

# Disorders of Phenylalanine and Tetrahydrobiopterin Metabolism

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## 1.1 Introduction

Hyperphenylalaninemia, a disorder of phenylalanine catabolism, is caused primarily by a deficiency of the hepatic apoenzyme phenylalanine-4-hydroxylase (PAH) or by one of the enzymes involved in its cofactor biosynthesis (GTP cyclohydrolase I, GTPCH; and 6-pyruvoyl-tetrahydropterin synthase, PTPS) or its regeneration (dihydropteridine reductase, DHPR; and pterin carbinolamine-4 $\alpha$ -dehydratase, PCD). Tetrahydrobiopterin (BH<sub>4</sub>) is known to be the natural cofactor for PAH, tyrosine-3-hydroxylase, and tryptophan-5-hydroxylase. The latter two are key enzymes in the biosynthesis of the neurotransmitters, dopamine and serotonin. Thus, any cofactor defect will result in a deficiency of biogenic amines accompanied by hyperphenylalaninemia. Similarly, because phenylalanine is a competitive inhibitor of both tyrosine and tryptophan hydroxylases, depletion of catecholamines and serotonin occurs in untreated patients with PAH deficiency. Both groups of hyperphenylalaninemias (PAH and BH<sub>4</sub> deficient) are heterogeneous disorders varying from severe, e.g., classical phenylketonuria (PKU), to mild, benign, and transient forms. Because of the different clinical and biochemical severities in this group of diseases, the terms “severe” or “mild” will be used based upon the need to treat or not treat patients.

For the pterin defects, symptoms may manifest during the first weeks of life but usually are noted at about 4 months of age. Birth is generally uneventful, except for an increased incidence of prematurity and lower birth weights in severe PTPS deficiency.

Two disorders of BH<sub>4</sub> metabolism may present without hyperphenylalaninemia. These are Dopa-responsive dystonia (DRD; Segawa disease) and sepiapterin reductase (SR) deficiency. While DRD is caused by a mutation in the GTPCH gene and is inherited in an autosomal dominant manner, SR deficiency is an autosomal recessive trait. Both diseases evidence severe biogenic amines deficiencies. DRD usually presents with a dystonic gait and diurnal variation. At least two reports describe heteroallelic patients with DRD suggesting a wide spectrum of GTPCH variants.

The simplicity and reliability of the “Guthrie” test made it the basis for newborn screening programs around the world. Today, the Guthrie screen-

ing test has been replaced by powerful tandem mass spectrometry techniques or by enzymatic methods. Once hyperphenylalaninemia has been detected, a sequence of quantitative tests (see Sect. 8 Diagnostic flow-chart) enables the differentiation between variants, i.e. classical PKU, BH<sub>4</sub>-responsive PKU, and BH<sub>4</sub> deficiencies. Because the BH<sub>4</sub> deficiencies are actually a group of diseases which may be detected because of hyperphenylalaninemia, but not simply and routinely identified by neonatal mass screening, selective screening for a BH<sub>4</sub> deficiency is essential in every newborn with even slightly elevated phenylalanine levels. Screening for a BH<sub>4</sub> deficiency should be done in all newborns with plasma phenylalanine levels greater than 120 μmol/l (2 mg/dl), as well as in older children with neurological signs and symptoms.

The two forms of BH<sub>4</sub> deficiency without hyperphenylalaninemia are detectable only by investigations for neurotransmitter metabolites and pterins in CSF. In DRD, a phenylalanine loading test, a trial with L-Dopa, and enzyme activity measurement in cytokine-stimulated fibroblasts are confirmatory for the diagnosis. SR deficiency can be definitely diagnosed only by an enzyme assay of cultured fibroblasts.

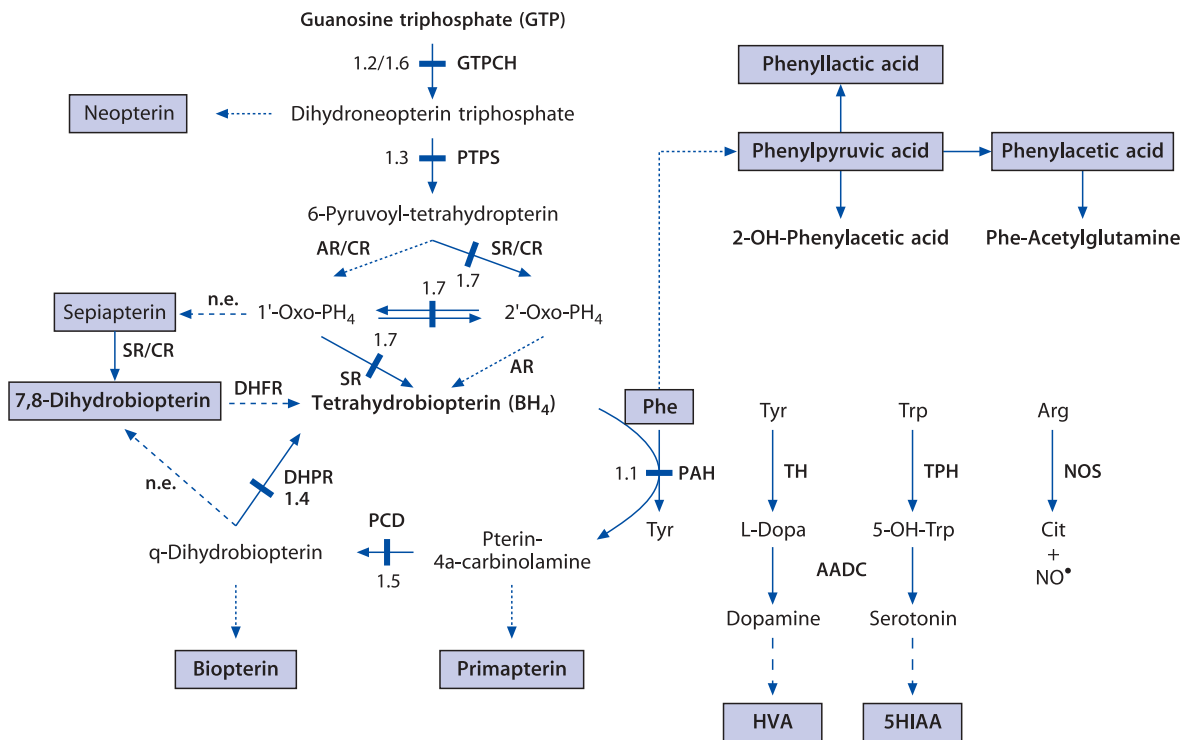
The goals of treatment are to control hyperphenylalaninemia by dietary restriction of phenylalanine (in PAH deficiency) or BH<sub>4</sub> administration (in GTPCH and PTPS deficiency), and to restore neurotransmitter homeostasis by the oral administration of dopamine and serotonin precursors (L-Dopa and 5-hydroxytryptophan, respectively) in BH<sub>4</sub> deficiencies. Late detection and introduction of treatment leads to irreversible brain damage. In contrast to patients with classical PKU, patients with BH<sub>4</sub> deficiencies show progressive neurological deterioration despite treatment with phenylalanine-restricted diets. DRD and SR patients benefit from L-Dopa/Carbidopa substitution (for the relevant literature see [1–18]).

## 1.2 Nomenclature

No.	Disorder	Tissue distribution	Chromosomal localisation	Mc-Kusick
With hyperphenylalaninemia				
1.1	Phenylalanine-4-hydroxylase (PAH) deficiency (classical PKU)	liver (kidney)	12q22–24.1	261600
1.2	GTP cyclohydrolase I (GTPCH) deficiency	liver, brain, kidney, lymphocytes	14q22.1–22.2	233910
1.3	6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency	liver, brain, kidney, lymphocytes, erythrocytes, fibroblasts	11q22.3–23.3	261640
1.4	Dihydropteridine reductase (DHPR) deficiency	all tissues	4p15.3	261630
1.5	Pterin carbinolamine-4 $\alpha$ -dehydratase (PCD) deficiency (Primapterinuria)	liver, kidney, intestine	10q22	264070
Without hyperphenylalaninemia				
1.6	Dopa-responsive dystonia (DRD) Autosomal dominant GTPCH deficiency <sup>a</sup> (Segawa disease, hereditary progressive dystonia)	liver, brain, kidney, lymphocytes	14q22.1–22.2	600225
1.7	Sepiapterin reductase deficiency (SR)	all tissues	2p14–p12	182185

<sup>a</sup> Compound heterozygotes described in two cases.

## 1.3 Metabolic Pathway



**Fig. 1.1.** Biosynthesis and regeneration of tetrahydrobiopterin including possible metabolic defects and catabolism of phenylalanine. 1.1=phenylalanine-4-hydroxylase (PAH); 1.2/1.6=GTP cyclohydrolase I (GTPCH), 1.3=6-pyruvoyl-tetrahydropterin synthase (PTPS), 1.4=dihydropteridine reductase (DHPR), 1.5=pterin-4 $\alpha$ -carbinolamine dehydratase (PCD), 1.7=sepiapterin reductase SR, carbonyl reductase (CR), aldose reductase (AR), dihydrofolate reductase (DHFR), aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), nitric oxide synthase (NOS). Pathological metabolites used as specific markers in the differential diagnosis are marked in squares. n.e.=non-enzymatic

## 1.4 Signs and Symptoms

**Table 1.1.** Phenylalanine-4-hydroxylase deficiency (classical PKU)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic clinical findings	Odor (urine and body)	±	+	+	+	+
	Lighter pigmentation in family constellation	+	+	+	+	+
Routine Lab Special laboratory	Mental retardation		+	+	+	+
	FeCl <sub>3</sub>	+	+	+	+	+
	MRI brain		±	+	+	+
	EEG		±	±	±	±
	Phe (P, U, CSF)	↑	↑	↑	↑	↑
	5HIAA, HVA (CSF)		↓-n	↓-n	↓	↓
	Phenylpyruvate (U) tetrahydrobiopterin loading test	n-↑ - <sup>c</sup>	↑ - <sup>c</sup>	↑ - <sup>c</sup>	↑ - <sup>c</sup>	↑ - <sup>c</sup>
G.I.	Vomiting <sup>a</sup>	±	±			
CNS	Microcephaly		+	+	+	+
	Mental retardation		+	+	+	+
	Irritability		±	±	±	±
	Seizures			±	±	
	Hypertonia		±	±	±	
Cardiac	Autism			±	±	± <sup>b</sup>
Dermatological	Ecematous rash	±	±	±	±	±

<sup>a</sup> Increased incidence of pyloric stenosis.

<sup>b</sup> Cardiac anomalies in infants of untreated maternal PKU.

<sup>c</sup> *Km* mutants of PAH presents with the positive loading test. 5HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid.

**Table 1.2.** GTPCH deficiency (17 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood
Characteristic clinical findings	Progressive psychomotor retardation despite treatment for PKU	±	+	+
	Feeding difficulties		+	+
Special laboratory	Phe (S)	n-↑	↑	↑
	Neopterin and biopterin (U, P, CSF), 5HIAA/HVA (CSF)		↓	↓
	tetrahydrobiopterin loading test		↓	↓
		+	+	+
CNS	Hypotonia/hypertonia	+	+	+
	Temperature instability	+	+	+
	Seizures – myoclonic		+	+
	Microcephaly	+	+	+
	Hypersalivation	+	+	+
	Mental retardation		+	+

5HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid.

**Table 1.3.** PTPS deficiency (257 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	
Characteristic clinical findings	Progressive mental and physical retardation despite dietary phenylalanine restriction	±	+	+	+	
Special laboratory	Birth weight	↓-n				
	Phe (S)	↑	↑	↑	↑	
	Abnormal pterins (U, P, CSF), high neopterin and very low biopterin	+	+	+	+	
	5HIAA/HVA (CSF)	↓	↓	↓	↓	
	Tetrahydrobiopterin loading test	+	+	+	+	
	MRI/CT scan – cortical atrophy	+	+	+	+	
	EEG – hypsarrhythmia	+	+	+	+	
	Respiratory	Pneumonia		+	+	+
		Sudden death		+	+	
Hair	Lighter pigmentation		+	+		
CNS	Myoclonic or tonic clonic seizures		+	+	+	
	Temperature instability	+	+	+		
	Hypersalivation	+	+	+	+	
	Lethargy and irritability		+	+		
	Hypotonia/hypertonia	+	+	+	+	
	Retardation and regression	+	+	+	+	
	Choreoathetosis		+	+		
Dermatological	Rash – eczema		+			

5HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid.

**Table 1.4.** DHPR deficiency (130 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence
Characteristic clinical findings	Progressive mental and physical retardation despite dietary phenylalanine restriction	±	+	+	+
Special laboratory	Phe (S)	↑	↑	↑	↑
	Abnormal pterins (U, P, CSF), normal neopterin and high biopterin	+	+	+	+
	Tetrahydrobiopterin loading test	+	+	+	+
	5HIAA/HVA (CSF)	↓	↓	↓	↓
	MRI/CT scan – brain calcification		+	+	+
	CT scan – cortical atrophy		+	+	+
	EEG – spike wave pattern	+	+	+	+
	EEG – hypsarrhythmia	+	+	+	
	Respiratory	Pneumonia	+	+	+
Hair	Lighter pigmentation		+	+	
CNS	Myoclonic seizures	+	+	+	
	Temperature instability	+	+	+	
	Microcephaly		+	+	+
	Hypersalivation	+	+	+	+
	Hypotonia/hypertonia	+	+	+	
	Retardation mental and physical		+	+	+
	Sudden death		+	+	
Dermatological	Chorea/athetosis		+	+	
	Rash – eczema		+	+	+

5HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid.

**Table 1.5.** PCD deficiency (22 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence
Characteristic clinical findings	No significant clinical abnormalities other than transient alterations in tone				
Special laboratory	Phe (S) <sup>a</sup>	↑	n-↑		
	Neopterin and primapterin (U)	↑	↑	↑	↑
	EEG - transient sharp waves	+	+		
	Tetrahydrobiopterin loading test	+	+	+	
CNS	Hypotonia/hypertonia	+	+		

<sup>a</sup> The plasma/serum Phe is quite variable and may rise to as high as 2100 μmol/l; however, it spontaneously resolves into normal ranges and blood levels do not correspond to phenylalanine intake and appear to be independently variable. This appears to be a benign condition that does not require ongoing treatment, however, in most cases treatment with tetrahydrobiopterin improves the transient symptoms.

**Table 1.6.** Dopa-responsive dystonia (DRD) – autosomal dominant GTPCH I deficiency (>400 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Characteristic features	Dystonia (lower limbs, trunk, arms, neck)		+	++	++	++
	Diurnal fluctuations of symptoms		±	+	+	±
	Parkinsonism (association of tremor, rigidity, bradykinesia)			±	±	+
Special laboratory	HVA (CSF)	↓	↓	↓	↓	↓
	5HIAA (CSF)	n-↓	n-↓	n-↓	n-↓	n-↓
	Neopterin and biopterin (CSF)	↓	↓	↓	↓	↓
	Phe loading test	±	±	±	±	±
Other extrapyramidal signs	Tremor				+	+
	Rigidity	±	+	+	+	+
	Neck tilting/poor head control		±	±	±	±
	Bradykinesia		±	±	±	±
	Hypo-/akinesia		±	±	±	±
	Oromandibular-oro-facial dyskinesia				±	±
	Dysphagia		±	±	±	±
Other neurological signs	Hyperreflexia	±	±	±	±	±
	Hypotonia (at onset)		±	±	±	±
	Hypertonia	±	±	±	±	±
	Spasticity	±	±	±	±	±
Postural and orthopedic complications	Scoliosis			±	±	
	Wry neck				±	
	Pes equinovarus			±	±	

**Table 1.7.** Sepiapterin reductase deficiency (5 patients)

System	Symptoms/markers	Neonatal	Infancy	Childhood	Adolescence	
Characteristic clinical findings	Progressive psychomotor retardation	++	++	++		
	Diurnal fluctuations of symptoms			±	±	
Special laboratory	5HIAA, HVA (CSF)	↓	↓	↓	↓	
	Neopterin (CSF)	n	n	n	n	
	Bioppterin, BH <sub>2</sub> (CSF)	↑	↑	↑	↑	
	Sepiapterin (CSF)	↑	↑	↑	↑	
	Phe loading test	±	±	±	±	
CNS	Microcephaly	+	+	+	+	
	Hypersomnolence				±	
	Motor and mental retardation	+	+	+	+	
	Hypersalivation	+	+	+	+	
	Dystonia	+	+	+	+	
	Hypotonia of the trunk	+	+	+	+	
	Hypertonia of the limbs	+	+	+	+	
	Seizures		±	±	±	
	Tremor		+	+	+	
	Extrapyramidal signs		+	+	+	
	Oculogyric crisis		±	±		
	MRI	Cortical atrophy			+	+

## 1.5 Reference Values

### ■ Serum, Urine, and CSF

Age	Phe (S) μmol/l	Neo (U) mmol/mol Creat	Bio (U) mmol/ mol Creat	Neo (S) nmol/l	Bio (S) nmol/l	Neo (CSF) nmol/l	Bio (CSF) nmol/l	5HIAA <sup>a</sup> (CSF) nmol/l	HVA <sup>a</sup> (CSF) nmol/l	5MTHF (CSF) nmol/l
newborns	<120	1.1–4.0	0.5–3.0	3–11	4–18	15–35	20–70	144–800	300–1000	64–182
0–1y	<80	1.1–4.0	0.5–3.0	3–11	4–18	12–30	15–40	114–336	295–932	64–182
2–4y	<80	1.1–4.0	0.5–3.0	3–11	4–18	9–20	10–30	105–299	211–871	63–111
5–10y	<80	1.1–4.0	0.5–3.0	3–11	4–18	9–20	10–30	88–178	144–801	41–117
11–16y	<70	0.2–1.7	0.5–2.7	3–11	4–18	9–20	10–30	74–163	133–551	41–117
>16y	<70	0.2–1.7	0.5–2.7	3–11	4–18	9–20	10–30	66–141	115–488	41–117

<sup>a</sup> See also Chap. 2 “Disorders of Neurotransmitter Metabolism”.

### ■ Amniotic Fluid, Amniocytes and Fibroblasts

	Age	Phe μmol/l	Neo nmol/l	Bio nmol/l	5-HIAA nmol/l	HVA nmol/l
Amniotic fluid	Fetus	<120	16–40	6–21	32–135	50–144
Amniocytes <sup>a</sup>	Fetus		<14 <sup>b</sup>	<115 <sup>b</sup>		
Fibroblasts <sup>a</sup>	Infants (0–12 m)		11–73 <sup>b</sup>	183–303 <sup>b</sup>		
	Children and adults		18–98 <sup>b</sup>	154–303 <sup>b</sup>		

<sup>a</sup> In cytokine-stimulated cells.

<sup>b</sup> pmol/mg.

### ■ Enzymes

Age	DHPR (RBC) mU/mg Hb	PTPS (RBC) μU/g Hb	SR (RBC) μU/mg protein	GTPCH (FB) <sup>a</sup> μU/mg protein	PTPS (FB) μU/mg protein	DHPR (FB) μU/mg protein	SR (FB) μU/mg protein
Fetus	2.3–3.8	35–77		1.5–1.9	3.0–3.3	5.8–8.8	
Newborns (0–1 m)	1.8–4.8	34–64					
Infants (0–12 m)	1.8–4.8		0.33–1.86	1.7–4.9	0.5–1.7	6.3–8.7	97–185
Children and adults	1.8–4.8	11–29	0.33–1.86	1.4–6.5	0.4–1.6	4.5–8.3	99–185

<sup>a</sup> In cytokine-stimulated cells.

## 1.6 Pathological Values/Differential Diagnosis

### ■ Plasma

Actual Phe <sup>a</sup> (μmol/l)	Neo (S) (nmol/l)	Bio (S) (nmol/l)
<200	3–11	4–18
200–600	2–32	12–46
600–1200	9–27	24–39

<sup>a</sup> Plasma neopterin and biopterin values depend strongly upon the actual hyperphenylalaninemia.

## ■ Plasma, Urine, and CSF

Variant	Phe (S) μmol/l	Neo (U) mmol	Bio (U) mol Creat	% Bio <sup>c</sup>	Neo (CSF) nmol/l	Bio (CSF)	5HIAA (CSF)	HVA (CSF)	5-MTHF (CSF)
1.1 PAH def. (classical)	>1200	1.2–19.8	0.5–7.9	~ 50	9–118	15–143	14–471	47–1174	n
1.1 PAH def. (atypical)	600–1200	1.2–14.5	0.6–5.3	~ 50	9–118	15–143	n	n	n
1.1 PHA def. (benign)	120–600	1.0–13.2	0.5–5.1	~ 50	n	n	n	n	n
1.2 GTPCH def.	120–1200 <sup>a</sup>	<0.2	<0.2	~ 50	0.05–3.0	1.5–7.5	61–183	15–48	n
1.3 PTPS def. (severe)	250–2500	5.0–51.2	<0.5	<5	47–402	1.0–16.0	5–113	5–223	n
1.3 PTPS def. (mild)	240–2200	5.0–51.2	<0.5	<5	25–230	13–56	93–420	249–998	n
1.4 DHPR def. (severe)	180–2500	0.5–23.2	3.8–25.6	>80	11–70	43–117	4–75	19–204	↓
1.4 DHPR def. (mild)	280–600	0.5–23.2	3.8–25.6	>80	11–70	43–117	21–66	n	↓-n
1.5 PCD def. (benign)	180–1200	4.1–22.5	0.7–1.5 <sup>b</sup>	<50	43–117	16–96	n	n	n
1.6 DRD	<120	n	n	~ 50	1.1–6.2	3.1–7.6	48–97	120–239	n
1.7 SR def. <sup>c</sup>	<120	n	n	~ 50	14–51	72–102 <sup>d</sup>	3–15	49–111	n

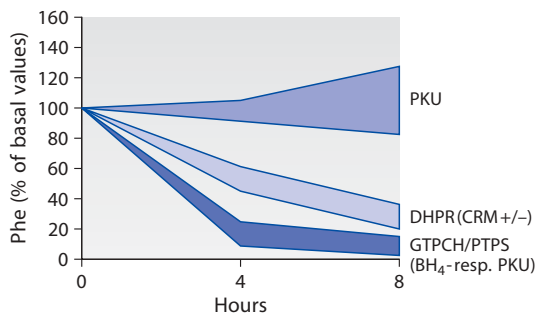
<sup>a</sup> Two patients were missed in the newborn screening due to the negative Guthrie test.

<sup>b</sup> Primapterin (7-Bio) ↑. <sup>c</sup> %Bio = 100 × Bio / (Neo + Bio). <sup>d</sup> 7,8-dihydrobiopterin ↑. <sup>e</sup> Sepiapterin (CSF) ↑.

### 1.7 Loading Tests

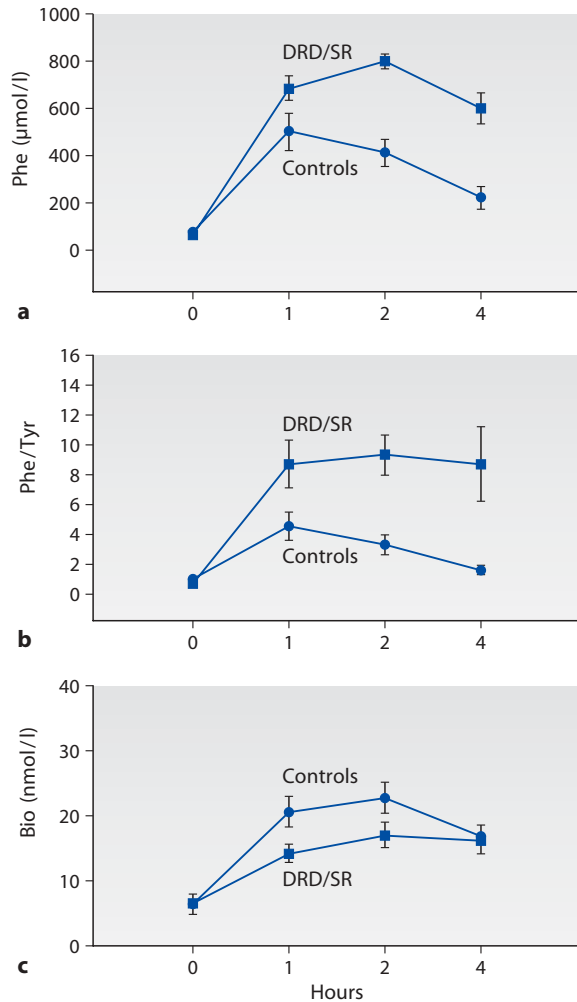
Recently, a patient with typical PKU was found to be BH<sub>4</sub>-responsive. This PKU variant is thought to have a *Km* variant. The response to 10 or 20 mg/kg of BH<sub>4</sub> was similar to patients with typical BH<sub>4</sub> deficiencies, however, additional blood sampling at 24 hours may be recommended.

A combined Phe (100 mg/kg) and BH<sub>4</sub> (20 mg/kg) loading test is sometimes difficult to interpret and is therefore not recommended.



**Fig. 1.2.** Typical results of a BH<sub>4</sub> loading test (20 mg/kg body weight) in patients with hyperphenylalaninemia. Tablets of synthetic cofactor (BH<sub>4</sub> supplied by Dr. Schircks Laboratories, Jona, Switzerland) were dissolved in 20 ml of water in dim light and administered at least 30 min before a meal. Blood was drawn before, and 4 and 8 hours after BH<sub>4</sub> loading. Patients with GTPCH and PTPS deficiencies show a rapid normalization of blood phenylalanine. Simultaneously, there is a transient increase in tyrosine levels 4 hours after the administration of BH<sub>4</sub>. Basal plasma Phe should be >400 μmol/l

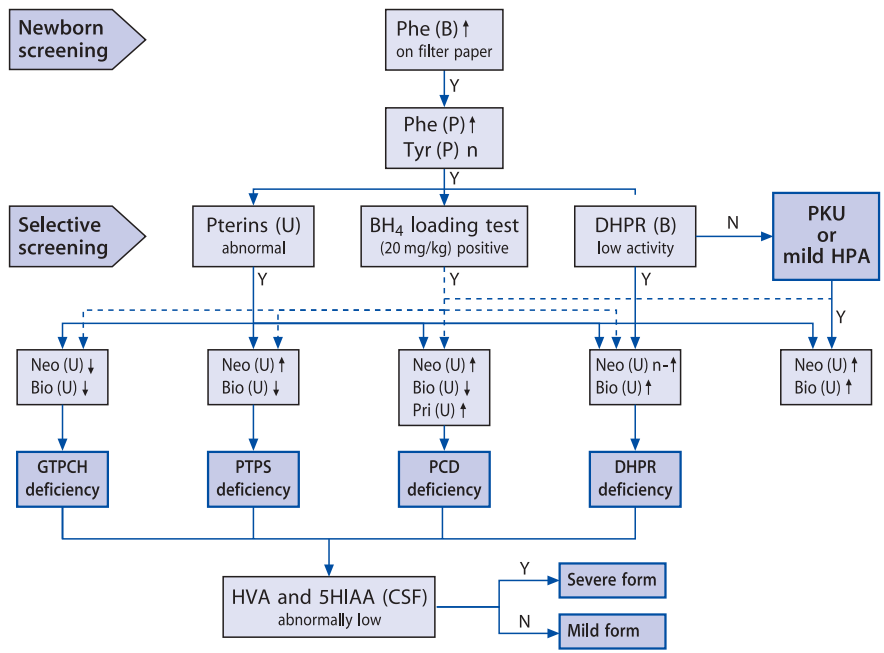
**Fig. 1.3.** Loading test with phenylalanine (100 mg/kg body weight) in patients *without* hyperphenylalaninemia. An oral loading with 100 mg/kg of L-phenylalanine was performed as previously described by Hyland et al. except that samples were drawn at baseline and 1, 2 and 4 hours after administration. In patients with DRD and SR disorders, plasma Phe, Phe/Tyr, and biopterin profiles are abnormal. Patients with DRD demonstrate increased plasma Phe levels between 1 and 2 hours after loading and biopterin is usually lower than 18 nmol/l (cut-off)



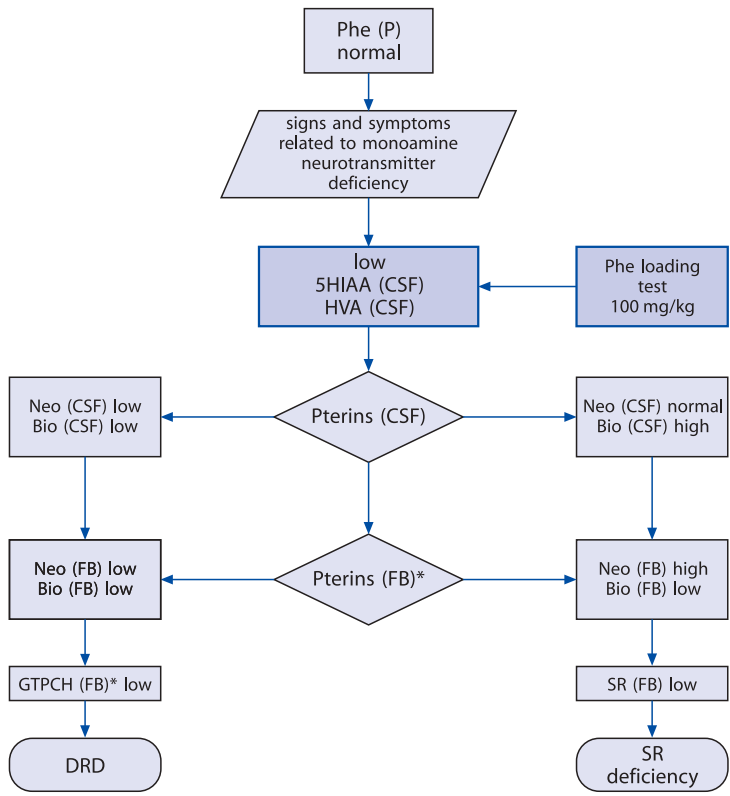
## 1.8 Diagnostic Flow-Chart

A positive Guthrie test for Phe should be repeated and confirmed by quantitative analysis. Screening for a  $\text{BH}_4$  deficiency should be done in all newborns with even slight hyperphenylalaninemia (plasma Phe  $>120 \mu\text{mol/l}$ ) as well as in older children without hyperphenylalaninemia but with neurological symptoms suggestive of a neurotransmitter deficiency. The following protocol is suggested:

1. Analysis of pterins in urine
2. Measurement of DHPR activity in blood from a Guthrie card
3. Analysis of phenylalanine and tyrosine in serum or plasma before and after a  $\text{BH}_4$  challenge.



**Fig. 1.4A.** Screening policy and a diagnostic flow-chart in the differential diagnosis of hyperphenylalaninemia variants. Phe: phenylalanine; Tyr: tyrosine; Neo: neopterin; Bio: biopterin; Pri: primappterin. Y: yes; N: no



**Fig. 1.4B.** Screening policy and a diagnostic flow-chart in the differential diagnosis of non-hyperphenylalaninemia variants (DRD and SR deficiency). Phe: phenylalanine; Neo: neopterin; Bio: biopterin; Y: yes; N: no; \* Cytokines-induced cells [13]

The first two tests are essential and will allow the differentiation between all variants with BH<sub>4</sub> deficiencies. With some limitations (DHPR def.), the BH<sub>4</sub> loading test is an additional useful diagnostic tool for the rapid discrimination between classical PKU and bipterin variants. This test is also useful for identifying the recently described BH<sub>4</sub>-responsive PAH deficiency. For the interpretation and determination of the various disorders based upon loading tests, see “Pathological values and differential diagnosis”.

DRD and SR deficiency both present without hyperphenylalaninemia, however a loading test with Phe (100 mg/kg/d) indicates impaired Phe metabolism under catabolic conditions. Initial CSF testing for neurotransmitter metabolites and pterins is essential and should be expanded by pterins production and enzyme activity measurements in cytokine-stimulated fibroblasts. Responsiveness to low dosage L-Dopa is highly indicative of DRD, however it may be also positive because of other disorders of neurotransmitter metabolism.

## 1.9 Specimen – Collection

Test	Preconditions	Material	Handling	Pitfalls
Phe	free diet	Guthrie card, serum/ plasma, CSF	keep cool (–20 °C)	
Neo	free diet, Phe in plasma high enough	random urine, spotted urine	keep cool, dark (–20 °C) oxidized sample <sup>a</sup> , dark, RT	infections (Neo ↑)
Bio		serum/plasma	keep cool, dark (–20 °C)	
BH <sub>4</sub> , BH <sub>2</sub>		CSF	EDTA tube (–20 °C)	
HVA	1 h before medication,	CSF	DTE/DETAPAC tube (–80 °C)	
5HIAA	withdraw first 0.5 ml		EDTA tube (–80 °C)	
5MTHF				
DHPR		erythrocytes from heparinized blood	frozen (–20 °C)	
		Guthrie card	RT	
		fibroblasts	RT	
		chorionic villi	frozen (–80 °C)	
PTPS	min. 50 mg before medication, no BH <sub>4</sub>	erythrocytes from heparinized blood	frozen (–20 °C)	
	min. 50 mg	chorionic villi	frozen (–80 °C)	
GTPCH		fibroblasts	RT	
SR		fibroblasts	RT	

RT: room temperature.

<sup>a</sup> Oxidized with MnO<sub>2</sub> at pH 1–2 [5].

## 1.10 Prenatal Diagnosis

Disorder	Material	Timing, trimester
1.1	fetal DNA	I
1.2	amniotic fluid, liver, DNA	II
1.3	amniotic fluid, liver, erythrocytes, amniocytes, DNA	II
1.4	CV	I
	amniotic fluid, liver, erythrocytes, amniocytes	II
	CV	I

## 1.11 DNA Analysis

DNA analysis is possible for all variants with BH<sub>4</sub> deficiency.

Disorder	Material	Method
1.1	Genomic DNA	PCR/RFLP/SSCP/Sequencing
1.2	Genomic DNA/FB-cDNA	PCR/DGGE/Sequencing
1.3	Genomic DNA/FB-cDNA	PCR/DGGE/Sequencing
1.4	Genomic DNA/FB-cDNA	PCR/RFLP/DGGE/Sequencing
1.5	Genomic DNA	PCR/Sequencing
1.6	Genomic DNA/FB-cDNA	PCR/DGGE/Sequencing
1.7	Genomic DNA/FB-cDNA	PCR/Sequencing

## 1.12 Initial Treatment

### ■ 1.1: PAH Deficiency (Classical PKU)

Both initial and long-term treatment consists of dietary restriction of phenylalanine intake and Phe-free amino acid supplementation.

### ■ 1.2 and 1.3: GTPCH and PTPS Deficiencies

While awaiting confirmation of the diagnosis by pterin and enzymatic analyses, these patients may be treated with BH<sub>4</sub> (2–5 mg/kg/day).

### ■ 1.4: DHPR Deficiency

Dietary phenylalanine restriction.

### ■ 1.5: PCD Deficiency

Dietary phenylalanine restriction.

### ■ 1.6: DRD

L-Dopa/Carbidopa.

### ■ 1.7: SR Deficiency

L-Dopa/Carbidopa.

The therapeutic protocol in BH<sub>4</sub> deficiencies *with* HPA is summarized below:

Therapy <sup>a</sup>	Age	Doses/day	PTPS/GTPCH deficiency	DHPR deficiency
Combined therapy				
Phenylalanine-low diet	initially	–	no	yes
or BH <sub>4</sub> (mg/kg/d)	initially	1–2	2–5	no
L-Dopa/Carbidopa	initially	3–4	1–3	1–3
(mg/kg/d)	<2y	3–4	4–7	4–7
	>2y	3–4	8–10	8–10
5-Hydroxytryptophan	initially	3–4	1–2	1–2
(mg/kg/d)	<2y	3–4	3–5	3–5
	>2y	3–4	6–8	6–8
Folinic acid (mg/d)	initially	1	no	10–20
BH <sub>4</sub> monotherapy				
BH <sub>4</sub> (mg/kg/d)	initially	1–2	5–20	no

<sup>a</sup> This information is subject to individual variations and may not represent the optimal therapeutic protocol.

The therapeutic protocol in a BH<sub>4</sub> deficiency *without* HPA is summarized below:

Therapy <sup>a</sup>	Age	Doses/day	DRD	SR deficiency
L-Dopa/Carbidopa (mg/kg/d)	initially	3–4	3–5	1–2
BH <sub>4</sub> (mg/kg/d)	initially	1–2	–	5–10

<sup>a</sup> This information is subject to individual variations and may not represent the optimal therapeutic protocol.

### 1.13 Comments

A diagnosis of hyperphenylalaninemia is usually based upon the confirmation of an elevated plasma phenylalanine level obtained on a normal diet following a positive newborn screening test. Early detection is desirable in order to introduce appropriate treatment and prevent mental retardation. Since a mother clears increased blood phenylalanine from her affected fetus transplacentally, an affected baby is born with normal blood phenylalanine levels. However, normal breast milk or formula feedings for as little as 24 hours is sufficient to raise the baby's blood phenylalanine sufficiently to trigger a positive test level ( $>120 \mu\text{mol/l}$ ). Even without feeding, due to catabolism, an infant will be found to have a positive screening test after 12 hours postnatally. The commonly used diagnostic methods are changing, and although the less economically advanced countries are still using the Guthrie screening test for both screening and confirmation of diagnosis, in most countries enzymatic tests are used for confirmation. This is also likely to change and the tandem mass spectrometer is becoming the method of choice for screening because the method allows for screening for multiple disorders and it is extremely cost effective with few false positives.

The duration of diet and outcome is best correlated, at the moment, with residual enzyme activity. Measurement of PAH activity in liver biopsies from PKU patients shows considerable variations in residual activity, ranging from  $\sim 1\%$  of normal in classical PKU to  $35\%$  of normal in non-PKU forms of hyperphenylalaninemia. An activity of  $5\%$  appears adequate for normal development without retardation.

One should be aware that there have been individuals with so called "severe" PKU mutations who have escaped retardation and all the other potential sequelae of PKU despite high blood Phe levels and very poor dietary control. It appears that the reason they have escaped the usual consequences of dietary indiscretions is that they have near normal brain Phe levels despite high blood Phe levels. A number of studies have now demonstrated considerable variability in blood vs. brain phe levels in PKU patients. Outcome in PKU appears to be related to the brain Phe levels. This, in all probability, will assume greater importance in making decisions about the strictness and duration of dietary control in the future. It has also been shown that the brain Phe levels of heterozygotes (parents) for PKU higher than are usually seen in PKU patients under very strict dietary control. This would suggest that control levels will probably be higher and safer than are now generally recommended.

A protocol for the diagnosis of  $\text{BH}_4$ -deficient hyperphenylalaninemia must be based upon both biochemical and clinical investigations. Symptoms may manifest during the first weeks of life, but usually are more commonly noted at about 4 months of age. However, careful reviews suggest that abnormal signs (poor sucking, decreased spontaneous movements,

“floppy baby”) may be noted even during the neonatal period. Birth is generally uneventful, except that there is a higher incidence of prematurity and lower birth weights in typical (severe) PTPS deficiency.

All variants with BH<sub>4</sub> deficiencies presenting with hyperphenylalaninemia have an autosomal recessive trait. Affected patients are homozygotes or compound heterozygotes, although there is some evidence for symptomatic heterozygosity in patients with PTPS deficiencies. Only Dopa-responsive dystonia and SR deficiency do not manifest with hyperphenylalaninemia and DRD is inherited as an autosomal dominant trait. The frequency of all autosomal recessive BH<sub>4</sub> deficiencies is uncertain, but, assuming they represent about 1–2% of all hyperphenylalaninemic patients, the combined frequency of BH<sub>4</sub> deficiencies is approximately 1:10<sup>-6</sup> live births. Data from newborn and selective screening programs reveal regional (demographic) variations in the frequency of BH<sub>4</sub> deficiencies. In the Piemonte region and also in the southern parts of Italy, 10% of all patients with hyperphenylalaninemia are accounted for by BH<sub>4</sub> deficiencies. In Turkey, the incidence is even higher (15%). In Taiwan, it is 19%, and Saudi Arabia has the highest incidence (66%).

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