

RESEARCH ARTICLE

Isolated Central Form of Tetrahydrobiopterin Deficiency Associated With Hemizyosity on Chromosome 11q and a Mutant Allele of PTPS

Nenad Blau,¹ Tanja Scherer-Oppliger,¹ Alessandra Baumer,² Mariluce Riegel,² Ana Matasovic,¹ Albert Schinzel,² Jaak Jaeken,³ and Beat Thöny^{1*}

¹Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Zürich, Switzerland

²Institute of Medical Genetics, University of Zürich, Zürich, Switzerland

³Department of Pediatrics, University of Leuven, Leuven, Belgium

Communicated by Richard G.H. Cotton

6-Pyruvoyl-tetrahydropterin synthase (PTS or PTPS) is involved in tetrahydrobiopterin (BH₄) biosynthesis, the cofactor for various enzymes including the aromatic amino acid hydroxylases. Inherited PTPS deficiency is a heterogeneous disease with different phenotypes leading to BH₄ depletion. The severe form of PTPS deficiency causes hyperphenylalaninemia and monoamine neurotransmitter deficiency, whereas the mild form gives rise to hyperphenylalaninemia only. From 228 patients with PTPS deficiency at least 32 different mutant alleles have been identified on its corresponding gene, located on chromosome 11q22.3-q23.3. Here we describe a new allele from a child with PTPS deficiency who exhibited a mild but transient form of hyperphenylalaninemia, yet was deficient in CSF monoamines. The patient was found to carry, on her genomic DNA and cDNA, a homozygous A>G transition, leading to PTPS codon alteration Tyr99 to Cys (Y99C). The mother and several members of the maternal family were carriers of the Y99C allele, also verified by the reduced PTPS enzyme activity in erythrocytes. By cytogenetic, molecular, and FISH analyses, a de novo deletion spanning from 11q14 to 11q23.3 on the patient's paternal chromosome was mapped, establishing hemizyosity of the Y99C allele. The PTPS mutation observed in this patient generates a novel phenotype with an apparently isolated central form of BH₄ deficiency. *Hum Mutat* 16:54–60, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: hyperphenylalaninemia; tetrahydrobiopterin deficiency; PTS; PTPS; 6-pyruvoyl-tetrahydropterin synthase; hemizyosity; neurotransmitter deficiency

DATABASES:

PTS – OMIM:261640; GDB:118856; HGMD:PTS

INTRODUCTION

6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (MIM# 261640) is a rare autosomal recessively inherited neurometabolic disorder characterized by early onset of truncal hypotonia, increased limb tone with pronated hand posture, hypersalivation, and swallowing difficulties [Blau et al., 2000]. It is the most common variant of tetrahydrobiopterin (BH₄)-dependent hyperphenylalaninemias. About 60% of all patients with BH₄ deficiency registered in the international BIODEF database are affected by mutations in the PTPS gene (PTS) [Blau et al., 1996]. In contrast to patients with classical PKU, which can be success-

fully treated with low-phenylalanine diet, patients with PTPS deficiency need different treatment [Ponzone et al., 1987]. They do not respond to the phenylalanine-restricted diet and, if not diagnosed early and treated properly (with L-Dopa/

Received 16 February 2000; accepted revised manuscript 10 April 2000.

*Correspondence to: Dr. Beat Thöny, Division of Clinical Chemistry and Biochemistry, University Children's Hospital, Steinwiesstrasse 75, CH-8032 Zürich, Switzerland. E-mail: bthony@kispi.unizh.ch

Contract grant sponsors: Swiss National Science Foundation; Sandoz Stiftung.

Carbidopa/5-hydroxytryptophan/BH₄), are subject to severe myoclonic epilepsy and mental retardation, often with progression to fatal outcome. Furthermore, several reports demonstrate partial or mild cases of PTPS deficiency [Dhondt et al., 1988; Hoganson et al., 1984; Hreidasson et al., 1982; Niederwieser et al., 1987; Scriver et al., 1987]; however, the pathophysiology and molecular background of different phenotypes are still not clear. Patients with the mild/peripheral type present with a normal CNS biogenic amines metabolism and need only correction of hyperphenylalaninemia by BH₄ administration. They account for about 17% of all PTPS-deficient patients.

BH₄ is the natural cofactor for phenylalanine, tyrosine, and tryptophan hydroxylases, as well as for all three isoforms of nitric oxide synthase (NOS) [Thöny et al., 2000]. It is synthesized from GTP by the sequence of reactions catalyzed by GTP cyclohydrolase I, PTPS, and sepiapterin reductase. The PTPS enzyme has a homohexameric structure composed of two trimers associated in a head-to-head fashion. It catalyzes the conversion of dihydroneopterin triphosphate to 6-pyruvoyl-tetrahydropterin [Nar et al., 1994; Ploom et al., 1999]. Patients with PTPS deficiency exhibit enzyme activity that is very low or absent in red blood cells (RBC) [Shintaku et al., 1988], or fibroblasts [Oppliger et al., 1997; Oppliger et al., 1995a,b], and is reduced to about 20% of controls in samples from obligate heterozygotes [Niederwieser et al., 1987; Scriver et al., 1987]. Enzyme activity is reported to be slightly higher in patients with the mild peripheral form than in those affected by the severe central form of PTPS deficiency. In the mild peripheral form of PTPS deficiency, CNS neurotransmitter homeostasis seems to be unchanged and BH₄ deficiency specifically affects the hepatic phenylalanine hydroxylation. The human gene, *PTS*, was localized to chromosome 11q22.3-q23.3, and consists of six exons encoding the 145 amino acids monomer [Kluge et al., 1996; Thöny et al., 1992]. So far, 32 different mutations in 84 patients have been detected either by RT-PCR from cultured primary skin fibroblasts and/or by screening exonic DNA [Imamura et al., 1999; Liu and Hsiao, 1996; Liu et al., 1998; Oppliger et al., 1995a,b; Scherer-Oppliger et al., 1999a,b; Thöny and Blau, 1997; Thöny et al., 1994; Yoo and Kim, 1997; Zekanowski et al., 1998]. Four patients with the mild peripheral form of PTPS deficiency were investigated on the molecular level (alleles R16C, K38X, N47D, D116G, K120X/361del14bp, and K129E). All other mutations are from the severe

central type of deficiency. The P87S mutant allele was found in 20 Chinese and Japanese patients, the N52S and D96N mutations also seem to be common in Asian populations (16 and five patients detected, respectively), while the D136V allele was detected in six Caucasian patients [Blau et al., 1996]. All other mutations were found in fewer than three patients. A homozygous I114V mutation was shown to be associated with mild hyperphenylalaninemia in a patient with generalized dystonia and diurnal fluctuation of symptoms [Hanihara et al., 1997].

We report here the enzymatic, cytogenetic, and molecular investigations of a family with a patient affected by a novel type of PTPS deficiency. A de novo deletion on chromosome 11 together with the hemizygous PTPS-Y99C mutant allele generated a phenotype consistent with BH₄ depletion predominantly in the central nervous system.

MATERIALS AND METHODS

Case Report

Patient IV-1 (BIODEF #277) [Blau and Dhondt, 2000] was born of Belgian parents after a normal term pregnancy. At birth, weight was 2,720 g (10th centile), length 48 cm (3rd centile) and head circumference 31.9 cm (<3rd centile). Birth was uneventful and the clinical examination of the girl was normal. She was formula fed. Neonatal metabolic screening revealed hyperphenylalaninemia (1,636 μmol/l; normal 43–100) for which dietary treatment was started. At the age of eight months she was referred to the University Hospital of Leuven for irritability and slowing of weight gain and motor development. EEG and MRI of the brain were normal; however, metabolic investigations suggested a defect in BH₄ metabolism. Urinary neopterin was increased (8.89 mmol/mol creat.; normal 1.1–4.4) and biopterin was below the normal range (0.46 mmol/mol creatinin; normal 0.5–3.0). In cerebrospinal fluid (CSF) neopterin was increased (184 nmol/l; normal 15–55) and biopterin was decreased (9.6 nmol/l; normal 12–40). 5-Hydroxyindoleacetic acid (5HIAA) in CSF was 97 nmol/l (normal 120–600) and homovanillic acid (HVA) was 264 nmol/l (normal 300–900 nmol/l). 6-Pyruvoyl-tetrahydropterin synthase was found to be deficient in erythrocytes (0.8 μU/g Hb; normal 11–29) and fibroblasts (0.05 μU/mg protein; normal 1.9–2.6).

Treatment with L-Dopa/10% Carbidopa (5.6–7.8 mg/kg/d) and 5-hydroxytryptophan (1.2–4.8 mg/kg/d) normalized CSF neurotransmitter metabolites

and neurological abnormalities disappeared. Dietary treatment was stopped and, under normal protein intake, plasma phenylalanine levels were only slightly increased (130–190 $\mu\text{mol/l}$).

The last control at the age of 6.5 years showed a healthy and bright girl with normal plasma phenylalanine levels and normal protein intake. Total IQ was 111 with verbal IQ 114, performance IQ 103, language understanding IQ 124, and memory IQ 107.

The mother (III-6) developed a progressive dystonia/Parkinsonian syndrome at the age of 32 years without intellectual deterioration. A therapeutic trial with L-Dopa/Carbidopa was ineffective. Dysarthria is intermittently present but there are no epileptic seizures. The neurological signs started at the left side and are still predominant on that side (she is left-handed). Routine laboratory investigations, including CSF, were normal. [^{123}I]-IBZM SPECT analysis revealed an asymmetric decreased uptake of the radioligand in the striatum compatible with D2 dopamine receptor deficiency [Eyskens et al., 1988]. The mother's father (II-3) shows the same clinical picture and is wheelchair bound. His neurological problems started on the right side of the body and are still predominant on that side (he is right-handed).

Biochemical Analyses and Mutation Detection

Cell culturing, enzymatic determination of PTPS activity, Western blot analysis, mutation detection on genomic DNA and on cDNA from RT-PCR products were described previously [Oppliger et al., 1997; Scherer-Oppliger et al., 1999b; Shintaku et al., 1988; Thöny et al., 1994].

Haplotype Analysis

The haplotype analysis of the critical region on 11q was carried out for the proband and her close relatives using genomic DNA extracted from fibroblasts and microsatellite primers purchased from Research Genetics (Huntsville, AL, USA). The localization and genetic distances of the markers compiled in Table 1 are derived from Généthon and Genome Database (GDB) linkage maps (<http://www.genethon.fr> and <http://www.gdb.org>). The microsatellite markers were used in polymerase chain reactions (PCR) performed in a reaction volume of 25 μl in standard mixture. Amplification was carried out in a Perkin-Elmer CetusTM thermocycler (PE Biosystems, Rotkreuz, Switzerland) for 35 cycles with denaturation at 94°C for 3 min for the first cycle only, and 30 sec for consecutive cycles, primer annealing ranging from 52

TABLE 1. Microsatellite Markers (GDB and Généthon Genetic Linkage Maps)

| Marker | Localization | Approximate genetic distance between markers |
|----------|---------------|--|
| PYGM | 11q13.1 | |
| D11S901 | 11q14 | 13.3 cM |
| D11S940 | 11q22 | 13.3 cM |
| D11S1339 | 11q22 | 1.7 cM |
| D11S927 | 11q22-q23 | 3.9 cM |
| D11S1356 | 11q23.3 | 9.9 cM |
| D11S4132 | 11q23.3 | 2.7 cM |
| D11S1353 | 11q23.3-q24.2 | 6.5 cM |
| D11S934 | 11q23.3-q24 | 4.0 cM |
| D11S912 | 11q23-q25 | 5.2 cM |

to 60°C according to the primers for 45 sec, and extension at 72°C for 1 min. The PCR products were separated on 6% denaturing polyacrylamide gels and visualized by silver staining using standard procedures [Ausubel et al., 1992].

Cytogenetic and FISH Investigations

Fibroblast cells derived from the proband and peripheral blood lymphocytes from her father (III-5) and sister (IV-2) were cultured using standard procedures. Metaphase chromosome spreads were stained with the QFQ technique (fibroblast preparation from proband) and GTG technique (lymphocyte preparations from the father and sister of the proband) according to standard protocols. FISH analysis was carried out as previously described, with 20 ng of λ -phage DNA containing a 3' segment of the PTS gene from $\lambda\text{HGPTS3-1}$ for in situ hybridization [Kluge et al., 1996].

RESULTS

Enzymatic Analysis of PTPS and Mutation Detection

Following urinary pterin and CSF neurotransmitter determinations, the diagnosis of PTPS deficiency in patient IV-1 was made. Subsequent enzymatic measurements revealed PTPS activity below 5% of normal in erythrocytes, and below 2% of normal in skin fibroblasts, which confirmed the initial diagnosis (see also Case Report above). Nevertheless, upon Western blot examination, we found PTPS cross-reactive material, although somewhat reduced amounts in comparison with control fibroblasts (not shown). Standard enzymatic as well as molecular genetic analyses, including cDNA and genomic DNA mutation detection for PTPS, were then performed in the patient and various family members on primary skin fibroblasts

and on peripheral blood cells (see also Fig. 1). RT-PCR and PTPS-cDNA analysis from fibroblasts of patient IV-1 revealed a single, homozygous A to G transition at nucleotide position 296 (296A>G), leading to an amino acid residue alteration Tyr 99 to Cys, Y99C allele, in the derived PTPS protein. On the genomic DNA, the patient was homoallelic for Y99C, as revealed by amplification and DNA sequence analysis of exon 5 (not shown) [Oppliger et al., 1997]. Unexpectedly, the father (III-5) exhibited normal enzyme activity in red blood cells, which was in accordance with the wild type DNA sequence of his PTPS-exon 5. On the other hand, the mother (III-6), her second daughter (IV-2, sister of patient), plus seven other members out of 22 from the maternal family were carriers of the Y99C allele, as revealed by amplification and DNA sequence analysis of exon 5. Heterozygosity of the Y99C-PTPS allele in these individuals was confirmed by the reduced PTPS activity in red blood cells (Fig. 1). Further cytogenetic and molecular analyses were applied to investigate the genetic background of the maternal Y99C-PTPS allele in the patient's genome.

Haplotype Analysis Using Microsatellite Markers

As the human gene encoding PTPS is located on chromosome 11q22.3-q23.3 [Kluge et al., 1996], a haplotype analysis of the critical region on 11q was carried out for the proband and her close relatives using microsatellite primers mapping to the critical region. The localization and genetic distances of the microsatellite markers given in Table 1 are derived from Génethon and Gene Database GDB linkage maps. Besides those indicated in Table 1, a large number of additional microsatellite markers not located in the deleted region were analyzed to support paternity (not shown). The microsatellite analysis revealed an interstitial deletion at 11q14-q23.3 on the paternally inherited chromosome 11 in the proband that

was evident by the four markers D11S901, D11S940 (Fig. 2), D11S1339, and D11S4132. The deletion spanning from marker D11S901 to D11S4132 encompassed the chromosomal region from 11q14 to q23.3 (see Table 1), covering about 23 Mb, and includes the paternal wild type allele for PTPS in patient IV-1. A summary of all haplotype results at the deletion site and flanking regions is depicted in Figure 3.

Karyotype and FISH Analyses

To rule out any type of rearrangement or larger deletion, a karyotype analysis was carried out for the patient IV-1, her sister (IV-2), and her father (III-5). At a 400 band level, a small interstitial deletion at distal 11q was suspected, but a confirmation beyond doubt could not be achieved due to the suboptimal resolution. At a 600–800 band level, neither an interstitial deletion nor a balanced translocation could be seen in GTG banded karyotypes from the father and the sister of the probanda (not shown). Furthermore, FISH analysis with a probe encompassing the human gene encoding PTPS gave normal signals on chromosome 11 for the sister and father, and only one signal for the patient IV-1 (not shown). These results not only confirmed the de novo deletion comprising the PTPS gene in the patient, but also lack of a balanced translocation in her father and sister.

DISCUSSION

Here we describe an unusual case of PTPS deficiency by coincidence of a maternally inherited mutation and a de novo deletion effecting the paternal PTPS-allele in the patient. Furthermore, as the patient exhibited only a transient hyperphenylalaninemia in the early neonatal period, but suffers persistently from cerebral neurotransmitter deficiency, this is the first case of PTPS deficiency presenting with an isolated central phenotype. This is also reflected in the current treatment of the patient that includes standard

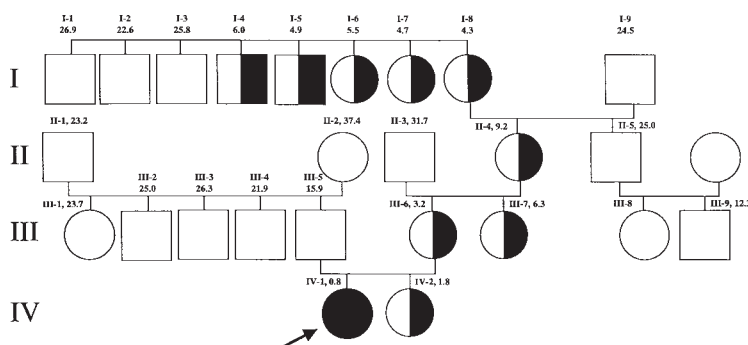


FIGURE 1. Pedigree of PTPS deficiency from the family presented in this study. Patient IV-1 is homozygous for the Y99C-PTPS allele and had enzyme activity in red blood cells that was < 5% of normal (0.8 μ U/mg Hb; normal values are between 11–29 μ U/mg Hb). Analysis of genomic DNA isolated from skin fibroblasts revealed that the patient's mother and sister plus seven other members of the maternal family were carriers of the Y99C-PTPS allele. Heterozygosity in these individuals is also reflected by the reduced PTPS enzyme activities in erythrocytes, as indicated after each individual's designations.

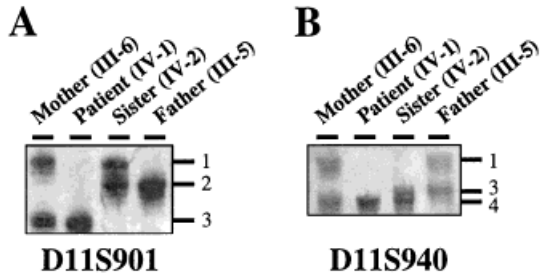


FIGURE 2. Microsatellite analysis for markers D11S901 (A) and D11S940 (B) for the proband, her sister, and both parents. The PCR products generated with the corresponding primers were separated on a 6% SDS-PAGE, and the alleles were numbered according to their size. For marker D11S901 (A), the lack of inheritance of the paternal allele (allele 2) is evident in the patient, whereas the two children inherited different alleles from their mother (alleles 1 and 3). For marker D11S940 (B), neither of the paternal alleles 1 and 3 are present in the patient, and the two children share the same allele 4 from the mother.

neurotransmitter precursor intake, but no BH₄, that is usually required to control the peripheral hyperphenylalaninemia. Two well-documented cases of PTPS deficiency with transient HPA have been described in the literature [Oppliger et al., 1997; Ponzone et al., 1990; Scherer-Oppliger et al., 1999b]. The first patient with a homozygous PTPS mutant allele K129E was clinically absolutely normal when treated with Deprenyl (MAO inhibitor) and showed normal PTPS immunoreactivity, but no enzyme activity, in primary fibroblasts and red blood cells [Oppliger et al., 1997]. In contrast

to its inactivity in these cells, the K129E-PTPS mutant was two–three fold more active than wild type PTPS when transfected into COS-1 cells or human hepatoma cell line Hep G2. The second patient exhibited compound heterozygosity with PTPS-mutant alleles N47D and D116G. He was treated with L-Dopa/Carbidopa/5-hydroxytryptophan in addition to BH₄ for the first 22 months of his life despite absence of any neurological abnormalities [Scherer-Oppliger et al., 1999b]. Transfection studies with single PTPS alleles in COS-1 cells showed that the N47D allele was inactive, while D116G had 66% of the wild type activity. Upon co-transfection of two PTPS alleles into COS-1 cells, the N47D allele had a dominant negative effect on both the wild type PTPS and the D116G mutant with relative reduction to about 20% of control values. In contrast to the new case reported here, both these previously described patients were initially treated with BH₄.

The development of such an isolated central phenotype (but also other transient forms of HPA) is enigmatic. The data presented from enzyme measurements with cell extracts conform very well to the DNA mutation analysis, i.e. for all carriers of the maternal family enzyme activity was reduced to the expected heterozygous levels [Scriver et al., 1987]. In addition, for the patient harboring hemizygotously the mutant PTPS, no enzyme activity was detected. Yet, upon recombinant expression of the Y99C-PTPS mutant in eukaryotic COS-1 cells or in the *E. coli* background, we found

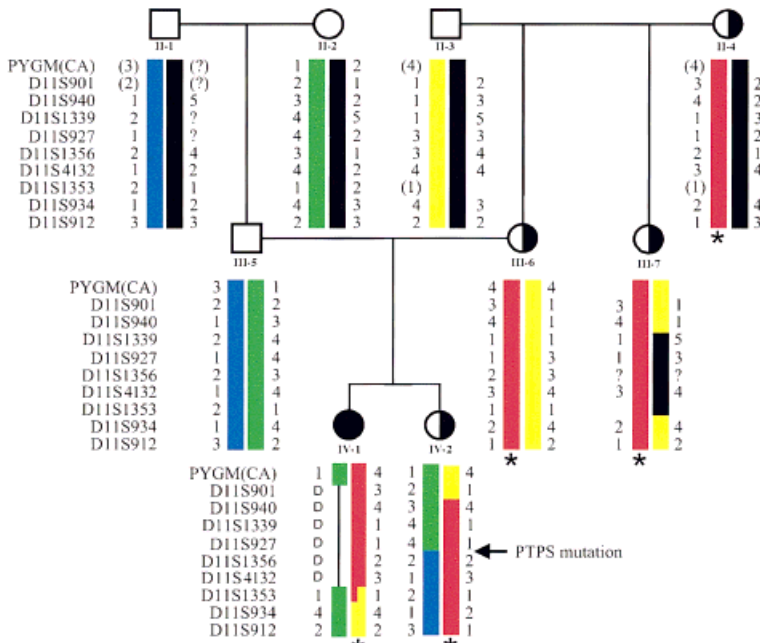


FIGURE 3. Schematic representation of the haplotypes on chromosome 11q for the proband, indicated with black circle, and her close relatives (see also Fig. 1). The haplotypes bearing the genomic PTPS mutation are indicated with an asterisk. The 11q deletion affecting the proband's paternal chromosome is indicated with "D" and a thin line. The localization and genetic distances of the microsatellite markers are indicated in Table 1. The alleles are numbered arbitrarily '1-5' according to their molecular size, whereby '1' represents the largest alleles (see also Fig. 2). Alleles shown in brackets were inferred from data based on the haplotypes of other family members. The position of the gene encoding PTPS is indicated (for IV-2) with an arrow.

no difference in specific enzyme activity when compared to the wild type enzyme (unpublished results). Obviously, more biochemical analyses need to be undertaken to potentially understand the physiology of this mutant enzyme.

As mentioned above, the genetic setup in patient IV-1 is rather unusual, as she carries an interstitial chromosomal deletion at 11q14-23.3. The deletion itself was not confidently demonstrable by cytogenetic investigations on metaphase chromosome spreads on fibroblasts. Furthermore, the presence of two alleles for three of the informative markers, D11S940, D11S1339, and D11S4132, as well as for D11S927 and D11S1356 in the father indicates the de novo nature of the deletion. The possibility of a balanced translocation in the father and sister was excluded karyotyping, and by fluorescent in situ hybridization (FISH) analysis using a clone containing the 3'-end of the gene encoding PTPS for in situ hybridization [Kluge et al., 1996]. Normal chromosome 11 specific signals were obtained for both the father and sister, whereas a single signal was seen for the patient, confirming hemizygoty for the Y99C-PTPS allele. The BH₄ deficiency in the proband can thus be explained by exposure of the recessive Y99C mutant allele inherited from the mother.

Deletions affecting the long arm of chromosome 11 are often associated with Jacobsen syndrome, also referred to as the 11q deletion syndrome [Feldman Lewanda et al., 1995; Michaelis et al., 1998; Pivnick et al., 1996; Stratton et al., 1994; Van Hemel et al., 1992]. Typical features of the syndrome include growth and psychomotor retardation, trigonocephaly, strabismus, inner epicanthic folds, short bulbous nose with anteverted nostrils, low set ears, abnormal fingers and toes, and a number of other anomalies, all of which show variability in their manifestation and severity. The majority of patients with clinical features of the Jacobsen syndrome have terminal deletions of 11q. A broad range of proximal breakpoints have been reported, although the vast majority of patients with Jacobsen syndrome have breakpoints at 11q23.3 (between markers D11S924 and D11S1341) [Penny et al., 1995]. Other patients have interstitial 11q deletions, translocations, and ring chromosomes [Klep-De Pater et al., 1984; Pivnick et al., 1996; Stratton et al., 1994; Van Hemel et al., 1992]. The 11q deletion in our patient extends beyond the Jacobsen syndrome common breakpoint at D11S924 and D11S1341. She shows, however, no signs of Jacobsen syndrome, thus her 11q deletion does not include the critical segment for

the syndrome. The degeneration of the basal ganglia affecting the proband's mother and her maternal grandfather appears to be an independent trait in the family.

ACKNOWLEDGMENTS

We thank M. Killen for help with the preparation of the manuscript. This work was supported by grants from the Swiss National Science Foundation (to N.B., A.S., and B.T.).

REFERENCES

- Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. 1992. Current protocols in molecular biology. John Wiley & Sons, Inc.: New York.
- Blau N, Barnes I, Dhondt JL. 1996. International database of tetrahydrobiopterin deficiencies. *J Inher Metab Dis* 19:8-14.
- Blau N, Dhondt JL. 2000. BIODEF: international database on tetrahydrobiopterin deficiencies. <http://www.unizh.ch/~blau/biodefl.html>.
- Blau N, Thöny B, Cotton RGH, Hyland K. 2000. Disorders of tetrahydrobiopterin and related biogenic amines. In: The metabolic and molecular bases of inherited disease. 8th ed. Scriver CR, Beaudet AL, Sly WS, Valle D, Vogelstein B, eds. New York: McGraw-Hill, in press.
- Blau N, Thöny B, Dianzani I. 2000. DIOMDB: database of mutations causing tetrahydrobiopterin deficiency. <http://www.unizh.ch/~blau/biomdb1.html>
- Dhondt JL, Meyer M, Malpuech G. 1988. Problems in the diagnosis of tetrahydrobiopterin deficiency. *Eur J Ped* 147:1988.
- Eyskens FJM, Booij J, Wevers RA, Nouwen A. 1988. A new progressive hereditary dystonia syndrome caused by a deficiency in D2 dopamine receptor. *J Inher Metab Dis* 21 (suppl 2):6.
- Feldman Lewanda ASM, Reid CS, Wang Jabs E. 1995. Two craniosynostotic patients with 11q deletions, and review of 48 cases. *Am J Med Genet* 59:193-198.
- Hanihara T, Inoue K, Kawanishi C, Sugiyama N, Miyakawa T, Onishi H, Yamada Y, et al. 1997. 6-Pyruvoyl-tetrahydropterin synthase deficiency with generalized dystonia and diurnal fluctuation of symptoms—a clinical and molecular study. *Movement Disorder* 12:408-411.
- Hoganson G, Berlow S, Kaufman S, Milstien S, Schuett V, Matalon R, Naylor E, Seifert W. 1984. Biopterin synthesis defects: problems in diagnosis. *Pediatrics* 74:1004-1011.
- Hreidasson S, Valle D, Holtzman N, Coyle J, Singer H, Kapatos G, Kaufman S. 1982. A peripheral defect in biopterin synthesis: a new mutant? *Pediatr Res* 16:192a.
- Imamura T, Okano Y, Shintaku H, Hase Y, Isshiki G. 1999. Molecular characterization of 6-pyruvoyl-tetrahydropterin synthase deficiency in Japanese patients. *J Hum Genet* 44:163-168.
- Klep-De Pater JM, De France HF, Bijlsma JB. 1984. Interstitial deletion of the long arm of chromosome. *J Med Genet* 11:224-226.
- Kluge C, Brecevic L, Heizmann CW, Blau N, Thöny B. 1996. Chromosomal localization, genomic structure and characterization of the human gene and a retropseudogene for 6-pyruvoyl-tetrahydropterin synthase. *Eur J Biochem* 240:477-484.
- Liu T-T, Hsiao K-J. 1996. Identification of a common 6-pyruvoyl-tetrahydropterin synthase mutation at codon 87 in Chinese phenylketonuria caused by tetrahydrobiopterin synthesis deficiency. *Hum Genet* 98:313-316.

- Liu TT, Hsiao KJ, Lu SF, Wu SJ, Wu KE, Chiang SH, Liu XQ, Chen RG, Yu WM. 1998. Mutation analysis of the 6-pyruvoyl-tetrahydropterin synthase gene in Chinese hyperphenylalaninemia caused by tetrahydrobiopterin synthesis deficiency. *Hum Mutat* 10:76–83.
- Michaelis RC, Velagaleti GVN, Jones C, Pivnick EK, Phelan MC, Boyd E, Tarleton J, et al. 1998. Most Jacobsen syndrome deletion breakpoints occur distal to FRA11B. *Am J Med Genet* 76:222–228.
- Nar H, Huber R, Heizmann CW, Thöny B, Bürgisser D. 1994. Three-dimensional structure of 6-pyruvoyl tetrahydropterin synthase, an enzyme involved in tetrahydrobiopterin biosynthesis. *EMBO J* 13:1255–1262.
- Niederwieser A, Shintaku H, Leimbacher W, Curtius H-C, Hyaneck J, Zeman J, Endres W. 1987. Peripheral tetrahydrobiopterin deficiency with hyperphenylalaninemia due to incomplete 6-pyruvoyl tetrahydropterin synthase deficiency or heterozygosity. *Eur J Pediatr* 146:228–232.
- Oppliger T, Thöny B, Leimbacher W, Scheibenreiter S, Brandt NJ, Heizmann CW, Blau N. 1995a. Mutation analysis in patients with 6-pyruvoyl-tetrahydropterin synthase deficiency. *Pteridines* 6:141–146.
- Oppliger T, Thöny B, Nar H, Bürgisser D, Huber R, Heizmann CW, Blau N. 1995b. Structural and functional consequences of mutations in 6-pyruvoyl-tetrahydropterin synthase causing hyperphenylalaninemia in humans—phosphorylation is a requirement for in vivo activity. *J Biol Chem* 270:29498–29506.
- Oppliger T, Thöny B, Kluge C, Matasovic A, Heizmann CW, Ponzone A, Spada M, Blau N. 1997. Identification of mutations causing 6-pyruvoyl-tetrahydropterin synthase deficiency in four Italian families. *Hum Mutat* 10:25–35.
- Penny LA, Dell'Aquila M, Jones MC, Bergoffen J, Cunniff C, Fryns J-P, Grace EG, Graham JM, Kousseff B, Mattina T, et al. 1995. Clinical and molecular characterization of patients with distal 11q deletions. *Am J Hum Genet* 56:676–683.
- Pivnick EK, Velagaleti GVN, Wilroy RS, Smith ME, Rose SR, Tipton RE, Tharapel AT. 1996. Jacobsen syndrome: report of a patient with severe eye anomalies, growth hormone deficiency, and hypothyroidism associated with deletion 11(q23q25) and review of 52 cases. *J Med Genet* 33:772–778.
- Ploom T, Thöny B, Lee S, Nar H, Leimbacher W, Huber R, Auerbach G. 1999. Crystallographic and kinetic investigations on the mechanism of 6-pyruvoyl-tetrahydropterin synthase. *J Mol Biol* 286:851–860.
- Ponzone A, Guardamagna O, Ferraris S, Biasetti S, Bracco G, Niederwieser A. 1987. Neurotransmitter therapy and diet in malignant phenylketonuria. *Eur J Pediatr* 146:93–94.
- Ponzone A, Blau N, Guardamagna O, Ferrero GB, Dianzani I, Endres W. 1990. Progression of 6-pyruvoyl-tetrahydropterin synthase deficiency from a peripheral into a central phenotype. *J Inher Metab Dis* 13:298–300.
- Scherer-Oppliger T, Leimbacher W, Blau N, Thöny B. 1999a. Serine 19 of human 6-pyruvoyl-tetrahydropterin synthase is a substrate for cGMP-protein kinase II-dependent phosphorylation. *J Biol Chem* 274:31341–31348.
- Scherer-Oppliger T, Matasovic A, Laufs S, Levy HL, Quackenbush EJ, Blau N, Thöny B. 1999b. Dominant negative allele (N47D) in a compound heterozygote for a variant of 6-pyruvoyl-tetrahydropterin synthase deficiency causing transient hyperphenylalaninemia. *Hum Mutat* 13:286–289.
- Scriver CR, Clow CL, Kaplan P, Niederwieser A. 1987. Hyperphenylalaninemia due to deficiency of 6-pyruvoyl-tetrahydropterin synthase. *Hum Genet* 77:168–171.
- Shintaku H, Niederwieser A, Leimbacher W, Curtius H-C. 1988. Tetrahydrobiopterin deficiency: assay for 6-pyruvoyl-tetrahydropterin synthase activity in erythrocytes, and detection of patients and heterozygous carriers. *Eur J Pediatr* 147:15–19.
- Stratton RF, Lazarus KH, Ritchie EJ, Bell AM. 1994. Deletion (11)(q14q21). *Am J Med Genet* 49:294–298.
- Thöny B, Leimbacher W, Bürgisser D, Heizmann CW. 1992. Human 6-pyruvoyl-tetrahydropterin synthase: cDNA cloning and heterologous expression of the recombinant enzyme. *Biochem Biophys Res Commun* 189:1437–1443.
- Thöny B, Leimbacher W, Blau N, Harvie A, Heizmann CW. 1994. Hyperphenylalaninemia due to defects in tetrahydrobiopterin metabolism: molecular characterization of mutations in 6-pyruvoyl-tetrahydropterin synthase. *Am J Hum Genet* 54:782–792.
- Thöny B, Blau N. 1997. Mutations in the GTP cyclohydrolase I and 6-pyruvoyl-tetrahydropterin synthase genes. *Hum Mutat* 10:11–20.
- Thöny B, Auerbach G, Blau N. 2000. Tetrahydrobiopterin biosynthesis, regeneration, and functions. *Biochem J* 347:1–16.
- Van Hemel JO, Eussen E, Wesby-van Saawy E, Oostra BA. 1992. Molecular detection of a translocation (Y;11)(q11.2;q24) in a 45,X male with signs of Jacobsen syndrome. *Hum Genet* 88:661–667.
- Yoo HW, Kim GH. 1997. Identification of mutations in Korean patients with atypical PKU caused by 6-pyruvoyl-tetrahydropterin synthase deficiency. *Am J Hum Genet* 61(suppl A):397.
- Zekanowski C, Nowacka M, Sendek E, Slowik M, Cabalska B, Bal J. 1998. Identification of mutations causing 6-pyruvoyl-tetrahydropterin synthase deficiency in Polish patients with variant hyperphenylalaninemia. *Mol Diagn* 3:237–239.