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Retrospective analysis of 19 patients with 6-Pyruvoyl Tetrahydropterin Synthase Deficiency: Prolactin levels inversely correlate with growth.

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Abstract

BACKGROUND.—Pyruvoyl Tetrahydropterin Synthase (PTPS) Deficiency is the most common form of BH4 deficiency resulting in hyperphenylalaninemia. It can have variable clinical severity and there is limited information on the clinical presentation, natural history and effectiveness of newborn screening for this condition.

METHODS.—Retrospective data (growth and clinical parameters, biochemical and genetic testing results, treatment) were collected from 19 patients with PTPS deficiency in different centers, to evaluate biochemical and clinical outcomes. Descriptive statistics was used for qualitative variables, while linear regression analysis was used to correlate quantitative variables.

RESULTS.—Patients with PTPS deficiency had an increased incidence of prematurity (4/18) with an average gestational age only mildly reduced (37.8±2.4 weeks) and low birth weight (−1.14±0.97 SD below that predicted for gestational age). With time, weight and height approached normal. values. All patients were identified by newborn screening for an elevated phenylalanine level. However, phenylalanine levels were normal in two whose testing was performed at or before 24 h of age. Sapropterin dihydrochloride treatment normalized phenylalanine levels. Molecular testing identified novel variants in the *PTS* gene, some of which present in more than one affected family. The neurotransmitter derivatives 5-hydroxyindoleacetic

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acid (5HIAA) and homovanillic acid (HVA) in the CSF were decreased in most cases except in 2 families with the peripheral form of PTPS deficiency. With time, HVA and 5HIAA became abnormally low in two of these patients requiring therapy. Prolactin (whose secretion is inhibited by dopamine) levels were elevated in several patients with PTPS deficiency and inversely correlated with the z-scores for height ($p<0.01$) and weight ($p<0.05$). Most patients with PTPS deficiency had delayed development early in life, improving around school age with IQs mostly in the normal range, with a small decline in older individuals. From a neurological standpoint, most patients had normal brain MRI and minor EEG anomalies, although some had persistent neurological symptoms.

DISCUSSION.—Patients with PTPS deficiency have not only an increased incidence of prematurity, but also decreased birth weight when corrected for gestational age. Hyperphenylalaninemia can be absent in the first day of life. Therapy with sapropterin dihydrochloride normalizes phenylalanine levels and neurotransmitter precursors can improve CSF neurotransmitter metabolites levels. Insufficient dopaminergic stimulation (as seen from elevated prolactin) might result in decreased height in patients with PTPS deficiency. Despite early delays in development, many patients can achieve independence in adult life, with usually normal neuroimaging and EEG.

Keywords

PTPS deficiency; hyperphenylalaninemia; newborn screening; neurotransmitter; prolactin; tetrahydrobiopterin; clinical outcome

Introduction.

Tetrahydrobiopterin (BH_4) deficiencies are caused by mutations in genes that encode enzymes required for BH_4 biosynthesis or regeneration [1]. BH4 is an essential cofactor for the aromatic amino acid hydroxylases phenylalanine hydroxylase (PAH), tyrosine-3-hydroxylase (TH) and tryptophan-5-hydroxylases (TPH1 and TPH2), as well as of the nitric oxide synthases (NOS). Deficiency of PAH activity causes hyperphenylalaninemia while TH and TPH deficiencies affect the synthesis of monoamine neurotransmitters in the brain [2].

BH_4 is synthesized by the human body by a series of reactions using the enzymes GTP cyclohydrolase I (GTPCH), 6-pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). Tetrahydrobiopterin provides electrons during the reaction required to hydroxylate substrates and, as a result, is oxidized to its hydroxyl compound pterin-4α-carbinolamine. Two enzymes pterin-4α-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR) are required to regenerate it to BH_4. Defects in any of these enzymes can cause BH4 deficiency [2, 3].

6-Pyruvoyltetrahydrobiopterin synthase (6-PTPS) deficiency is the most common form of BH_4 deficiency (65% of all cases). 6-PTPS catalyzes a magnesium- and zinc- dependent reaction that converts 7,8-dihydroneotriphosphate to 6-pyruvoyl-tetrahydropterin. This was initially discovered in patients with hyperphenylalaninemia and progressive neurological deterioration despite optimal metabolic control of phenylalanine levels [3].

Most patients with PTPS deficiency present severe manifestations with truncal hypotonia and increased limb tone with pronated hand posture. Loss of head control may precede the onset of a progressive neurological degenerative disease leading to hypertonicity, especially in the lower extremities. Difficulty in swallowing, oculogyric crises, somnolence, irritability, hyperthermia, seizures, and impaired neurophysiological development may occur. There may be bradykinesia and episodic “lead pipe” or “cogwheel” rigidity. Symptoms of generalized dystonia with marked diurnal fluctuations can occur in adult patients. Patients with the mild “peripheral” forms of PTPS deficiency may have normal CSF neurotransmitters in the first months of life, but can still progress to have very low CSF neurotransmitter levels between 1 and 2 years of age [4–7]. While these disorders were identified about 40 years ago [6], there are still gaps in knowledge regarding natural history and optimal treatment.

The study of the first 50 patients indicated that BH4 deficiencies affected about 2% of all hyperphenylalaninemic babies [8]. Usually their tolerance to dietary phenylalanine was high and their clinical symptoms responded to neurotransmitter precursors if started early in life. Diagnosis was accomplished by measurement of urinary pteridines and the activity of dihydropteridine reductase (DHPR) in blood spots [8].

The frequency of BH_4 deficiency varies greatly in different countries, with a high incidence (up to 1:11,000) of PTPS deficiency in China and Japan [9] [10]. Implementation of newborn screening decreased the median age of diagnosis [10] and therapy with sapropterin dihydrochloride (average 7.9 mg/kg per day) normalized phenylalanine levels in all patients with PTPS deficiency and reduced them in one patient with DHPR deficiency [5].

An international survey of 626 patients with BH_4 deficiencies indicated that some patients with GTPCH deficiency (mild variants and dominant forms) and all with SR deficiency had normal blood phenylalanine levels [11]. Up to 57% of neonates with BH_4 deficiencies had already symptoms at time of diagnosis, with hypotonia, delays in development, and age-dependent movement disorders. Therapy included a combination of L-dopa/carbidopa and 5-hydroxytryptophan in all conditions in addition to sapropterin dihydrochloride in PTPS and GTPCH deficiencies. Folinic acid and a low phenylalanine diet were utilized in DHPR deficiency [11].

To further complicate the issue, some patients with PTPS deficiency have only a mild defect, with no changes in CNS neurotransmitters. In Italy, a cohort of 19 patients with PTPS deficiency was divided into those with relatively normal cerebrospinal fluid (CSF) neurotransmitter metabolites (5-hydroxyindolacetic acid (5-HIAA) and homovanillic acid (HVA) (mild variant) and those with decreased levels (severe form) [4]. In the mild form, levels of CSF HVA remained in the normal range, but there was an asymptomatic mild decline of CSF 5-HIAA in all subjects after the second year of life. Patients with the severe form had a higher diagnostic level of blood phenylalanine compared to mild forms. Variants in the *PTS* gene predicted to cause a lesser impairment of PTPS enzyme activity were associated with milder biochemical abnormalities [4]. Therapy failed to fully restore neurotransmitter metabolites in the CSF in the severe form [4]. Patients with milder forms had normal neurological outcome, while most patients with the severe form were already

neurologically impaired at time of diagnosis. Early correction (<2 months of life) of monoamine neurotransmitter levels by medical therapy was associated with a better prognosis and a continuous improvement in the neuropsychiatric symptoms [12].

Here we present the natural history of a group of patients with PTPS deficiency collected from three different centers showing how the condition evolves over time and that some symptoms remain difficult to control with treatment. Our cohort includes some patients from a recent published Italian study and extends the analysis of data.

Patients and Methods.

This was a retrospective chart review of patients followed at three metabolic centers (San Paolo Hospital of Milan, University Hospital of Padua, University of Utah in Salt Lake City) for PTPS deficiency. It was approved by the University of Utah Institutional Review Board, from San Paolo Hospital Institutional Review Board and from University of Padua Review Board.

Medical records of 19 patients with PTPS deficiency in all centers were retrospectively reviewed, 9 from the University of Milan, 2 from the University of Padua and 8 from the University of Utah. Electronic and paper records were reviewed and pertinent data recorded (deidentified) in an excel spreadsheet.

Patients were identified either by an elevated Phe level at newborn screening, symptomatically or based upon family history.

The measurement of urine pterins (neopterin and biopterin) was performed in all patients. Italian patients also had a sapropterin dihydrochloride loading test (performed by giving a dose of 20 mg/kg of sapropterin with measurement of phenylalanine levels at baseline and after 4,8, 24, 32 and 48 h) for the rapid differentiation between classic PKU and BH_4 deficiency at time of initial diagnosis.

Patients with abnormal urine pterins had measurement of pterins and neurotransmitters metabolites in the CSF, to differentiate between severe and mild forms of BH_4 deficiency.

Some Italian patients had measurement of PTPS enzyme activity in fibroblasts in addition to DNA studies that were done in all patients to provide definitive confirmation of the specific deficiency.

Serum prolactin levels were measured in some patients to estimate deficiency of dopaminergic stimulation (prolactin levels increase with defective availability of dopamine). Serum prolactin levels were usually collected in the morning before the administration of monoamine neurotransmitter precursors.

Genotype information was collected and evaluated based upon publicly available databases (ClinVar®, HGMD®, UCSC Genome Browser®, GnomAD®, Exac®) or in the literature and missense variants not previously reported were evaluated using Sorting Intolerant From Tolerant (SIFT, https://sift.jcvi.org/www/SIFT_enst_submit.html) and Polymorphism Phenotyping Version v2.2.2r398 (POLYPHEN-2, https://genetics.bwh.harvard.edu/pph2/

index.shtml) software programs for variant evaluation (Table 3). Scores from these programs (Polyphen-2, score 0 to 1, with 1 being the most damaging; SIFT, score 0 to 1 with scores ≤ 0.05 indicating a damaging effect) were utilized to predict whether amino acid substitutions were tolerated or damaging.

Mutations were described according to official guidelines: (http://www.hgvs.org/mutnomen/).

For each individual, all available data regarding diagnostic evaluations, biochemical parameters, clinical symptoms at follow up and therapeutic interventions were collected.

Treatment in all patients with PTPS included correction of elevated blood phenylalanine levels by BH4 and replacement therapy with neurotransmitters precursors (L-dopa/Carbidopa and 5-hydroxytryptophan). Patients with the mild peripheral phenotype required only BH4 monotherapy to correct hyperphenylalaninemia.

Growth parameters, including height, weight, BMI and head circumference were collected at each visit. For analysis, they were stratified per age groups (0 – 6 months, 6 – 12 months, 12 – 18 months, 18 – 24 months, 24 – 36 months, 3 –4 years, 4–5 years, 5–6 years, 6–7 years, 7–8 years, 8–9 years, 9–10 years, 10–11 years, 11–12 years, 12–13 years, 13–14 years, 14–15 years, 15–16 years, 16–17 years, 17–18 years, 18–25 years, >25 years). Data were standardized using WHO Anthro® and PediTools® software to eliminate any possible sex-related bias.

Data were tabulated and analyzed using Excel Statistics Software 2016®. Data were summarized using descriptive statistics. Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were reported as frequencies. Linear regression analysis was used to correlate continuous variables.

RESULTS

Demographics.

Table 1 summarizes the demographics of all participants at the time of data cut-off (1 April 2019). Nineteen patients (8 males, 42%, and 11 females, 58%) with confirmed PTPS deficiency were followed at the San Paolo Hospital in Milan, Italy (9 patients, 4 males and 5 females), the University of Padua, Italy (2 patients, 1 male and 1 female), and the University of Utah, Salt Lake City UT, USA (8 patients, 3 males and 5 females). Age ranged from 11 months to 42 years, with an average age of 15.9±10.8 years (Table 1). Three patients (8, 10 and 19) had a previously affected sibling and were identified and treated earlier than other patients. Of the patients in Table 1, patients 5, 7 and 8 have already been partially reported [4, 12].

There was an increased incidence of prematurity among patients with PTPS deficiency (4/18) with 4 patients (1, 6, 13, 14 in Table 1) having a birth weight 2 SD or more below average for gestational age. The average weight z-score was −1.14 ±0.97 after correction for gestational age and gender (Table 1). Average gestational age was 36.9±3.2 weeks for males

(average birth weight 2.407±0.797 kg) and 38.5±1.5 weeks for females (average birth weight 2.647±0.500 kg).

All patients were identified by newborn screening for an elevated Phe level (Fig. 1). However, patients 13 and 19 had their first newborn screen collected at 24 h of age with normal Phe (73 and 90 μmol/L) and tyrosine level (57 and 47 μmol/L) and completely normal Phe/Tyr ratio (1.3 and 1.9), respectively. In these two patients, the second newborn screening collected at 8 days of life identified the elevated Phe levels (1,203 and 1,099 μmol/L in patients 13 and 19, respectively). Phenylalanine levels increased steadily with age of sample collection (Fig. 1), reflecting both the increased supply of phenylalanine with the diet and, possibly, the disappearance of the BH_4 cofactor provided by the mother to the fetus through the placenta [13]. Phe levels in patient 19 were normal at day one of life, but started to increase at 3 days of life and all affected patients had elevated phenylalanine after this age. In all patients identified by the first newborn screen, the sample was collected after 72 h of life and all of them had elevated phenylalanine. Therefore, newborn screening cards collected before 48 h of life can potentially miss cases of PTPS deficiency.

After initiation of sapropterin dihydrochloride treatment, phenylalanine levels normalized (30–90 μmol/L) in all patients, with the exception of patient 18 in whom Phe levels were consistently mildly increased (80–120 μmol/L) despite continuation of sapropterin dihydrochloride therapy. DNA sequencing in this case identified heterozygosity for a pathogenic variant (c.516G>T (p.Gln172His)) in the *PAH* gene, which likely explained the persistent mild elevation of phenylalanine levels.

A rise in phenylalanine levels was observed when sapropterin dihydrochloride therapy was omitted for various reasons (non-compliance, lack of insurance coverage, temporary drug unavailability) and returned promptly to normal upon restarting therapy (data not shown). In some patients, mild increases of phenylalanine just above the normal range caused by lack of sapropterin dihydrochloride therapy were associated with the onset of non-specific symptoms (inattention, irritability) that disappeared upon re-administration of sapropterin dihydrochloride.

Molecular and enzymatic findings.

Table 2 summarizes PTPS enzyme activity in patients' erythrocytes and the results of molecular analysis of the *PTS* gene encoding PTPS. Enzyme assay was performed only in a few patients, being substituted by molecular testing in younger patients. PTPS enzyme activity, when performed, was always below the normal range (Table 2). Given the limited number of patients in which enzyme activity was performed, genotype-activity correlation could not be assessed.

DNA sequencing identified 23 variants in the *PTS* gene. Variants were identified in 36 of 38 predicted abnormal alleles. One patient (15 in table 2) did not have any variants in the coding region of the *PTS* gene. However, parents were related and the *PTS* gene was in the region of loss of heterozygosity as ascertained with cytogenomic SNP (Single Nucleotide Polymorphism) microarrays. In addition, whole gene sequencing at an outside institution

identified a putative causal variant in the promoter region of the *PTS* gene that is currently being tested for pathogenicity in cellular models.

Of the 23 variants identified, 13 had been previously reported [12, 14–16] and 10 were novel (in Bold in table 2). Among all variants, 16 were missense, 2 frameshift causing the premature insertion of a stop codon, and 4 affected non-coding regions with 3 presumably affecting splicing (Table 3). Of the 19 patients, 4 were homozygotes (patients 9, 11, 14 and 15) while the others were compound heterozygotes (Table 2). Five variants (c.84–3C>G (IVS1–3C>G), c.164–1G>C (IVS2–1G>C), c.245A>G (p.Glu82Gly), c.308T>C (p.Val103Ala), c.370G>T (p.Val124Leu)) were observed in more than one family (two/three families each), with the remaining variants being restricted to single families. c.308T>C (p.Val103Ala) is the most common variant in our cohort and it was only present in Italian patients. The second most common variant, c.245A>G (p.Glu82Gly), was only seen in patients from the USA.

Of the 10 novel variants, 5 were missense (p.Ser15Ala, p.Met80IIe, p.Glu82Gly, p.Val103Ala, p.Leu127Val) and 5 were located outside the coding region (c.164–1G>C, c.164–37dupC, c.187–37insC, c.244–8G>C, g.3760_3816del57). Missense variants were evaluated using SIFT (Sorting Intolerant From Tolerant) and POLYPHEN-2 (Polymorphism Phenotyping Version v2.2.2r398). The c.308T>C (p.Val103Ala) was present in 2 siblings (patient 7 and 8, previously published), in patient 1 and in patient 4 [4]. The amino acid substitution is predicted to be benign by Polyphen2, but so is at least one known pathogenic variant (p.Pro87Leu). The novel variant c.245A>G (p.Glu82Gly) was found in 2 siblings (patient 13 and 19) and in patient 2. *In silico* predictions suggest that the amino acid substitution is well tolerated. However, the nucleotide change (A>G) occurs 2 nucleotides inside the new exon and likely affects splicing. Of the other 3 missense variants, only p.Leu127Val is predicted to be pathogenic. Of the novel variants outside the coding region, only c.164–1G>C affects a canonical splicing site, with all other having an unknown mechanism of possible pathogenicity.

In the patients in whom enzymatic activity was measured (Table 2), there was no clearcut correlation between genotype and function, although the highest residual enzymatic activity was found in patients carrying missense variants (p.His49Arg, p.Val103Ala, p.Leu127Val).

Biochemical abnormalities.

All patients with PTPS deficiency had abnormal neopterin and/or biopterin/tetrahydrobiopterin in urine and/or CSF (Table 4). Results of neopterin and biopterin are reported in absolute units or as percent biopterin of total pterins with the normal range indicated at the bottom of the table. Neopterin was increased and biopterin (or the percent biopterin) was decreased in all urine samples analyzed before treatment. CSF was analyzed in all patients with abnormal biopterin screen. In five patients the two studies (urine and CSF) were performed at the same time (patients 1, 5, 7, 9, 11). Biopterin was reduced in the CSF of most patients with an increase in neopterin.

The neurotransmitter derivatives HVA and 5HIAA were decreased in the CSF of 13 patients with PTPS deficiency at time of diagnosis (Table 4). Patient 11 had only a mild decrease of

5HIAA, while neurotransmitter derivatives were within the normal range in 5 patients, 4 of whom had an initial diagnosis of the peripheral form of PTPS deficiency. Patients 7 and 8 had normal CSF neurotransmitter metabolites at birth. However, they developed daytime somnolence at 2–3 years of age and repeated CSF analysis demonstrated low 5HIAA and HVA, for which they were started on neurotransmitter precursors at low dose [17].

There was no strict correlation between reduced BH_4 in the CSF and reduced neurotransmitter precursors, although patients with BH_4 below the limit of detection (<5) all had very low neurotransmitter levels (Table 4).

In Italian patients the BH4 loading test was also performed with the administration of 20 mg/kg of sapropterin dihydrochloride at 1–2 months of age. This caused prompt normalization of Phe levels in all patients (not shown).

Therapy.

Table 5 summarize the current therapy in all patients at time of the last follow up. The dose of sapropterin dihydrochloride ranged from 1.2 to 41 mg/kg per day.

Patients 4 and 17 had normal level of CSF neurotransmitter metabolites (5HIAA and HVA) and received only sapropterin dihydrochloride supplementation. In addition to sapropterin dihydrochloride, all other patients (17/19) received levodopa/carbidopa, with 16 of them also receiving 5-hydroxytryptophan (5-HTP) and 8 of them also receiving other medications (MAO B inhibitors, dopamine agonists, peripheral COMT inhibitors). The dose of levodopa ranged from 1.4 to 12.7 mg/kg per day (average 6.00±2.71 mg/kg per day); carbidopa ranged from 0.33 to 5.25 mg/kg (average 1.94±1.72 mg/kg per day), in part reflecting the different ages of the patients and the spectrum of severity of PTPS deficiency.

Sixteen of our 19 patients received 5-hydroxytryptophan (4.23±2.02 mg/kg per day, range 0.3–8.4 mg/kg per day). Patient 9 was not treated with 5-HTP due to gastrointestinal side effects and an early age. The average dose of 5-HTP in our patients was similar to that reported in a previous survey [11].

Trihexyphenidyl was given to one patient because of involuntary movements resulting in their disappearance. Three patients received selegiline to minimize fluctuations in L-dopa effects. Patient 6 receives a peripheral COMT inhibitor (entacapone) and nine patients receive folinic acid supplements to prevent central 3-methyltetrahydrofolate deficiency. Non-compliance with therapy (patient 4) due to a psychiatric disorder resulted in significant clinical deterioration.

Outcome.

Many children with PTPS deficiency were small at birth even after correcting for prematurity (Table 1). Given the small sample size, we evaluated Z-scores to enable combining males and female patients. After birth, their weight was below average in the first 2 years of life before normalizing with Z-score overlapping the mean (Fig. 2A). Similarly, the height was below average at birth although still overlapping with the mean (Fig. 2B). We had very few values for head circumference that did not allow statistical analysis for all time

points (Fig. 2C). Overall, growth parameters had only minimal variations from the average and normalized with time.

We evaluated the correlation between average prolactin levels (excluding values in the neonatal period before therapy) and height, weight and BMI (measured as Z-scores) in patients with PTPS deficiency (Fig. 3). Even if levels of prolactin vary during the day, we tried to collect samples before the administration of L-Dopa in the morning. Our analysis revealed an inverse correlation between average levels of prolactin and weight (Fig. 3A, $p=0.04$), height (Fig. 3B, $p=0.005$), but not body mass index (Fig. 3C). Our data suggest that poor dopaminergic stimulation might results in decreased height and resultant weight in patients with PTPS deficiency.

Most patients with PTPS deficiency had delayed early development with low developmental quotient (DQ) and/or intelligence quotient (IQ) (Fig. 4), but they improved around school age with IQs mostly in the normal range (Fig. 4). However, many patients had residual neurological problems and, in many cases, behavioral or psychiatric comorbidities (Table 6).

Table 6 reports in narrative form neurological and functional outcomes of patients with PTPS deficiency. Most patients had delayed early development, but improved with time, with 3 of 6 adults (patients 1,3 and 4) being partially independent and 3 mostly independent (patients 2,5 and 6). One female patient (patient 2) had three normal children while continuing therapy with neurotransmitters and sapropterin dihydrochloride during pregnancy. Among the 13 younger patients, five (7,8,10, 12 and 17) have normal or near normal development, 6 (11, 13, 14,15, 18, and 19) are mildly behind with speech being the most affected area, and 2 (9 and 16) have intellectual disability, dystonic posture and movements, oculogyric crisis and drooling. It is interesting that patients with peripheral deficiency seemed to be behind as well, indicating that current testing might not be fully predictive of neurotransmitter deficiency.

About 50% of PTPS patients can have neuroradiological abnormalities such as delayed myelination, periventricular hyperintensities, brain atrophy, and in one case brain calcifications [12]. Eleven of our 19 patients had at least one brain MRI (Table 6) with mostly normal results, with a few having periventricular hyperintensities and/or dilation of lateral ventricles (patients 1,3,5 in Table 6) as seen by others [18]. EEG, usually obtained for the presence of neurological symptoms showed few minor anomalies. Patient 4 had absence seizures at age 7, these improved with antiepileptic drugs (valproic acid first, and then lamotrigine). Discontinuation of therapy for fear of weight gain (she developed an eating disorder) caused onset of new focal seizures. Patient 9 had two episodes of generalized seizures with cyanosis for which levetiracetam was started. Overall, epilepsy was less frequent then in other Italian cohorts [12].

Dopamine deficiency can cause parkinsonism, dystonic and involuntary movements, while serotoninergic deficiency is responsible for sleep disturbance, mood dysregulation and temperature instability [19]. Patient 1 still has motor (fatigue, dysarthria, abnormal gait, dystonic crisis) and behavior difficulties (anxiety attacks, sleep disorder). Patient 2 had chorea and psychologic issues (temper tantrum, depression, hyperactivity) as a child, but as

an adult experiences mild symptoms. Patient 3 has occasional dystonic episodes and perioral tremors, in addition to obsessive-compulsive disorder. Patient 4 had focal seizures and developed anorexia after stopping all medications. Patient 5 has occasional mild fatigue and headache at night, but is otherwise normal. Patient 6 has issues in fine motor dexterity and attention, as well as in working memory and speech. Neurological symptoms are also present in younger patients, but are harder to define. Most younger patients with PTPS deficiency require help in school and supportive therapies (occupational, physical and speech therapy), as did the older individuals during childhood.

DISCUSSION

6-Pyruvoyltetrahydrobiopterin synthase (6-PTPS) deficiency is the most common form of BH_4 deficiency. Here we report novel patients from Italy and the USA with the oldest being more than 40 years old (Table 1). An increased incidence of prematurity among patients with PTPS deficiency is well described [11]. Here we find that our population also had decreased birth weight when corrected for gestational age adding to the possible prenatal effects of this condition. The small size corrected with time, with patients eventually achieving normal growth parameters (Fig. 2).

All cases of PTPS deficiency were identified by newborn screening, but phenylalanine levels can be normal (<90 umol/L) in patients with severe PTPS deficiency if collected too early in life (<48 h of life) (Fig. 1) and reinforce the need to collect additional samples in premature or sick infants as part of almost all newborn screening protocols.

PTPS deficiency is caused by heterogeneous pathogenic variants in the *PTS* gene (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PTS) located in 11q23.1 (MIM*612719). DNA testing identified known and novel pathogenic variants in the *PTS* gene (Tables 2 and 3), with some occurring in more than one individual and in some cases in independent families. One patient had variants outside the coding region, indicating that not all variants can be identified with routine DNA sequencing.

PTPS deficiency causes hyperphenylalaninemia and depletion of the monoamine neurotransmitters of the central nervous system [2, 13]. Our standard therapy is the correction of elevated blood phenylalanine levels by sapropterin dihydrochloride and supplementation with neurotransmitter precursors (levodopa with carbidopa, 5-hydroxytryptophan). While even the lower dose of sapropterin dihydrochloride normalized plasma phenylalanine levels, doses >20 mg/kg per day were the only ones capable of increasing BH_4 in the CSF of patients with very low baseline levels (not shown). The relatively high dose of sapropterin dihydrochloride necessary for detection in the CSF is likely related to its poor penetration into the brain as compared to other organs [2]. By contrast, the BH_4 precursors sepiapterin and dihydrobiopterin can enter cells more efficiently using equilibrative nucleoside transporters (mostly hENT2) [20] and administration of sepiapterin can increase CSF BH_4 levels in healthy volunteers [21]. Prevention of BH_4 deficiency attenuates dopamine depletion rat ventral mesencephalic slice cultures [22], but we do not know whether the presence of BH_4 in the CSF can improve endogenous production of neurotransmitters in humans.

With the most recent patients in the USA, levodopa-carbidopa in infants were compounded with higher amounts of carbidopa to fully inhibit peripheral levodopa metabolism (carbidopa/levodopa are compounded both at 10 mg/ml) and resulting in fewer gastrointestinal side-effects. Treatment is usually started with 1 mg/kg per day of levodopa divided into 5–6 doses, increasing the dose every 7 days by 0.5 mg/kg per day to a target of 5–15 mg/kg per day. After 1 year of age, we provide lower amounts of carbidopa (carbidopa is compounded at 5 mg/ml with levodopa at 10 mg/ml) and reduce carbidopa further to 25% of levodopa after 5 years of age and then even further. The average dose of levodopa used by our patients is about one half than the one recommended by a recent guideline (10 mg/kg per day in PTPS deficiency [23]), but similar to that reported in a previous survey of European patients [11]. Six of our patients received long-acting dopamine-agonists such as pramipexole or ropinorole. In all of our patients, the dosage of these drugs was relatively low (6.3–18 μg/kg per day) and well below the dose associated with impulse control disorders (30–33 μg/kg per day) in patients with PTPS deficiency [24]. At the same time, the use of long-acting dopamine-agonists probably reduced the requirements for levodopa-carbidopa at least in some of our patients.

There are no formal recommendations for the dose of 5-hydroxytryptophan to use in PTPS deficiency, but the dose is usually a little lower than the dose of levodopa [23]. While it is difficult to assess clinically the adequacy of dosing, review of CSF studies in patients who received more than 2 mg/kg per day of 5-hydroxytryptophan show values of HIAA within the normal range.

Additional therapies, if clinically indicated, include the use of monoamine oxidase B (MAO B) inhibitors, dopa agonists, anticholinergic agents, peripheral catechol-O-methyltransferase (COMT) inhibitor and folinic acid [23]. Treatment is usually titrated based on symptoms, serum prolactin levels and CSF neurotransmitter derivatives measured by lumbar puncture.

All patients had abnormal CSF pterin metabolites and in most cases reduced levels of neurotransmitter metabolites (Table 4), requiring therapy with neurotransmitter precursors (Table 5). Patients with the mild peripheral form of PTPS deficiency require only sapropterin dihydrochloride monotherapy to correct hyperphenylalaninemia. Despite variability in the treatment of individual patients, the dosage of the drugs was, on average, similar to that reported in other studies [11].

Despite therapy, many patients had complications with neurological and developmental problems (Table 6 and Fig. 3). A high frequency of neurological, psychological, and psychiatric symptoms has been reported in patients with PTPS deficiency symptoms [4, 23]. In our cohort, 50% (3/6) of adult patients had mild to moderate psychiatric symptoms (Table 6) as in other studies [25]. On average, our patients were mildly or moderately delayed in development with no worsening over time (Fig. 3). However, a small decline was seen in older individuals and we do not know whether this represents the effect of poor compliance with therapy, inappropriate/late therapies early in life or progression of the disease. Previous studies found a median IQ of 80 (range 36.3–112) with normal developmental quotients (DQs) early in life only in about 2/3 of patients with PTPS deficiency from China, with late treatment being associated with lower scores [10]. A survey of a large number of patients

identified “retardation” in about 30% of neonates, 40% of infants, and more than 60% of children with PTPS deficiency [11]. Overall, the neurodevelopmental involvement in our cohort shows a gradual improvement especially when patients remain on continuous treatment started early in life.

Height is the resultant of several variables, the main of which are genetic factors inherited from both parents. Genetic and metabolic disorders can impair growth by providing a decreased genetic potential, reducing the production of growth-related hormones or affecting the utilization of nutrients required for growing. Patients with PTPS deficiency can have central dopamine deficiency that results in elevated levels of prolactin. The increase in serum prolactin levels is used, with other parameters, to monitor neurotransmitter precursor therapy in patients with PTPS deficiency [26]. In our cohort, growth parameters (weight and height) inversely correlated to average prolactin levels (Fig. 3), suggesting that low dopaminergic stimulation might impair growth.

Short stature and defective growth hormone secretion in response to arginine and insulin has been reported in one patient with PTPS deficiency [27] and dopamine-responsive growth-hormone deficiency and central hypothyroidism have been reported in another with sepiapterin reductase deficiency, a brain-specific disorder of biopterin synthesis [28]. Our data on a limited population of patients with PTPS deficiency further support a connection between proper dopamine production (with suppression of prolactin secretion in the brain) and growth.

Given the variability in therapies across centers, the range of severity of PTPS deficiency, the large age range (1–42 years of age), and the evolution of therapies evolving over time, it is difficult to determine which treatment modality works the best. However, the residual presence of significant complications in our populations of patients with PTPS deficiency and others [4, 5, 10–12, 18, 24] highlight the need for more effective therapies in PTPS deficiency. The ideal therapy should minimize the fluctuations of neurological stimulation seen with the current drugs to reduce the frequency and severity of long-term complications.

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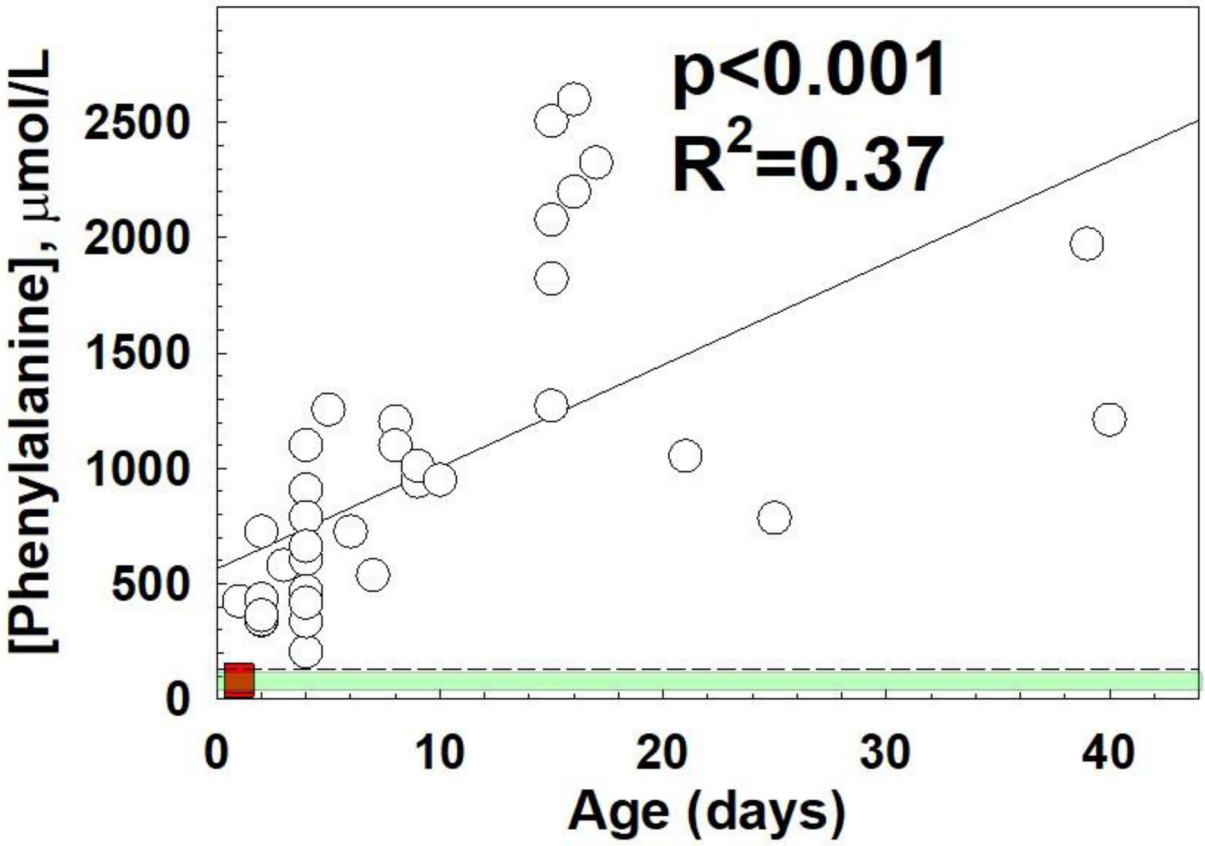

Figure 1.

Phenylalanine levels (measured in newborn screening blood spots or by plasma amino acids) in infants with PTPS deficiency as a function of age before initiation of treatment. The green shaded area represents the normal range for phenylalanine in newborns. The dashed line represents the cut-off for newborn screening. The two squares represent normal values of phenylalanine in two infants whose first newborn screening card was collected at one day of life, the empty circles abnormal values measured in other infants or after 1 day of life in the infants represented with a square.

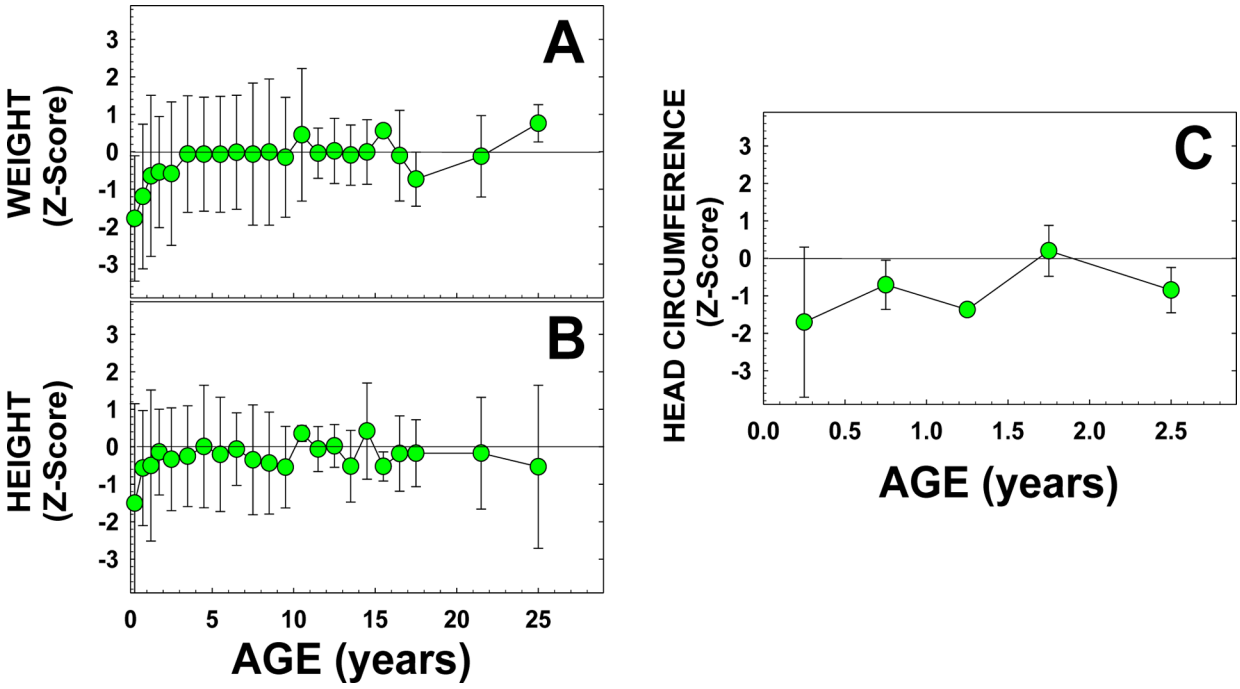

Figure 2.

Z-scores for anthropometric data in patients with PTPS deficiency. Scores for weight (A), height (B), and head circumference (C) were averaged at specific age points and are shown with their confidence intervals. Note the overlap of the confidence intervals with the 0 for all parameters after 1 year of age.

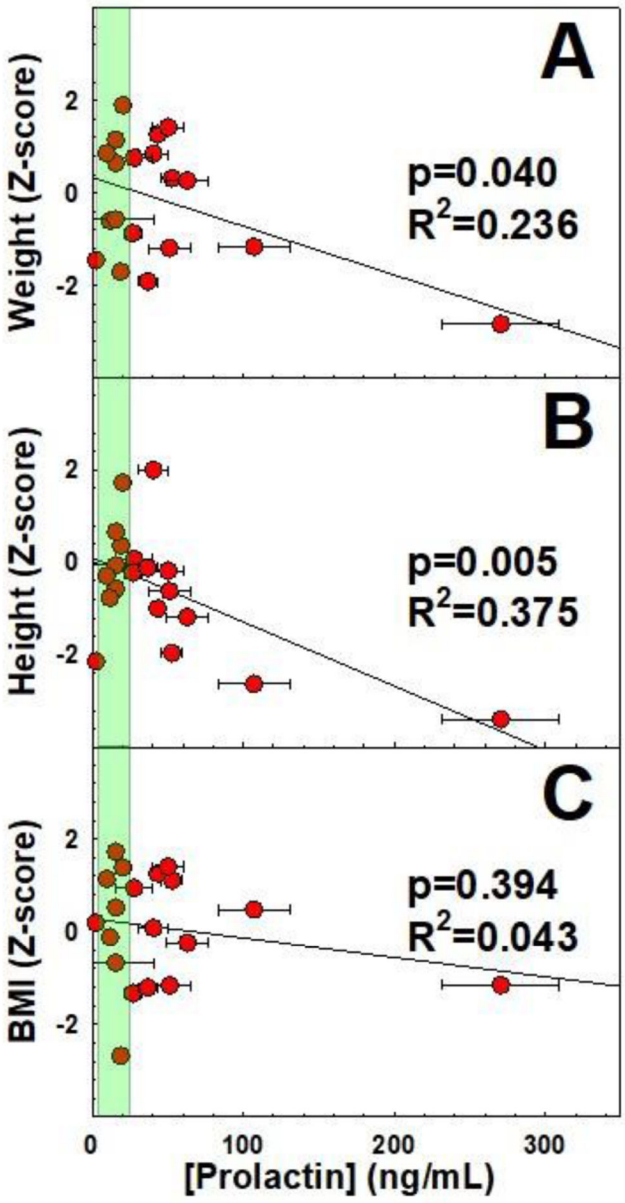

Figure 3.

Correlation between average prolactin levels (excluding those in the neonatal period) and weight, height and BMI (Z-scores) in patients with PTPS deficiency. The line represents a linear regression to the data with the parameters indicated. The green area represents the normal range for prolactin levels.

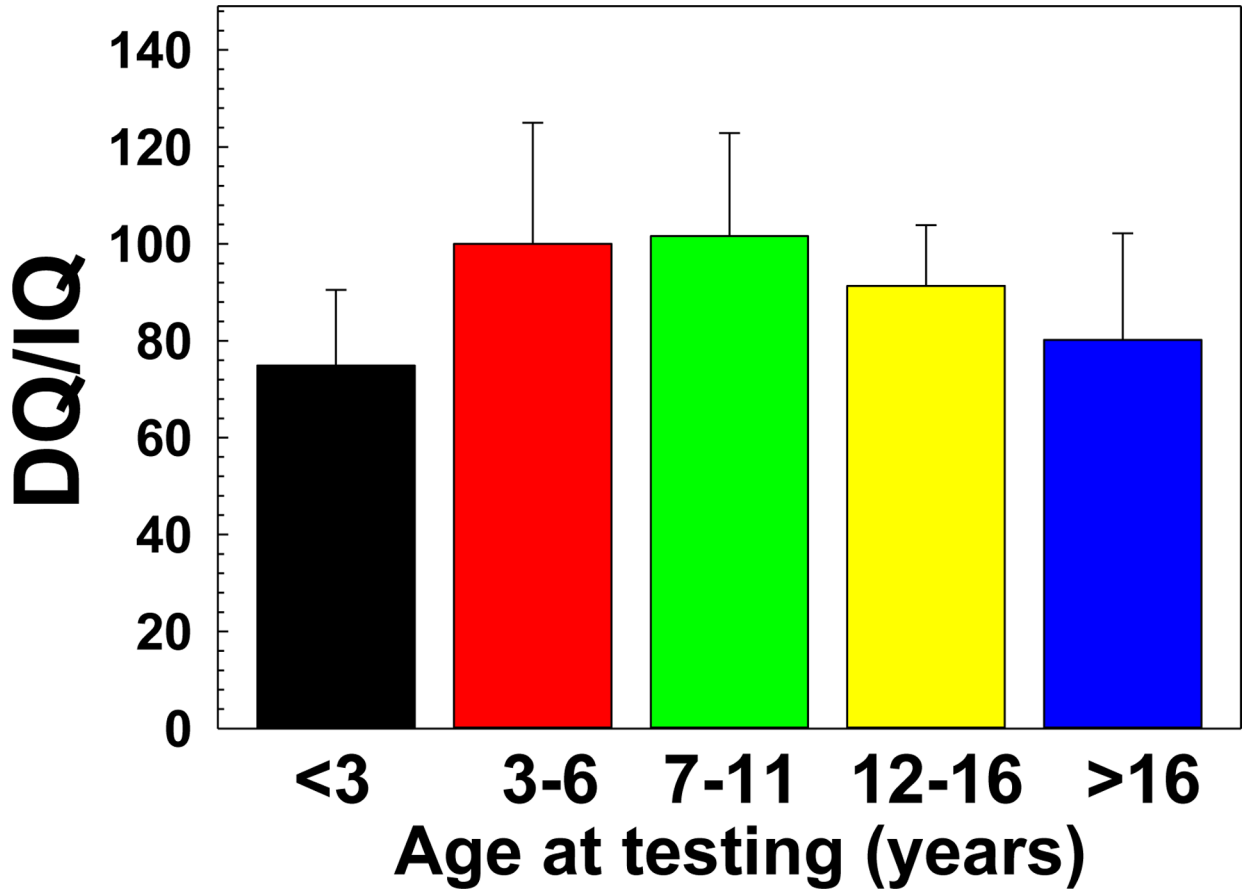

Figure 4.

DQ/IQ levels in patients with PTPS deficiency at different ages. Patients had initially a low DQ followed by an improvement in the pre-school period and an IQ mostly in the normal range during school, with a small decline in the later age.

DQ: Development quotient; IQ: Intelligence quotient.

Table 1.

Demographics of patients with PTPS deficiency (data cut-off 1 April 2019).

PATIENT	AGE (years)	GENDER	NATION	GESTATIONAL AGE (w)	BW (g)	Z-score
1	42.34	M	ITA	40	2520	−2.41
2	33.20	F	USA	40	3628	0.41
3	31.42	M	ITA	40	2830	−1.66
4	22.28	F	ITA	37	2600	−0.53
5	21.44	M	ITA	38	2750	−0.92
6	20.14	F	USA	40	2126	−3.11
7	14.53	F	ITA	38	2800	−0.55
8	12.26	F	ITA	40	3070	−0.72
9	12.11	F	ITA	38	2750	−1,12
10	11.99	F	ITA	38.3	2810	−0.65
11	10.30	M	ITA	39	3440	0,3
12	9.48	F	ITA	37.3	2365	−1.24
13	9.45	M	USA	34	1351	−2.17
14	8.75	F	USA	40	2495	−2.09
15	7.20	F	USA	36	1825	−1.93
16	7.06	M	USA	38	3175	0.04
17	6.53	M	ITA	31.3	1450	−0.54
18	5.23	F	USA	Unknown	Unknown	Unknown
19	0.95	M	USA	34.9	1740	−1.71
AVERAGE	15.1±10.8			37.8±2.5	2540±639	−1.14 ±0.97

Table 2.

Molecular and enzymatic findings of patients with PTPS deficiency.

PATIENT	PTPS activity	Variant 1	Variant 2
1		c.317C>T (p.Thr106Met)	**c.308 C>T (p.Val103Ala)**
2		[2] c.84–3C>G (IVS1–3C>G)	c.245A>G (p.Glu82Gly)
3		**c.240G>T (p.Met80IIe)**	**c.164–37dupC (IVS2–37dupC)**
4	**1.72**	**c.308T>C (p.Val103Ala)**	c.379C>G (p.Leu127Val)
5	**0.34**	c.393delA (p.K131fs)	g.3760_3816del57 (IVS2-762-718)
6		c.155A>G (p.Asn52Ser)	c.73C>G (p.Arg25Gly)
7	**<0.01**	c.260C>T (p.Pro87Leu)	**c.308T>C (p.Val103Ala)**
8	**<0.5**	c.260C>T (p.Pro87Leu)	**c.308T>C (p.Val103Ala)**
9		**c.187–37insC (IVS3–37-insC)**	**c.187–37insC (IVS3–37-insC)**
10		c.393delA (p.K131fs)	**g.3760_3816del57 (IVS2-762-718)**
11		c.84–3C>G (IVS1–3C>G)	c.84–3C>G (IVS1–3C>G)
12	**<0.01**	c.146A>G (p.His49Arg)	**c.244–8G>C (IVS4–8G>C)**
13		**c.245A>G (p.Glu82Gly)**	c.315–2 A>G (IVS5–2 A>G)
14		c.200C>T (p.Thr67Met)	c.200C>T (p.Thr67Met)
15		Negative Exome [1]	Negative Exome [1]
16		[3] c.118_121del M TG (p.Phe40Glyfs)	**c.43T>G (p.Ser15Ala)**
17		c.370G>T (p.Val124Leu)	**c.164–1G>C (IVS2–1G>C)**
18		c.259C>T (p.Pro87Ser)	c.286G>A (p.Asp96Asn)
19		c.245A>G (p.Glu82Gly)	c.315–2 A>G (IVS5–2 A>G)
Mean	**11–29 μU/g Hb**		

[1] Homozygous by descent for the genetic region containing the PTS gene and for a variant in the promoter region that is still being characterized.

[2] Activates a cryptic splice site causing a 12 bp deletion (p.del K29-S32) and an unstable protein.

[3] This variation was previously reported as c.116_119delTGTT (p.Phe40Glyfs*18).

Table 3.

Variants in the PTS gene of patients with PTPS deficiency. VUS= Variant of uncertain significance. For variants not previously characterized, the predicted effect of missense variants on function is also reported using commonly available software (SIFT and Polyphen2).

VARIANT	PT	EFFECT	ClinVar	REFERENCES	GnomAD frequency	PREDICTED EFFECT (SIFT, PolyPhen)
c.43T>G	16	p.Ser15Ala	339121	none	0.00022 (28/125568)	Benign 0.059
c.73C>G	6	p.Arg25Gly	463153	Likely pathogenic	0.00001 (1/125568)	Probably damaging 0.998
c.84-3C>G	2,11	IVS1-3C>G	553378	Likely pathogenic	0.000236 (45/190752)	
c.118_121delTTTG	16	p.Phe40Glyfs	550850	Pathogenic	0.000004 (1/247390)	
c.146A>G	12	p.His49Arg	558152	VUS	0.000016 (2/125568)	Probably damaging 0.999
c.155A>G	6	p.Asn52Ser	479	Pathogenic	0.000116 (29/250558)	Probably damaging 0.999
c.164-1G>C	1,17	VS2-1G>C	none			
c.164-37dupC	3	VS2-37dupC	none			
c.187-37insC	9	VS3-37-insC	none			
c.200C>T	14	p.Thr67Met	463151	Pathogenic	0.000068 (17/251424)	Probably damaging 1.000
c.240G>T	3	p.Met80IIe	none			Benign 0.003
c.244-8G>C	12	(IVS4-8G>C)	none			
c.245A>G	13,19,2	D.Glu82Gly	none	Splice variant		Benign 0.024
c.259C>T	18	p.Pro87Ser	480	Pathogenic	0.000024 (3/125568)	Probably damaging 0.895
c.260C>T	7,8	p.Pro87Leu	553829	Likely pathogenic	0.000008 (2/251448)	Benign 0.306
c.286G>A	18	p.Asp96Asn	484	Pathogenic	0.00003 (4/125568)	Probably damaging 0.985
c.308T>C	1,7,8,4	p.Val103Ala	none			Benign 0.005
c.315-2A>G	13,19	VS5-2A>G	558308	Likely pathogenic	none	
c.317C>T	1	p.Thr106Met	552175	Pathogenic	0.00016 (5/30948)	
c.370G>T	1,17	p.Val124Leu	556231	VUS	0.000072 (18/250678	Benign 0.000
c.379C>G	4	p.Leu127Val	none			Probably damaging 0.999
c.393delA	5,9	p.K131fs	556173	Likely pathogenic	0.000016 (4/250742)	
g.3760_3816del57	5,9	IVS2-762-718del	485	Pathogenic	none	

Table 4.

Urine and CSF pterins and CSF neurotransmitters in patients with PTPS deficiency. Phenylalanine levels are reported only if obtained concomitantly with other CSF measurements.

PATIENT	URINE NEOPTERIN	URINE BIOPTERIN	% BIOPTERIN	BLOODPHE[1]	NEOPTERIN (CSF)	BH_4 (CSF)	5HIAA (CSF)	HVA (CSF)
1	6.45	0.17		50.2	54	3.6	7.6	24.8
2			0.8–2.6	1200	96	<5	11.5	21.7
3	133	10			133	10	16	252
4	15.5	0.17			50	32	235	553
5	38	0			69	8.5	31	81
6[3]					64	5	119	199
7	13.34	0		190	81.3	30.7	383	475
8	21.4	0		1052	58.9	18.4	301	568
9	44.1	0.2		422	69	5	13	22
10	18.08	0			105	5.8	96	301
11	6.5	0.15			55	19	156	349
12	24.89	0.03		274	492	4.4	44.3	202
13			0		216	<5	6	7
14	7.52	0.28			272	12	41	129
15	11.3	0.66		544	284	<5	<5	26
16			1.6		61	33	35	132
18	6.3	0.26			8.15	10.6	241	459
18[2]	5.98	0.18		106	106	14	434	>1000
19			0		>300	<5	<5	12
Normal Range	***1.02–4.46 μmol/mol creatinine***	***0.46–1.17 μmol/mol creatinine***	***19–60***	***30–90 μmol/L***	***7–75 nmol/L***	***40–105 nmol/L***	***208–2259 nmol/L***	***337–1299 nmol/L***

[1] at time of CSF sampling.

[2] performed while on sapropterin dihydrochloride and neurotransmitter precursors after adoption.

[3] Original report of urine testing was not available

Table 5.

Therapy in patients with PTPS deficiency with dosage at time of last clinical evaluation. Doses are in mg/kg. Patients not receiving drugs were omitted from the computation of averages.

PT	AGE (y)	Weight (kg)	L-DOPA mg/kg per ay	CARBIDOPA mg/kg per day	5-OH Trp mg/kg per day	Sapropterin dihydrochloride mg/kg per day	Others
1	42.34	75.3	3.3	0.33	2.7	4.7	Folinic acid (4 mg/d)
2	33.20	68.3	11.7	2.49	7.7	5.9	Trihexyphenidyl (2 mg/d)
3	31.42	85	5.3	1.33	3.5	4.7	Folinic acid (8.5 mg/d)
4	22.28	46	0	0	0	5.9 (up to 2014)	Folinic acid (5 mg/d), Valproic acid, Lamotrigine
5	21.44	87.1	8.6	1.65	4.6	1.2	Folinic acid (4 mg/d),
6	20.14	53	6.6	1.65	8.4	3.8	Pramipexole (0.885 mg/d), Entacapone (400 mg/d). Folinic acid (10 mg/d). Growth Hormone
7	14.53	51.5	3.5	0.35	3.5	3.5	
8	12.26	29.7	3.5	0.35	3.5	3.5	
9	12.11	50	4.5	1.13	0	18	Pramipexole (0.9 mg/d) Levetiracetam (1500 mg/d)
10	11.99	45.7	8.2	0.82	3.3	5.5	Folinic acid (4mg/d)
11	10.30	77	1.4	0.34	0.32	13	Folinic acid (8.5mg/d) Benzerazide
12	9.48	19.8	5	0.5	3	5	
13	9.45	20.2	10.5	5.25	4.4	20	Selegiline (15mg/d) Ropinorole (0.235 mg/d)
14	8.75	18.8	5.5	2.75	4	21	Pramipexole (0.25 mg/d)
15	7.20	21.3	8.5	5	6.3	39	Pramipexole (135μg/d) Folic Acid (10 mg/d)
16	7.06	16.2	4.3	1.1	3.3	20	Selegiline (5 mg/d)
17	6.53	21.1	0	0	0	2.4	
18	5.23	14.4	7.5	2.86	6.1	27.8	Folinic acid (15 mg/d)
19	0.95	7.3	5.1	5.1	2.9	41	
		MEAN	5.84	1.96	4.2	14.8	mg/kg per day
		SD	2.72	1.78	2.09	12.6	
		Range	1.4–12.7	0.3–5.25	0.3–8.4	1.2–41	

Table 6.

Functional outcome of patients with PTPS deficiency.

PT	AGE (y)	SEX	DEVELOPMENT	Brain MRI	EEG	CURRENT STATUS
1	42.3	M	NA	Mild periventricular enhancement (29 y	diffuse bilateral theta waves (20 y)	Partially Independent. Dysartria, abnormal gait, occasional cervical dystonia. Anxiety, fatigue, sleep disorder.
2	33.2	F	Mild speech delays, behavior issues up to age 6	Normal (12 y)	Normal	Independent, employed, married with 3 sons. Episodes of tiredness
3	31.5	M	Borderline IQ, ADHD, poor memory	Generous lateral ventricles with periventricular enhancement (16 y)	Normal	Mild ID, partially independent, works and lives with parents. OCD. Dystonic episodes, perioral tremor, hypersalivation, sleep problems.
4	22.3	F	NA	Normal (8y)	Short patterns of large delta waves with sharp spikes on anterior regions (21 y)	Partially Independent, normal IQ. Focal seizures with medication discontinuation. Eating disorder.
5	21.4	M	Mild global delays (2.4 y)	Peritrigonal hyperintensity (3.5y); normal (18y).	Sharp waves in fronto-central temporal regions (4 y)	Independent, normal IQ. Arms postural tremor. Works, Mild fatigue/ headache at night.
6	20. 1	F	Mild speech delay, stuttering, earning and processing	NA	NA	Independent. Attends college. Issues with attention, working memory, fine motor dexterity, reading and comprehension
7	14.5	F	disorders Normal DQ, low range (13 m)	NA	Normal (7 y)	Appropriate development, doing well in school, dance
8	12.3	F	Mild hypotonia (7m)	NA	NA	Appropriate development, normal IQ. Introverted, poor eater, sleep problems, easy fatigability
9	12.11	F	Mild hypotonia, global delays. Tremors, dystonia, oculogyric crisis. Poor sleep	Normal	Normal	Severe intellectual disability, dystonia, hypersalivation, sleep problems, scoliosis. Epilepsy (2 episodes) treated with Levetiracetam.
10	11.9	F	Global delays	NA	Normal	Normal development, doing well at school, playing basketball
11	10.3	M	Normal	NA	NA	Borderline IQ, clumsiness. ADHD. Sleep problems, binge eating episodes.
12	9.4	F	Mild hypotonia early in life	Normal (1 m)	Normal	Attends normal school. Emotional dysregulation with fluctuation of speech, sleep, appetite and energy levels with drug therapy
13	9.4	M	Mild global delays more marked in speech	Normal with normal spectroscopy (4 y)	NA	Requires help in normal school. 2 years behind. Issues with speech, patience, focus and memory
14	8.8	F	Mild global delays	NA	NA	Normal school with support for speech. Motor tics. Insomnia
15	7.2	F	Mild global delays	Normal (1 m)	Normal	One year behind in normal school, requires speech and motor therapies. Easy fatigability, dyskinesia, incoordination when tired
16	7	M	Moderate global delays	Macrocephaly, mile Chiari I malformation, hypoplastic vermis	Normal	Severe global developmental delays, not toilet trained, abnormal gait, sleep problems. Variable compliance with medications

Abbreviations. IEP: Individualized Educational Program. **OT**: Occupational Therapy. **PT**: Physical Therapy. **ST**: Speech Therapy. **ADHD**: Attention Deficit hyperactivity Disorder. **OCD**: obsessive-compulsive disorder. **ID**: intellectual disability.