; DRD), initially described as Segawa disease [Segawa et al., 1976], together with SR deficiency [Bonafé et al., 2001] present both without elevated plasma phenylalanine levels in infancy, and thus, in contrast to classical BH₄ deficiencies, can not be detected through the newborn screening for phenylketonuria (PKU).

The clinical course of the illness is similar in untreated patients with severe forms of GTPCH, PTPS, and DHPR deficiencies. The variable but common symptoms are mental retardation, convulsions (grand mal or myoclonic attacks), disturbance of tone and posture, drowsiness, irritability, abnormal movements, recurrent hyperthermia without infections, hypersalivation, and swallowing difficulties. The prominent phenotype in *adGTPCH* deficiency is childhood onset, limb-predominant dystonia. The DRD phenotype may be encompassed by a number of atypical presentations,

including Parkinsonism, spastic paraplegia, and a presentation mimicking athetoid cerebral palsy. Dystonia results from a reduction of BH₄ production, responsible for low cerebrospinal fluid (CSF) dopamine concentrations and reduced striatal tyrosine hydroxylase protein concentrations and activity. The clinical features of patients with SR deficiency combine signs and symptoms found both in classical BH₄ deficiencies and an *adGTPCH* deficiency.

In 1997, we published a first *Mutation Update* with different disease-causing mutations, 19 in *GCHI* and 16 in *PTS* from almost 50 patients [Thöny and Blau, 1997]. Since then, biochemical, clinical, and DNA data of patients with BH₄ deficiencies have been periodically updated and tabulated in the BIODEF and BIOMDB databases (available at www.bh4.org). Correspondingly, in the new and extended version of a *Mutation Update/Database* for BH₄ deficiencies presented herein, we tabulated, and discussed to some extent, data from these two electronic databases, including all types of genetic deficiencies with 193 mutant alleles identified in several hundred patients.

Mutations Within the *GCHI* Gene

Cloning of the human GTPCH-cDNA and structural analysis of the human *GCHI* gene enabled detection of the first mutations, two missense R184H and M211I and one nonsense mutation Q110X, causing BH₄-deficient HPA (Blau et al., 1995).

TABLE 1. BH₄-Metabolic Enzymes, Their Corresponding Genes, OMIM Entries, and Chromosomal Location

Enzyme	E.C. number	OMIM	Gene symbol	Number of exons	Chromosome location
GTPCH	3.5.4.16	233910; 600225	<i>GCHI</i>	6	14q22.1–q22.2
PTPS	4.6.1.10	261640	<i>PTS</i>	6	11q22.3–q23.3
SR	1.1.1.153	182125	<i>SPR</i>	3	2p13
PCD/DCoH	4.2.1.96	264070;126090	<i>PCBD</i>	4	10q22
DHPR	1.6.99.7	261630	<i>QDPR</i>	7	4p15.3

References: Brown and Dahl [1987]; Ichinose et al. [1994]; Thöny et al. [1994a, 1995a].

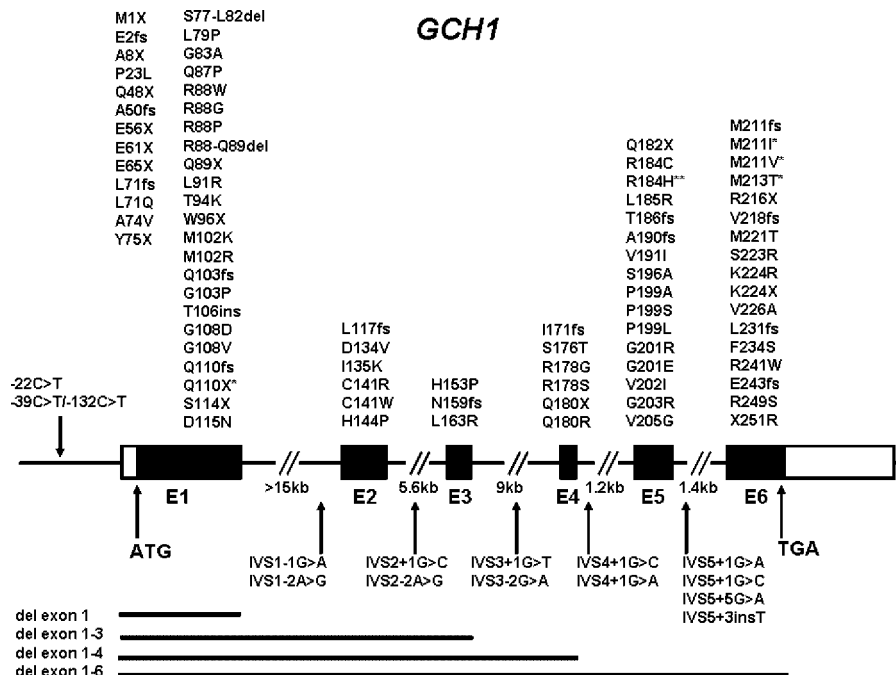


FIGURE 1. Genomic structure and location of mutations in the human *GCHI* gene. *Mutations detected in patients presenting with HPA; **Mutation detected in patients presenting both with and without HPA.

disease-causing mutant alleles isolated from approximately 250 PTPS-deficient patients. These mutations are distributed across all six exons and the first three introns, and no hotspots for mutations are found, although two mutations, N52S and P87S, appear to be relatively frequent in the Asian population. Among the exonic mutations there are one insertion (R16ins), five deletions, and 33 substitutions. The four splice-site mutations are distributed in the first three introns, two of them lead to skipping of exon 3 (IVS2–7T>A and IVS3+1G>A), whereas IVS1–323A>T leads to insertion of a putative pseudoexon as revealed by analyzing the corresponding cDNA (unpublished results). Note that the E81E mutation is not a polymorphism, but rather leads to a splicing defect and skipping of exon 4 (see also below).

A total of 27 mutant alleles in PTS (~63%) are associated with HPA and the severe or central phenotype, i.e., presenting with catecholamine and serotonin neurotransmitter deficiency. The other approximately 37% of mutations does not fit the expected phenotype with PHA and neurotransmitter deficiency. They either cause only HPA, the so called mild or peripheral phenotype (R16C, L26F, K40fs, Y99X, Y113C, K121fs, and V124L), or a mixed phenotype with HPA and severe or mild neurotransmitter deficiency, depending on the individual patient background (R25G), or an isolated transient form of HPA (N47D, D116G, and N138H), an isolated form of HPA with progression to a central phenotype (K129E), or an isolated central form associated with hemizyosity (Y99C). Of three mutations we have no information on whether they cause only HPA or the severe combination with neurotransmitter deficiency (F40L, N72L, and G144R).

Expression data are available from approximately one-half of all mutations as single PTPS alleles (see Supplementary Table S2). However, there is no correlation from these limited data available with the patients' phenotypes. Furthermore, one has to bear in mind that the mutant PTPS can be present as homozygous or more commonly as compound heterozygous alleles (see Supplementary Table S2), and that mutant alleles, when present in the compound heterozygous state, may influence each other. For instance, the N47D allele is present in a compound heterozygous state with D116G and was found to influence its allelic counterpart when coexpressed in COS-1 cells by exhibiting a dominant negative effect on D116G (and also on wild-type PTPS). In at least one other case, the mutant allele R16C seems not to be fully active as it cannot be phosphorylated, i.e., it is not recognized by a protein kinase modifier. Taken together, it is not clear whether expression studies will elucidate a correlation between genotype and phenotype, a situation that is reminiscent to a number of other genetic diseases with poor genotype–phenotype correlation.

MUTATIONS WITHIN THE *SPR* GENE

SR deficiency was the most recent discovery of a genetic disorder of BH₄ metabolism, inherited in an autosomal recessive fashion and characterized by monoamine neurotransmitter deficiency without HPA [Bonafé et al., 2001]. The finding of a defective cofactor biosynthesis without HPA led to the postulation of an alternative pathway for BH₄ in peripheral tissues [Blau et al., 2001a]. Since this first observation of two patients, 12 additional affected subjects have been described (Supplementary Table S3) [Abeling et al., 2003; Elzaouk et al., 2002; Neville et al., 2005; Steinberger et al., 2004]. Thus far, seven different disease-causing alleles are known, four located in exon 2 (Q119X, R150fs, R150G, and P163L), one in exon 3 (K251X), one in intron 2 (IVS2–2A>G), and one in a 5'-untranslated region (UTR)

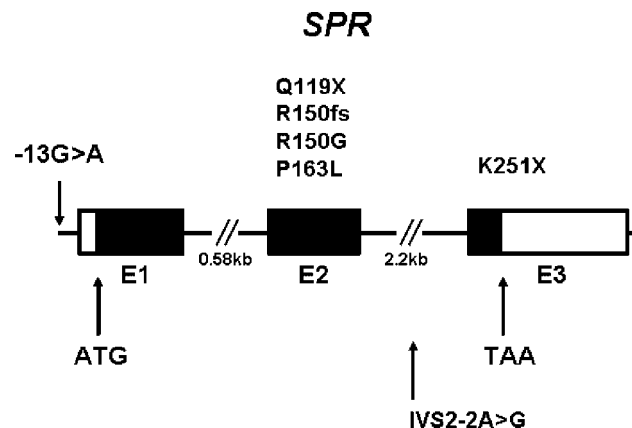


FIGURE 3. Genomic structure and location of mutations in the human *SPR* gene.

(–13G>A) (Fig. 3). Whereas two of the four mutations in exon 2 cause premature stop codons, the other two generate an amino acid exchange. Enzymatic activity in primary fibroblasts from patients are available for four mutant *SPR* alleles that are present in homozygous or heterozygous state (Q119X, R150fs, R150G, and –13G>A) and are all, with exception of –13G>A, completely inactive. Fibroblasts from patient with the –13G>A mutations showed 62% residual activity and 39% of the protein, compared with the wild-type control [Steinberger et al., 2004]. Recombinant expression of mutant *SPR* gene in *E. coli* was also tested for R150G, resulting in an inactive protein [Bonafé et al., 2001].

Thus far 15 patients with SR deficiency have been identified; however, it is believed that there are probably more. Such affected individuals might be relatively difficult to diagnose since they do not show abnormal plasma phenylalanine levels as in classical BH₄ deficiency, which is commonly detected by newborn screening programs. Besides, such patients have a broad variation of signs and phenotypes, which makes differential diagnosis even more difficult. As an initial laboratory diagnosis the determination of dopamine and serotonin metabolites from cerebrospinal fluids is recommended [Blau et al., 2001a]. The IVS2–2A>G mutation at the splice site consensus sequence in intron 2 of the *SPR* gene was found to be the sole causative (founder) mutation in a number of Maltese patients initially diagnosed as DRD [Farrugia and Felice, 2004].

Mutations Within the *PCBD* Gene

PCD/DCoH was originally detected as a contaminant in a preparation of rat PAH as a consequence of its ability to stimulate the BH₄-dependent hydroxylation of phenylalanine [Kaufman, 1970]. This stimulating protein was subsequently purified from rat liver [Huang et al., 1973] and its activity was shown to be due to the catalysis of dehydration of the 4a-carbinolamine intermediate. The human PCD/DCoH was found to be encoded by *PCBD*, which is located on chromosome 10q22 [Thöny et al., 1994a] and is composed of four exons [Citron et al., 1993; Thöny et al., 1995b]. Later, a human paralog was annotated on chromosome 5, DCoH2 or DCoH α , and blast searches identified in addition two pseudogenes on chromosomes 2 and 17 [Rose et al., 2004]. The exon structures coding for the two human paralogs, PCD/DCoH and DCoH2, are identical and contain four exons (for a more detailed comparison of human genes and pseudogenes see Bossow et al. [1998]).

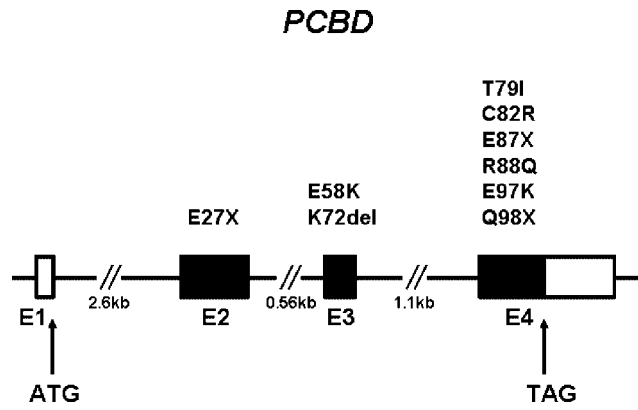


FIGURE 4. Genomic structure and location of mutations in the human *PCBD* gene.

So far, nine different mutations have been detected in patients with PCD deficiency [Thöny et al., 1998a, b], most of them located in exon 4 (Fig. 4; Supplementary Table S4). All these mutations are associated with a benign form of BH₄ deficiency, characterized by persistent urinary excretion of 7-substituted biopterin (primapterin or primapterinuria) and transient HPA [Curtius et al., 1988]. Most of the mutations so far recognized in patients with PCD deficiency are either a single amino acid change or a stop codon (see above). Only C82R has been successfully expressed as a soluble form, and was found to have 40% of normal activity. A biopsy of duodenal mucosa from a patient with homozygous E97K mutation had 17% of normal activity [Ayling et al., 2000]. Since subjects with mutant PCD have only a transient form of HPA and to our knowledge no transcription factor HNF-1 α -related expression defects, it was proposed based also on recent experimental evidence that this may at least in part be explained by a compensatory activity of DCoH2 [Hevel et al., 2006].

Mutations Within the *QDPR* Gene

DHPR deficiency is an autosomal recessive diseases, and the corresponding gene, *QDPR*, contains seven exons. So far, the 29 different reported mutant alleles are spread over all coding exons

and two introns: 17 missense mutations, three nonsense mutations, three frameshift mutations, four splice-site mutations, and two insertions (Fig. 5; Supplementary Table S5). Two mutations were found to be associated with a mild form of DHPR deficiency, i.e., affecting only serotonin metabolism in the brain (G151S and F212C) [Blau et al., 1992].

In vitro expression of mutation has been performed only in a very few cases, and in some cases the activity was surprisingly high (p.W108G and p.Y150C). The p.Y150C mutant protein was found to cause only a partial decrease in enzyme activity and in combination with the G23D mutation an intermediate phenotype. The G23D mutation itself was shown to cause a dramatic decrease in enzyme activity when expressed recombinantly in *E. coli*. A number of mutations located in exon 1 (G17R, G18D, and G23D) were proposed to affect the nicotinamide adenine dinucleotide (NADH) binding.

DHPR deficiency, though the BH₄ biosynthesis and recycling pathways are both intact, leads commonly to progressive mental and physical retardation despite dietary phenylalanine restriction, and to other serious clinical symptoms including basal ganglia calcification and sudden death. Such unexpected clinical severity may be explained by two concomitant disease mechanisms, accumulation of H₂-biopterin which is a strong inhibitor of the aromatic amino acid hydroxylases [Heales and Hyland, 1990] (and leading to low L-Dopa and 5-OH-tryptophan) and NOS, and low cerebral 5-methyltetrahydrofolate, which may disturb neural development [Blau et al., 2001a; Ramaekers and Blau, 2004]. Whereas accumulation of H₂-biopterin is a direct consequence of DHPR deficiency, the reason for low 5-methyl-tetrahydrofolate is not clear but maybe linked to the impaired reduction of 5,10-methylene-tetrahydrofolate to 5-methyl-tetrahydrofolate [Kaufman, 1991].

SNPs and Genomic Variants in the *BH₄*-Metabolizing Genes

The Ensembl Gene Variation Report database GeneSNPView (www.ensembl.org; v37) provides detailed information about SNPs as genetic variation, including nonsynonymous SNPs with amino acid changes and synonymous SNPs with no amino acid changes, but also others such as regulatory region, 5'- and 3'-UTRs, frameshift, stop lost and stop gained, splice site, and intronic

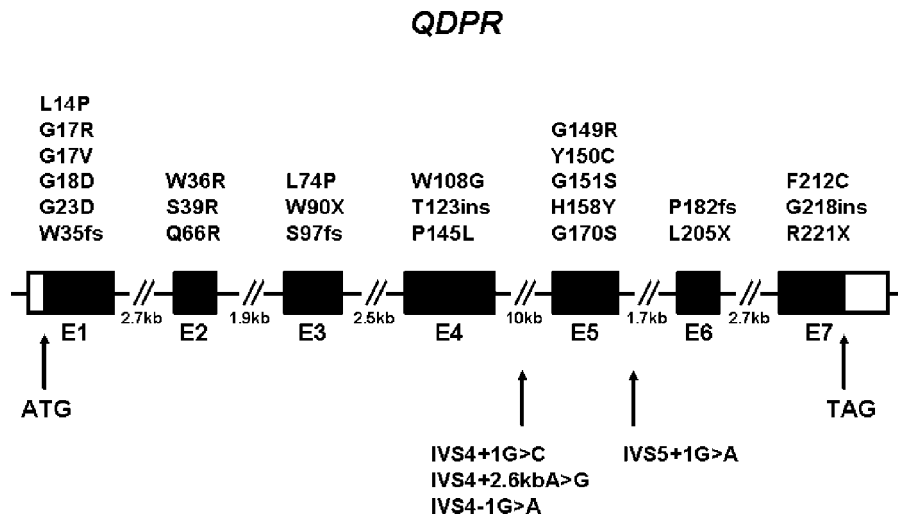


FIGURE 5. Genomic structure and location of mutations in the human *QDPR* gene.

TABLE 2. SNPs in BH₄ Metabolizing Genes

Gene ^a	ID ^b	Allele or nucleotide aberration	Location in gene	SNP type	Amino acid exchange	Comment	References
GCHI	rs28458175	G/A (*)	Exon 1	5'-UTR	—	41 nt upstream of ATG start codon	—
GCHI	rs987	c.993T>C	Exon 6	3'-UTR	—	—	—
GCHI	rs841	c.995C>T (*)	Exon 6	3'-UTR	—	—	—
GCHI	rs10136966	c.1429C>T (*)	Exon 6	3'-UTR	—	—	—
GCHI	rs10151500	c.1894C>T (*)	Exon 6	3'-UTR	—	—	—
GCHI	rs17128017	c.2667A>G (*)	Exon 6	3'-UTR	—	—	—
GCHI	rs6572984	c.2717C>A (*)	Exon 6	3'-UTR	—	—	—
PTS	rs17851591	c.309G>T	Exon 5	Synonymous coding	p.V103V	Not validated	—
PTS	rs17851590	c.342C>G	Exon 6	Nonsynonymous coding	p.I114M	Not validated	—
PTS	rs3211484	c.534T>G	Exon 6	3'-UTR	—	—	—
PTS	rs3176952	c.689C>A	Exon 6	3'-UTR	—	—	—
SPR	rs1876487	T/G (*)	Exon 1	5'-UTR	—	—	—
PCBD	rs2630336	g.4643C>A (*)	Exon 1	5'-UTR	—	—	—
PCBD	rs827237	g.4557G>A (*)	Exon 1	5'-UTR	—	—	—
PCBD	rs11554325	c.111C>T (*)	Exon 2	Nonsynonymous coding	p.R31C	Not validated	—
PCBD	rs9712	c.371G>A	Exon 4	3'-UTR	—	—	—
PCBD	rs15279	c.670A>G (*)	Exon 4	3'-UTR	—	—	—
QDPR	rs11556809	c.73C>G	Exon 1	Nonsynonymous coding	p.R25G	Not validated	—
QDPR	rs25186608	c.96C>T	Exon 1	Synonymous coding	p.A32A	No effect	Smooker and Cotton [1995]
QDPR	rs12645938	c.255C>T (*)	Exon 3	Synonymous coding	p.C85C	No effect	Romstad et al. [2000]
QDPR	rs3733570	c.345G>A (*)	Exon 4	Synonymous coding	p.S115S	No effect	Howells et al. [1990]
QDPR	rs2597775	c.396G>A (*)	Exon 4	Synonymous coding	p.L132L	No effect	Romstad et al. [2000]
QDPR	rs3733569	c.408C>T (*)	Exon 4	Synonymous coding	p.G136G	No effect	—
QDPR	rs1049581	c.955T>C	Exon 7	3'-UTR	—	—	—
QDPR	rs1049582	c.956G>A	Exon 7	3'-UTR	—	—	—
QDPR	rs11556811	c.1137C>T	Exon 7	3'-UTR	—	—	—
QDPR	rs1049600	c.1138A>G	Exon 7	3'-UTR	—	—	—
QDPR	rs1049601	c.1143C>T	Exon 7	3'-UTR	—	—	—
QDPR	rs699460	c.1203A>C (*)	Exon 7	3'-UTR	—	—	—
QDPR	rs1031327	c.1245C>T (*)	Exon 7	3'-UTR	—	—	—
QDPR	rs10604	c.1357T>G	Exon 7	3'-UTR	—	—	—
QDPR	rs699459	c.1417A>G (*)	Exon 7	3'-UTR	—	—	—
QDPR	rs1031326	c.1425A>G (*)	Exon 7	3'-UTR	—	—	—

^aAccording to the Ensembl Gene Variation Report database GeneSNPView (www.ensembl.org; v.37); GCHI gene ENSG00000131979, GCHI transcript region ENST0000254299; PTS gene ENSG00000150787, PTS transcript region ENST0000280362; SPR gene ENSG00000116096, SPR transcript region ENST0000234454; PCBD gene ENSG00000166228, PCBD transcript region ENST0000292929; QDPR gene ENSG00000151552, and QDPR transcript region ENST0000281243.

^bAccession numbers in Genome Sequence Database: see Supplementary Tables S1–S5; a star (*) indicates that in the Ensembl Database the reverse sequence is indicated.

variants. Based on this database, in Table 2, we compiled the exonic SNPs in all five BH₄ metabolizing genes, including 5'- and 3'-UTRs, plus the synonymous coding and nonsynonymous coding SNPs.

For *GCHI* a total of 192 SNPs are reported. This includes 184 intronic variants spread over all five introns and one not validated genomic T/C ambiguity in the “regulatory region,” besides the seven tabulated SNPs in the 5'- and 3'-UTRs.

For the *PTS* gene, 16 SNPs are reported in intronic regions 1–5, and two SNPs in the 3'-UTR. Furthermore, exon 5 contains one synonymous coding SNP (c.309G>T, p.V103V), and exon 6 one nonsynonymous coding SNP (c.342C>G, p.I114M). The coding I114M SNP coincides with the I114V mutation with HPA and neurotransmitter deficiency (severe, central phenotype). Since this is not a validated polymorphism, the consequences are not known.

The intronic variant g.2666A>T (or c.[84-322A>T]+[84-322A>T]) in *PTS*, that was detected only recently in a PTPS patient, is not listed in the SNP database but in Table S2. The patient exhibited only this single homozygous genomic mutation, inherited from the consanguineous parents, and RT-PCR analysis of primary dermal fibroblasts revealed two mRNA species, a wild-type and one containing a 79-nucleotide-long pseudoexon exactly between exons 1 and 2 (r.[=,83_84ins84-313_84-235]; D. Meili and B. Thöny, unpublished results). Moreover, the pseudoexon contained the essential splicing signals AG and GT dinucleotides at the intron–pseudoexon and pseudoexon–intron junctions, respectively. We thus hypothesized that the g.2666A>T (c.[84-322A>T]+[84-322A>T]) mutation is a potential deep-intronic variation at the splice acceptor site of this pseudoexon, leading to an extra exon in the PTPS-mRNA [Pagani and Baralle, 2004]. Also not in Table 2 with the SNPs, but rather in Table S2, is the E81E allele, as it turned out to be a nonneutral synonymous mutation associated with altered splicing rather than a silent mutation [Imamura et al., 1999]. The corresponding G-to-A transition was at the third base of codon 81 (E81E), and was also the last nucleotide in exon 4 on the genomic *PTS*-DNA. Upon analyzing the PTPS mRNA, it turned out that the E81E mutation affected the splice donor site of exon 4 and caused a splicing error by skipping of exon 4.

The *SPR* gene contains one nonvalidated genomic T/G ambiguity in the “regulatory region” and two SNP variants in intron 2.

The *PCBD* gene contains two SNPs in the 5'-UTR and two in the 3'-UTR, and one nonsynonymous coding nonvalidated SNP is reported in exon 2 (c.111C>T, p.R30C). In addition, 17 SNPs are spread over all four introns.

The human *QDPR* gene contains three nonvalidated genomic G/T or G/C ambiguities in the “regulatory region,” 10 SNPs in the 3'-UTR, and a total 113 SNPs in the six introns. Furthermore, six exonic SNPs are found, two in exon 1 (c.73C>G, p.R25G and c.96C>T, p.A32A), one in exon 3 (c.255C>T, p.C85C), and three in exon 4 (c.345G>A, p.S115S; c.396G>A, p.L132L; c.408C>T, p.G136G). Only p.R25G is a nonsynonymous coding SNP with unknown validation, whereas the other five are synonymous coding SNPs that were validated and/or reported to have no effect.

Diagnostic and Clinical Relevance

Presently, the major advantage of mutation analysis is in the prenatal diagnosis of GTPCH, PTPS, SR, and DHPR deficiencies causing monoamine neurotransmitter deficiency and/or HPA. Using PCR-based mutation analysis, Liu et al. [1998] diagnosed a

fetus at risk for PTPS deficiency to be a carrier of the N52N mutation. In another prenatal diagnosis in a family with a child with PTPS deficiency the fetus was found to be heterozygous for the T67M mutation (our unpublished results). For DHPR deficiency, prenatal diagnosis was performed using a RFLP linkage analysis [Chiou et al., 1995; Dahl et al., 1988] and denaturing gradient gel electrophoresis (DGGE) analysis of the *QDPR* gene [Kalkanoglu et al., 2001].

It should be emphasized that in order to identify also deep intronic variants, as for instance the c.84–323A>T allele in the *PTS* gene, or exonic mutations that affect pre-mRNA splicing, including cryptic splice sites and nonneutral synonymous sites near or at splice junctions, as for instance the E81E allele in the *PTS* gene, mRNA analysis bears an important advantage over only analyzing exons and adjacent regions in the genomic DNA. Moreover, it was reported that exon skipping association with point mutations other than those of the nonsense type, i.e., missense or silent mutations, has been frequently observed, but is also often ignored [Cartegni et al., 2002; Pagani and Baralle, 2004; Chamary et al., 2006]. As primary dermal fibroblasts can be used for analyzing the mRNA of all five BH₄-metabolizing genes that are presented here, we suggest as a general strategy for mutation detection to search by sequencing the RT-PCR products from patients' fibroblasts, followed by sequencing the corresponding exon(s) from genomic DNA.

As it is also the case for other diseases than BH₄ deficiency, not only identification but also the correct classification of mutations in terms of their actual mechanism of gene inactivation is essential for understanding of structure–function relationships in the corresponding protein. This will hopefully help to predict genotype–phenotype relationships in the future.

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Supplementary Material for the article:

Thöny and Blau, *Human Mutation*

Mutations in the BH₄-Metabolizing Genes GTP Cyclohydrolase I, 6-Pyruvoyl-Tetrahydropterin Synthase, Sepiapterin Reductase, Carbinolamine-4a-Dehydratase, and Dihydropteridine Reductase

Beat Thöny and Nenad Blau*

Supplementary Tables S1 to S5

Supplementary Table S1. Mutations in the *GCHI* Gene

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype ^b	Comment (% activity of wild-type)	Patients' number in BIODEF ^c	References
-22C>T	substitution	-22C>T	5'-UTR		DRD	decreased transcription/translation (?)	(hetero)	(Tassin, et al., 2000)
-39C>T / -132C>T	substitution	-39C>T / -132C>T	5'-UTR		DRD	decreased transcription/translation (?)	(hetero)	(Bandmann, et al., 1998)
del exon 1	deletion	?	exon 1	?	DRD	loss of protein	(hetero)	(Klein, et al., 2002)
del exon 1-3	deletion	?	exon 1-3	?	DRD	loss of protein	(hetero)	(Klein, et al., 2002)
del exon 1-4	deletion	?	exon 1-4	?	DRD	loss of protein	(hetero)	(Hagenah, et al., 2005)
del exon 1-6	deletion	?	exon 1-6	?	DRD	loss of protein	(hetero)	(Hagenah, et al., 2005)
M1X	substitution	c.3G>C	exon 1	p.M1X	DRD	no translation initiation	(hetero)	(Tamaru, et al., 1998)
E2fs	insertion	c.3_4insGG	exon 1	p.E2fsX65	DRD	frame shift and premature stop codon	(hetero)	(Ichinose, et al., 1994)
A8X	substitution	c.22_23GC>TA	exon 1	p.A8X	DRD		(hetero)	(Hagenah, et al., 2005)
P23L	substitution	c.68C>T	exon 1	p.P23L	DRD	see also mutation D115N	(hetero)	(de la Fuente-Fernandez, 1997) (Jarman, et al., 1997) (Steinberger, et al., 2000)
Q48X	substitution	c.142C>T	exon 1	p.A48Q	DRD	frame shift and protein truncation	(hetero)	(Hong, et al., 2001)
A50fs	deletion	c.149delC	exon 1	p.A50fsX16	DRD	frame shift and protein truncation	(hetero)	(Furukawa, et al., 1998b)
E56X	substitution	c.166G>T	exon 1	p.E56X	DRD	frame shift and protein truncation (erroneously published as c.165G>T)	(hetero)	(Bandmann, et al., 1998)
E61X	substitution	c.181G>T	exon 1	p.E61X	DRD	frame shift and protein truncation	(hetero)	(Nitschke, et al., 1998) (Steinberger, et al., 2000)
E65X	substitution	c.193G>T	exon 1	p.E65X	DRD	frame shift and protein truncation	(hetero)	(Furukawa, et al., 1996)
L71fs	deletion	c.212delT	exon 1	p.L71fsX8	DRD	frame shift and protein	(hetero)	(Furukawa, et al., 1998b)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patients' number in BIODF ^c	References
L71Q	substitution	c.212T>A	exon 1	p.L71Q	DRD	(erroneously published as L71E)	(hetero)	(Bandmann, et al., 1998)
A74V	substitution	c.221C>T	exon 1	p.A74V	DRD		(hetero)	(Bandmann, et al., 1998)
Y75X	substitution	c.225C>A	exon 1	p.Y75X	DRD	premature stop codon	(hetero)	(Steinberger, et al., 2000)
S77-L82del 1998b)	deletion	c.229_246del18	exon 1	p.S77_L82delSILSSL	DRD	DRD 6 amino acid deletion	aberrant protein by a (hetero)	(Furukawa, et al.,
L79P	substitution	c.236T>C	exon 1	p.L79P	DRD		(hetero)	(Ichinose, et al., 1995b)
G83A	substitution	c.248G>C	exon 1	p.G83A	DRD		(hetero)	(Bandmann, et al., 1998)
Q87P	substitution	c.260A>C	exon 1	p.Q87P	DRD		(hetero)	(Klein, et al., 2002)
R88W	substitution	c.262C>T	exon 1	p.R88W	DRD	no activity when expressed in bacteria	(hetero)	(Ichinose, et al., 1994) (Bandmann, et al., 1996)
R88G	substitution	c.262C>G	exon 1	p.R88G	DRD		(hetero)	(Hagenah, et al., 2005)
R88P	substitution	c.263G>C	exon 1	p.R88P	DRD		(hetero)	(Bandmann, et al., 1996)
R88-Q89del	deletion	c.261_266delIGCGGCA	exon 1	p.R88_Q89delIRQ	DRD		(hetero)	(Tassin, et al., 2000)
Q89X	substitution	c.265C>T	exon 1	p.Q89X	DRD	premature stop codon	(hetero)	(Hoenicka, et al., 2001)
L91R	substitution	c.272T>G	exon 1	p.L91R	DRD		(hetero)	(Hagenah, et al., 2005)
T94K	substitution	c.281C>A	exon 1	p.T94K	DRD		(hetero)	(Markova, et al., 1999) (Markova, et al., 2000)
W96X	substitution	c.288G>A	exon 1	p.W96X	DRD	premature stop codon	(hetero)	(Sasaki, et al., 1998)
M102K	substitution	c.305T>A	exon 1	p.M102K	DRD		(hetero)	(Illarioshkin, et al., 1998)
M102R	substitution	c.305T>G	exon 1	p.M102R	DRD		(hetero)	(Nishiyama, et al., 2000)
Q103fs	deletion	c.309delG	exon 1	p.Q103fsX14	DRD	frame shift and protein truncation	(hetero)	(Steinberger, et al., 1998) (Steinberger, et al., 2000)
G103P	substitution	c.308A>C	exon 1	p.G103P	DRD		(hetero)	(Hagenah, et al., 2005)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patients' number in BIODEF ^c	References
T106ins	insertion	c.315_316insTTC	exon 1	p.F105_T106insF	DRD		(hetero)	(Hagenah, et al., 2005)
G108D	substitution	c.323G>A	exon 1	p.G108D	DRD		(hetero)	(Furukawa, et al., 1998a)
G108V	substitution	c.323G>T	exon 1	p.G108V	DRD		(hetero)	(Hirano and Ueno, 1999)
Q110fs	insertion	c.328_329insA	exon 1	p.Q110fsX13	DRD	frame shift and protein truncation	(hetero)	(Furukawa, et al., 1998b)
Q110X	substitution	c.328C>T	exon 1	p.Q110X	HPA, severe, central DRD	premature stop codon	#209 (hetero) (hetero)	H. Ichinose, unpublished (Saunders-Pullman, et al, 2004)
S114X	substitution	c.341C>A	exon 1	p.S114X	DRD	premature stop codon	(hetero)	(Furukawa, et al., 1996)
S114X	substitution	c.341C>G	exon 1	p.S114X	DRD	premature stop codon	(hetero)	(Jeong, et al., 1998)
D115N	substitution	c.343G>A	exon 1	p.D115N	DRD	variant of DRD, compound heterozygote without HPA	(hetero)	(Jarman, et al., 1997)
IVS1-1G>A	substitution	c.344-1G>A	intron 1	p.D115fsX7	DRD	skipping of exon 2 and premature stop codon	(hetero)	(Furukawa, et al., 1998b)
IVS1-2A>G	substitution	c.344-2A>G	intron 1	p.D115fsX7	DRD	skipping of exon 2 and premature stop codon	(hetero)	(Weber, et al., 1997) (Steinberger, et al., 2000)
L117fs	deletion	c.351delA	exon 2	p.L117fsX13	DRD	frame shift and protein truncation	(hetero)	(Furukawa, et al., 1998b)
D134V	substitution	c.401A>T	exon 2	p.D134V	DRD		(hetero)	(Ichinose, et al., 1994) (Bandmann, et al., 1996)
I135K	substitution	c.404T>A	exon 2	p.I135K	DRD		(hetero)	(Brique, et al., 1999)
C141R	substitution	c.421T>C	exon 2	p.C141R	DRD		(hetero)	(Nishiyama, et al., 2000)
C141W	substitution	c.423T>G	exon 2	p.C141W	DRD		(hetero)	(Illarioshkin, et al., 1998) (Markova, et al., 2000)
H144P	substitution	c.431A>C	exon 2	p.H144P	DRD		(hetero)	(Hirano, et al., 1996) (Hirano, et al., 1999) (Tamaru, et al., 1998)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patients' number in BIODEF ^c	References
IVS2+1G>C	substitution	c.453+1G>C	intron 2	p.D115fsX7	DRD	skipping of exon 2 and premature stop codon	(hetero)	(Hirano, et al., 1995)
IVS2-2A>G	substitution	c.454-2A>G	intron 2	p.V152fsX5	DRD	splice site mutation, protein truncation	(hetero)	(Weber, et al., 1997)
H153P	substitution	c.458A>C	exon 3	p.H153P	DRD		(hetero)	(Bandmann, et al., 1996)
N159fs	deletion	c.477delC	exon 3	p.N159fsX13	DRD	frame shift and protein truncation	(hetero)	(Steinberger, et al., 2000)
L163R	substitution	c.488T>G	exon 3	p.L163R	DRD		(hetero)	(Steinberger, et al., 2000)
IVS3+1G>T	substitution	c.509+1G>T	intron 3	p.R170fsX26	DRD	skipping of exon 4 and premature stop codon	(hetero)	(Steinberger, et al., 2000)
IVS3-2G>A	substitution	c.510-2G>A	intron 3	p.R170fsX26	DRD	skipping of exon 4 and premature stop codon	(hetero)	(Furukawa, et al., 1998b)
I171fs	deletion	c.512_524del13	exon 4	p.I171fsX16	DRD	frame shift and protein truncation	(hetero)	(Ichinose, et al., 1995b)
S176T	substitution	c.527G>C	exon 4	p.S176T	DRD		(hetero)	(Illarioshkin, et al., 1998) (Markova, et al., 2000)
R178G	substitution	c.532A>G	exon 4	p.R178G	DRD		(hetero)	(Hagenah, et al., 2005)
R178S	substitution	c.534A>C	exon 4	p.R178S	DRD		(hetero)	(Beye, et al., 1997)
R178S	substitution	c.534A>T	exon 4	p.R178S	DRD		(hetero)	(Tassin, et al., 2000)
Q180X	substitution	c.538C>T	exon 4	p.Q180X	DRD	protein truncation	(hetero)	(Tassin, et al., 2000) (Nishiyama, et al., 2000)
Q180 R	substitution	c.539A>G	exon 4	p.Q180R	DRD		(hetero)	(Tassin, et al., 2000)
IVS4+1G>C	substitution	c.541+1G>C	intron 4	p.V181fsX5	DRD	skipping of exon 5 and premature stop codon	(hetero)	(Steinberger, et al., 2000)
IVS4+1G>A	substitution	c.541+1G>A	intron 4	-	DRD	protein truncation	(hetero)	(Hagenah, et al., 2005)
Q182X	substitution	c.544C>T	exon 5	p.Q182X	DRD	protein truncation	(hetero)	(Steinberger, et al., 1998) (Steinberger, et al., 2000)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patients' number in BIODEF ^c	References
R184C	substitution	c.550C>T	exon 5	p.R184C	DRD		(hetero)	(Hagenah, et al., 2005)
R184H	substitution	c.551G>A	exon 5	p.R184H	HPA, severe, central DRD	no activity when expressed in bacteria	#208 (homo) (hetero)	(Ichinose, et al., 1995a) (Kikuchi, et al., 2004)
L185R	substitution	c.554T>G	exon 5	p.L185R	DRD		(hetero)	(Furukawa, et al., 2000)
T186fs	substitution	c.557C>A	exon 5	p.V181fsX112	DRD	frame shift, exon 5 and partial exon 6 skipping, protein truncation	(hetero)	(Hirano, et al., 1997) (Imaiso, et al., 1998)
A190fs	deletion	c.568delG	exon 5	p.A190fsX1	DRD	frame shift and protein truncation	(hetero)	Furukawa et al. unpublished
V191I	substitution	c.571G>A	exon 5	p.V191I	DRD		(hetero)	(Bandmann, et al., 1998)
S196A	substitution	c.586G>T	exon 5	p.S196A	DRD		(hetero)	(Steinberger, et al., 1999)
P199A	substitution	c.595C>G	exon 5	p.P199A	DRD, recessive		(hetero)	(Nardocci, et al., 2003)
P199S	substitution	c.595C>T	exon 5	p.P199S	DRD		(hetero)	(Hjermind, et al., 2004)
P199L	substitution	c.596C>T	exon 5	p.P199L	DRD		(hetero)	(Tassin, et al., 2000)
G201R	substitution	c.601G>A	exon 5	p.G201R	DRD		(hetero)	(Hagenah, et al., 2005)
G201E	substitution	c.602G>A	exon 5	p.G201E	DRD	no activity when expressed in bacteria	(hetero)	(Ichinose, et al., 1994) (Bandmann, et al., 1996)
V202I	substitution	c.604G>A	exon 5	p.V202I	DRD		(hetero)	(Hagenah, et al., 2005)
G203R	substitution	c.607G>A	exon 5	p.G203E	DRD		(hetero)	(Bandmann, et al., 1996)
V205G	substitution	c.614T>G	exon 5	p.V205G	DRD		(hetero)	(Garavaglia, et al., 2004)
IVS5+1G>A	substitution	c.626+1G>A	intron 5	p.V181fsX5	DRD	exon 5 skipping and protein truncation	(hetero)	(Hirano, et al., 1998)
IVS5+1G>C	substitution	c.626+1G>C	intron 5		DRD		(hetero)	(Garavaglia, et al., 2004)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patients' number in BIODEF ^c	References
IVS5+5G>A	substitution	c.626+5G>A	intron 5		DRD	splicing defect	(hetero)	(Steinberger, et al., 2000)
IVS5+3insT	substitution	c.626+3insT	intron 5		DRD	splicing defect	(hetero)	(Steinberger, et al., 2000)
M211fs	deletion	c.631_632delAT	exon 6	p.M211fsX37	DRD	frame shift and protein truncation	(hetero)	(Bandmann, et al., 1998) (Furukawa, et al., 1998b)
M211I	substitution	c.633G>A	exon 6	p.M211I	HPA, severe, central	no activity when expressed in bacteria	#265 (homo)	(Blau, et al., 1995) (Ichinose, et al., 1995a) (Hirano and Ueno, 1999)
M211V	substitution	c.631A>G	exon 6	p.M211V	HPA, severe, central		#269 (homo)	(Bandmann, et al., 1998)
M213T	substitution	c.638T>C	exon 6	p.M213T	HPA, severe, central		#351 (homo)	(Touma, et al., 2000)
R216X	substitution	c.646C>T	exon 6	p.R216X	DRD	protein truncation	(hetero)	(Bandmann, et al., 1996)
V218fs	insertion	c.654insT	exon 6	p.V218fsX31	DRD	frame shift and protein truncation	(hetero)	(Hagenah, et al., 2005)
M221T	substitution	c.662T>C	exon 6	p.M221T	DRD		(hetero)	(Bezin, et al., 1998) (Furukawa, et al., 1998a)
S223R	substitution	c.669C>A	exon 6	p.S223R	DRD		(hetero)	(Hagenah, et al., 2005)
K224R	substitution	c.671A>G	exon 6	p.K224R	DRD		(hetero)	(Bandmann, et al., 1996)
K224X	substitution	c.670A>T	exon 6	p.K224X	DRD		(hetero)	(Jarman, et al., 1997)
V226A	substitution	c.677T>C	exon 6	p.V226A	DRD		(hetero)	(Klein, et al., 2002)
L231fs	deletion	c.693delG	exon 6	p.L231fsX14	DRD	frame shift and protein truncation	(hetero)	Garavaglia et al. 2004
F234S	substitution	c.701T>C	exon 6	p.F234S	DRD		(hetero)	(Bandmann, et al., 1996)
R241W	substitution	c.721C>T	exon 6	p.R241W	DRD,		(hetero)	(Bandmann, et al., 1998)
E243fs	insertion	c.726_727insTTCCC	exon 6	p.E243fsX4	DRD	frame shift and protein truncation	(hetero)	(Uncini, et al., 2004)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patients' number in BIODÉF ^c	References
R249S	substitution	c.747C>G	exon 6	p.R249S	DRD, recessive	low GTPCH activity in leukocytes, wild-type activity when expressed in bacteria	(homo)	(Hwu, et al., 1999)
X251R	substitution	c.751T>C	exon 6	p.X251Rins34X285	DRD	protein extension	(hetero)	(Romstad, et al., 2003)

^a Accession numbers in Genome Sequence Data Base: U19523.1 for human cDNA (the numbering starts with 1 at A at the ATG-start codon); for published fragments of human gDNA see D38603.1, U19256.1, U19257.1, U19258.1, U19259.1, and D38602.1

^b DRD, Dopa-responsive dystonia

^c BIODÉF database: www.bh4.org; homo, homozygote; hetero, compound heterozygote

Supplementary Online Table S2. Mutations in the *PTS* Gene

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODÉF ^b	References
R16ins	insertion	g.932_933insGGC c.45_46insGGC	exon 1	p.S15_R16insG	HPA, severe, central	no activity as recombinant protein (erroneously published as S15_R16insR)	#49 (hetero)	(Dudsek, et al., 2001)
R16C	substitution	g.933C>T c.46C>T	exon 1	p.R16C	HPA, mild, peripheral	phosphorylation deficiency, reduced activity	#121 (hetero)	(Thöny, et al., 1994b) (Oppliger, et al., 1995)
A22G	substitution	g.952C>G c.65C>G	exon 1	p.A22G	HPA, severe, central		(homo)	(Preusse, et al. 2001)
R25G	substitution	g.960C>G c.73C>G	exon 1	p.R25G	HPA, severe/mild		#84 (hetero) #114 (hetero) #284 (hetero)	(Liu, et al., 1998) (Liu, et al., 2001b)
R25Q	substitution	g.961G>A c.74G>A	exon 1	p.R25Q	HPA, severe, central	reduced activity as recombinant protein	#252 (homo)	(Thöny, et al., 1994b) (Oppliger, et al., 1995)
L26F	substitution	g.965G>T c.78G>T	exon 1	p.L26F	HPA, mild, peripheral	no activity as recombinant protein	#247 (hetero)	(Dudsek, et al., 2001)
IVS1-3C>G	substitution/ deletion	c.84-3C>G	intron1	p.K29_S32delKFLS r.84_95del12	HPA, severe, central	splice site mutation and cryptic splice site usage leading to a 4 codon-deletion; protein unstable (also termed delK29-S32)	#62 (hetero) #109 (hetero) #274 (homo) #275 (homo) #516 (homo)	(Oppliger, et al., 1997) Romstad, online submission Thöny and Blau, unpublished
IVS1-323A>T	substitution	g.2666A>T c.[84-322A>T]+[84-322A>T]	intron 1	p.S28fsX6 r.[=,83_84ins84-313_84-235]	HPA, severe, central	two mRNA species: wt and pseudo-exon insertion of 79 nucleotides	#503 (homo)	Thöny et al., unpublished
E35G	substitution	g.3008A>G c.104A>G	exon 2	p.E35G	HPA, severe, central		#98 (hetero) #281 (hetero)	(Zekanowski, et al., 1998)
N36K	substitution	g.3012C>G c.108C>G	exon 2	p.N36K	HPA, severe, central		#226 (hetero) #364 (hetero)	(Zekanowski, et al., 1998)
L40fs	deletion	g.3020_3023delTGTT c.116_119delTGTT	exon 2	p.F40fsX16	HPA, mild, peripheral	frame shift and protein truncation, initially described as K38X	#115 (hetero)	(Liu, et al., 2001a)
F40fs	deletion	g.3022_3025delTTTG c.118_121delTTTG	exon 2	p.F40fsX16	HPA, severe, central	frame shift and protein truncation	(hetero)	(Preusse, et al. 2001)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODF ^b	References
F40L	substitution	g.3024>T>G c.120T>G	exon 2	p.F40L	HPA		(hetero)	(Liu, et al., 2001b)
N47D	substitution	g.3043A>G c.139A>G	exon 2	p.N47D	HPA, transient	dominant negative allele ; inactive in COS-1 cells	#302 (hetero)	(Scherer-Oppliger, et al., 1999)
N52S	substitution	g.3059A>G c.155A>G	exon 2	p.N52S	HPA, severe, central	frequent among Asian population	#29,30,42,44,45, 63,85,86,283,321, 324,353-355,402, 410,426-428,430, 432-434,455 (hetero, homo)	(Liu and Hsiao, 1996) (Yoo and Kim, 1997) Thöny and Blau, unpublished Chien, online submission (Liu, et al., 2001b)
IVS2-7T>A	substitution, deletion	g.4592T>A c.164-7T>A	intron 2	p.K55fsX1 r.164_186del exon 3	HPA, severe, central	exon 3 skipping causing frame shift and protein truncation/degradation (also termed K54X)	#16 (hetero)	(Oppliger, et al., 1995) (Kluge, et al., 1996)
V56M	substitution	g.4601G>A c.166G>A	exon 3	p.V56M	HPA, severe, central		#114 (hetero) #173 (hetero) #356 (hetero)	(Hsiao and Liu, 1996) (Liu, et al., 1998) (Liu, et al., 2001b)
V57del	deletion	g.4604_4606delGTG c.169_171delGTG	exon 3	p.V57del	HPA, severe, central	single amino acid deletion, protein inactive and degraded	#16 (hetero) #62 (hetero) #82 (hetero)	(Oppliger, et al., 1995) (Oppliger, et al., 1997) (Liu, et al., 2001a)
IVS3+1G>A	substitution	g.4622G>A c.186+1G>A	intron 3	p.K55fsX1 r.164_186del exon 3	HPA, severe, central	exon 3 skipping causing frame shift and protein truncation/degradation	#370 (hetero)	(Liu, et al., 2001a) (Liu, et al., 2001b)
T67M	substitution	g.5030C>T c.200C>T	exon 4	p.T67M	HPA, severe, central	degraded in fibroblasts, 60% activity in COS-1	#64 (hetero) #281 (hetero) #341 (hetero) #386 (homo) #411 (hetero) #450 (homo)	(Oppliger, et al., 1997) (Zekanowski, et al., 1998) (Dudsek, et al., 2001) Thöny and Blau, unpublished (Liu, et al., 2001b)
V70D	substitution	g.5039T>A c.209T>A	exon 4	p.V70D	HPA, severe, central		#356 (hetero)	(Liu, et al., 1998)
N72L	substitution	g.5046T>A c.216T>A	exon 4	p.N72L	HPA			Zekanowski, online submission

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODEF ^b	References
L76F	substitution	g.5056C>T c.226C>T	exon 4	p.L76F	HPA, severe, central		#369 (hetero)	(Liu, et al., 2001a)
E81E	substitution	g.5073G>A c.243G>A	exon 4	splicing defect r.187_243del	HPA, severe, central	skipping of exon 4, no PTPS activity detectable	(homo)	(Imamura, et al., 1994)
P87S	substitution	g.7569C>T c.259C>T	exon 5	p.P87S	HPA, severe, central	50% active when expressed in COS-1, frequent among Asian population	#29,30,40,43,45, 82,84-86,271,284, 320-323,353,354, 370,371,411,429 431,435,460 (hetero, homo)	(Liu and Hsiao, 1996) (Yoo and Kim, 1997) (Imamura, et al., 1999) Chien, online submission (Liu, et al., 2001a)
P87L	substitution	g.7570C>T c.260C>T	exon 5	p.P87L	HPA, severe, central	protein degraded in patient's fibroblasts, reduced activity when expressed in COS-1 cells (36%)	#48 (homo) #90 (hetero) #285 (hetero) #341 (hetero) #347 (homo)	(Oppliger, et al., 1995) Thöny and Blau, unpublished (Dudsek, et al., 2001)
D96N	substitution	g.7596G>A c.286G>A	exon 5	p.D96N	HPA, severe, central	reduced activity in COS-1 (10%)	#39,43,271,320 369,432,435,460 (all hetero)	(Hsiao and Liu, 1996) (Yoo and Kim, 1997) (Imamura, et al., 1999) Chien, online submission (Liu, et al., 2001a)
Y99C	substitution	g.7606A>G c.296A>G	exon 5	p.Y99C	HPA, isolated, central		#277 (hemi)	(Blau, et al., 2000)
Y99X	substitution	g.7607C>A c.297C>A	exon 5	p.Y99X	HPA, mild, peripheral		#419 (homo) #24 (hetero)	Thöny and Blau, unpublished (Preusse, et al. 2001)
F100V	substitution	g.7608T>G c.298T>G	exon 5	p.F100V	HPA, severe, central		#98 (hetero)	(Zekanowski, et al., 1998)
A101V	substitution	g.7612C>T c.302C>T	exon 5	p.A101V	HPA, severe, central		#24 (hetero)	(Preusse, et al. 2001)
T106M	substitution	g.7825C>T c.317C>T	exon 6	p.T106M	HPA, severe, central	protein degraded in patient's fibroblasts	#173 (hetero) #298 (hetero) #372 (homo) (homo)	(Liu, et al., 2001b) (Hsiao and Liu, 1996) Thöny and Blau, unpublished (Yoo and Kim, 1997)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODF ^b	References
Y113C	substitution	g.7846A>G c.338A>G	exon 6	p.Y113C	HPA, mild, peripheral		#375 (homo) #376 (homo) #377 (homo)	(Meli, et al., 1999) Thöny and Blau, unpublished
I114V	substitution	g.7848A>G c.340A>G	exon 6	p.I114V	HPA, severe, central		(homo)	(Ashida, et al., 1994)
D116G	substitution	g.7855A>G c.347A>G	exon 6	p.D116G	HPA, transient		#302 (hetero)	(Scherer-Opplinger, et al., 1999)
V121fs	deletion	g.7869_7882del14 c.361_374del14	exon 6	p.V121fsX3	HPA, mild, peripheral	protein truncation (also termed K120X or K120fs)	#121 (hetero)	(Thöny, et al., 1994b)
V124L	substitution	g.7878G>T c.370G>T	exon 6	p.V124L	HPA, mild, peripheral	no activity as recombinant protein in COS-1 cells	#247 (hetero)	(Dudsek, et al., 2001)
G125R	substitution	g.7881G>A c.373G>A	exon 6	p.G125R	HPA, severe, central		#7 (homo)	(Preusse, et al. 2001)
K129E	substitution	g.7893A>G c.385A>G	exon 6	p.K129E	HPA, peripheral to central	protein stably active depending on the cellular background	#111 (homo)	(Opplinger, et al., 1997)
K131fs	deletion	g.7901delA c.393delA	exon 6	p.K131fsX19	HPA, severe, central	frame shift, out of frame extension	#298 (hetero)	Thöny and Blau, unpublished
D136V	substitution	g.7915A>T c.407A>T	exon 6	p.D136V	HPA, severe, central	degraded in fibroblasts (30% active in COS-1)	#4,5,49,64,99, 226,285,364,378 (hetero, homo)	(Opplinger, et al., 1997) (Zekanowski, et al., 1998) (Dudsek, et al., 2001) Thöny and Blau, unpublished
D136G	substitution	g.7915A>G c.407A>G	exon 6	p.D136G	HPA, severe, central		#49 (hetero)	(Dudsek, et al., 2001)
N138H	substitution	g.7920A>C c.412A>C	exon 6	p.N138H	HPA, transient		#122 (homo)	(Preusse, et al. 2001)
G144R	substitution	g.7938G>C c.430G>C	exon 6	p.G144R	HPA		(hetero)	(Liu, et al., 2001b)

^a Accession numbers in Genome Sequence Data Base: L76259.1 for human gDNA; M97655.1 for human cDNA (for the cDNA numbering starts with 1 at A at the ATG-start codon)

^b BIODF database: www.bh4.org; homo, homozygote; hetero, compound heterozygote; hemi, hemizygote

Supplementary Table S3. Mutations in the *SPR* Gene

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change)	Patient's phenotype ^b	Comment (% activity of wild-type)	Patient's number in BIODÉF ^c	References
-13G>A	substitution	g.359G>A	5'-UTR		DRD	62% in fibroblasts	(hetero)	(Steinberger, et al., 2004)
Q119X	substitution	g.1303_1304TC>CT c.354_355TC>CT	exon 2	p.Q119X	NT low, no HPA	no activity in fibroblasts	#360 (homo)	(Bonafé, et al., 2001)
R150fs	deletion	g.1397_1401delAGAAC (cDNA not detectable)	exon 2	p.R150fsX2	NT low, no HPA	not expressed in fibroblasts; (also termed N149X)	#229 (hetero) #439 (homo)	(Bonafé, et al., 2001) Thöny & Blau, unpublished
R150G	substitution	g.1397A>G c.448A>G	exon 2	p.R150G	NT low, no HPA	no activity in fibroblasts or as recombinant protein	#229 (hetero) #373 (homo)	(Bonafé, et al., 2001) (Elzaouk, et al., 2002)
P163L	substitution	g.1437C>T c.488C>T	exon 2	p.P163L	NT low, no HPA		#447 (homo)	(Abeling, et al., 2003)
K251X	substitution	g.587A>T c.751A>T	exon 3	p.K251X	NT low, no HPA		#492 (homo)	Thöny and Blau, unpublished
IVS2-2A>G		c.596-2A>G	intron 2		no HPA		#509 (homo) #510 (homo) #511 (homo) #512 (homo) #513 (homo) #514 (homo) #515 (homo)	(Farrugia and Felice, 2004) (Neville, et al., 2005)

^a Accession numbers in Genome Sequence Data Base: AB017547.1 and AB017548.1 for human gDNA; M76231.1 for human cDNA (for the cDNA numbering starts with 1 at A at the ATG-start codon)

^b NT, neurotransmitter metabolites; HPA, hyperphenylalaninemia

^c BIODÉF database: www.bh4.org; homo, homozygote; hetero, compound heterozygote

Supplementary Table S4. Mutations in the *PCBD* Gene

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change ^b	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODÉF ^c	References
E27X	substitution	g.7367G>T c.102G>T	exon 2	p.E27X	transient HPA, peripheral	allele associated with R88Q, initially described as E26X	#329 (homo)	(Thöny, et al., 1998b)
E58K	substitution	g.8025G>A c.195G>A	exon 3	p.E58K	transient HPA, peripheral	initially described as E57K	#210 (hetero)	Thöny & Blau, unpublished
K72del	deletion	g.8066_8068delCAA c.236_238delCAA	exon 3	p.K72del	transient HPA, peripheral	insoluble when expressed in <i>E. coli</i> , initially described as K71del	#210 (hetero)	Thöny & Blau, unpublished
T79I	substitution	g.9191C>T c.259C>T	exon 4	p.T79I	transient HPA, peripheral	insoluble when expressed in <i>E. coli</i> , initially described as T78I	#211 (homo) #212 (homo)	(Thöny, et al., 1998a) (Thöny, et al., 1998b)
C82R	substitution	g.9199T>C c.267T>C	exon 4	p.C82R	transient HPA, peripheral	40% of wild-type activity when expressed in <i>E. coli</i> , initially described as C81R	#270 (hetero)	(Citron, et al., 1993) (Rebrin, et al., 1995) (Köster, et al., 1995)
E87X	substitution	g.9214G>T c.282G>T	exon 4	p.E87X	transient HPA, peripheral	insoluble when expressed in <i>E. coli</i> initially described E86X	#270 (hetero) #273 (homo) #344 (homo) #394 (homo) #395 (homo)	(Citron, et al., 1993) (Thöny, et al., 1998a) Thöny & Blau, unpublished
R88Q	substitution	g.9218G>A c.286G>A	exon 4	p.R88Q	transient HPA, peripheral	allele associated with E27X, initially described as R87Q	#329 (homo)	(Thöny, et al., 1998b)
E97K	substitution	g.9244G>A c.312G>A	exon 4	p.E97K	transient HPA, peripheral	17% of wild-type activity in duodenal mucosa, initially described E96K	#216 (homo) #306 (homo) #319 (hetero)	(Thöny, et al., 1998b) (Ayling, et al., 2000)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BLODEF ^b	References
Q98X	substitution	g.9247C>T c.315C>T	exon 4	p.Q98X	transient HPA, peripheral	insoluble when expressed in <i>E. coli</i> , initially described Q97X	#263 (homo) #264 (homo) #272 (homo) #319 (hetero)	(Thöny, et al., 1998b) (Thöny, et al., 1998a)

^a Accession numbers in Genome Sequence Data Base: L41560.1 for human gDNA; L41559.1 and NM_000281.2 for human cDNA (for the cDNA numbering starts with 1 at A at the ATG-start codon); ^b BLODEF database: www.bh4.org; homo, homozygote; hetero, compound heterozygote

Supplementary Table S5. Mutations in the *QDPR* Gene

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODEF ^b	References
L14P	substitution	c.41T>C	exon 1	p.L14P	HPA, severe, central		#134 (homo)	(Smooker, et al., 1999)
G17R	substitution	c.49G>C	exon 1	p.G17R	HPA, severe, central	affected NADH binding	#282 (homo)	(Romstad, et al., 2000)
G17V	substitution	c.50G>T	exon 1	p.G17V	HPA, severe, central		#151 (hetero)	(Smooker, et al., 1999)
G18D	substitution	c.53G>A	exon 1	p.G18D	HPA, severe, central	affected NADH binding	#374 (homo)	(Romstad, et al., 2000)
G23D	substitution	c.68G>A	exon 1	p.G23D	HPA, severe, central	affected NADH binding; no activity in fibroblasts	#118 (homo) #132 (homo) #150 (homo) #174 (hetero) #202 (homo) #335 (homo)	(Dianzani, et al., 1998) (Romstad, et al., 2000)
W35fs	deletion/insertion	c.105delGgta/insAC	exon 1	p.W35fsX?	HPA, severe, central	putative protein truncation	#304 (homo)	(Romstad, et al., 2000)
W36R	substitution	c.106T>C	exon 2	p.W36R	HPA, severe, central	inactive in vitro	#149 (homo)	(Ikeda, et al., 1997)
S39R	substitution	c.115A>C	exon 2	p.S39R	HPA, severe, central		(hetero)	(Chen, et al., 1998)
Q66R	substitution	c.197A>G	exon 2	p.Q66R	HPA, severe, central	potentially altered stability	#403 (homo)	(Romstad, et al., 2000)
L74P	substitution	c.221T>C	exon 3	p.L74P	HPA, severe, central		#201 (?)	(Smooker and Cotton, 1995)
W90X	substitution	c.270G>A	exon 3	p.W90X	HPA, severe, central	truncated protein	#246 (homo) #334 (homo) #381 (homo)	(Romstad, et al., 2000) (Romstad, et al., 2000) (Romstad, et al., 2000)
S97fs	deletion	c.291delC	exon 3	p.S97fsX100	HPA, severe, central	truncated protein	#258 (homo)	(Romstad, et al., 2000)
W108G	substitution	c.322T>G	exon 4	p.W108G	HPA, severe, central	putative dimerization defect	#135 (homo)	(Dianzani, et al., 1993)
T123ins	insertion	c.366_367insACT	exon 4	p.A122_T123insT	HPA, severe, central	putative dimerization defect	#163 (homo)	(Howells, et al., 1990a) (Smooker, et al., 1993)
P145L	substitution	c.434C>T	exon 4	p.P145L	HPA, severe, central	mutation results in proteolysis	#145 (hetero)	(Smooker, et al., 1993)
IVS4+1G>C	substitution	c.436+1G>C	intron 4	splicing defect	HPA, severe, central	skipping of exons 4 and 5	#140 (homo)	(Smooker and Cotton, 1995) (Smooker, et al., 1999)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODÉF ^b	References
IVS4+2.6kbA>G	substitution/ insertion	c.436_437ins152bp	intron 4	splicing defect	HPA, severe, central	generation of an extra exon; protein truncation	#136 (homo) #137 (homo) #259 (homo)	(Ikeda, et al., 1997)
IVS4-1G>A	substitution	c.437-1G>A	intron 4	splicing defect	HPA, severe, central	skipping of exon 5	#131 (homo)	(Smooker, et al., 1999)
G149R	substitution	c.445G>A	exon 5	p.G149P	HPA, severe, central	affected dimerization	#253 (homo)	(Romstad, et al., 2000)
Y150C	substitution	c.449A>G	exon 5	p.Y150C	HPA, intermediate	affected catalysis	#174 (hetero) #188 (homo) #342 (homo)	(Dianzani, et al., 1998) (Zhang, et al., 1996) (Romstad, et al., 2000)
G151S	substitution	c.451G>A	exon 5	p.G151S	HPA, mild		#198 (homo)	(Blau, et al., 1992)
H158Y	substitution	c.472C>T	exon 5	p.H158Y	HPA, severe, central	unstable when expressed in bacterial cells	#153 (homo) #165 (homo)	(Smooker, et al., 1993) (Dianzani, et al., 1993)
G170S	substitution	c.508G>A	exon 5	p.G170S	HPA, severe, central	erroneously published as G170S	#193 (homo) #194 (homo)	(Smooker, et al., 1993) Liu et al. unpublished
IVS5+1G>A	substitution	c.545+1G>A	intron 5	splicing defect	HPA, severe, central	skipping of exon 5	#128 (homo) #289 (hetero)	(Smooker and Cotton, 1995) (Dianzani, et al., 1993) Smooker et al. 1999
P182fs	deletion	c.546delG	exon 6	p.P182fsX188	HPA	frame shift and premature stop and/or exon 5 skipping	(homo)	(Smooker and Cotton, 1995)
L205X	substitution	c.614T>G	exon 6	p.L205X	HPA, severe, central	protein truncation	#145 (hetero)	(Smooker, et al., 1993)
F212C	substitution	c.635T>G	exon 7	p.F212C	HPA, mild		#175 (homo)	(Dianzani, et al., 1998) (Blau, et al., 1992)
G218ins	insertion	c.653_654insGGATCACAG	exon 7	p.G218_K219insITG	HPA, mild		#289 (hetero)	(Dianzani, et al., 1998)

Mutant designation	Mutant type	Nucleotide aberration in cDNA or gDNA ^a	Location in gene	Predicted protein or RNA change	Patient's phenotype	Comment (% activity of wild-type)	Patient's number in BIODÉF ^b	References
R221X	substitution	c.661C>T	exon 7	p.R221X	HPA, severe, central	truncated protein	#151 (hetero) #182 (homo) #255 (homo) #260 (homo) #300 (homo) #390 (homo)	(Dianzani, et al., 1998) (Romstad, et al., 2000)

^a Accession numbers in Genome Sequence Data Base: X04882.1 or NM_000320.1 for human cDNA (the numbering starts with 1 at A at the ATG-start codon); for published fragments of human gDNA see AJ006239.1, AJ006240.1, AJ006241.1, AJ006242.1, AJ006243.1, AJ006244.1, and AJ006245.1

^b BIODÉF database: www.bh4.org; homo, homozygote; hetero, compound heterozygote