Disorders of Phenylalanine and Tetrahydrobiopterin Metabolism

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Contents

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1.1 Introduction

Hyperphenylalaninemia (HPA), a disorder of phenylalanine catabolism, is caused primarily by a deficiency of the hepatic phenylalanine-4-hydroxylase (PAH) or by one of the enzymes involved in its cofactor tetrahydrobiopterin (BH4) biosynthesis (GTP cyclohydrolase I (GTPCH) and 6-pyruvoyl-tetrahydropterin synthase (PTPS)) or regeneration (dihydropteridine reductase (DHPR) and pterin-4a-carbinolamine dehydratase (PCD)) (Blau et al. 2001). BH4 is known to be the natural cofactor for PAH, tyrosine-3-hydroxylase, and tryptophan-5-hydroxylase as well as all three isoforms of nitric oxide synthase (NOS) (Werner et al. 2011), the latter two being the key enzymes in the biosynthesis of the neurotransmitters dopamine and serotonin. Thus, with two exceptions (see below) any cofactor defect will result in a deficiency of biogenic amines accompanied by HPA. Because phenylalanine is a competitive inhibitor of the uptake of tyrosine and tryptophan across the blood-brain barrier and of the hydroxylases of tyrosine and tryptophan, depletion of catecholamines and serotonin occurs in untreated patients with PAH deficiency. Both groups of HPA (PAH and BH4 deficiency) are heterogeneous disorders varying from severe, e.g., classical phenylketonuria (PKU), to mild and benign forms (see Sect. 1.4). Because of the different clinical and biochemical severities in this group of diseases, the terms "severe" or "mild" will be used based upon the type of treatment and involvement of the CNS. For the BH4 defects, symptoms may manifest during the first weeks of life but usually are noted within the first half year of life. Birth is generally uneventful, except for an increased incidence of prematurity and lower birth weights in severe PTPS deficiency (Opladen et al. 2012).

Two disorders of BH4 metabolism may present without HPA. These are dopa-responsive dystonia (DRD; Segawa disease) (Segawa 2011) and sepiapterin reductase (SR) deficiency (Friedman et al. 2012). While DRD is caused by mutations in the GTPCH gene and is inherited in an autosomal dominant manner, SR deficiency is an autosomal recessive trait. Both diseases evidence severe biogenic amine deficiencies. DRD

N. Blau et al. (eds.), *Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases*,
DOI 10.1007/978-3-642-40337-8_1, © Springer-Verlag Berlin Heidelberg 2014

usually presents with a dystonic gait and diurnal variation, while many patients with SR deficiency have initial diagnosis of cerebral palsy. At least two reports describe heteroallelic patients with DRD suggesting a wide spectrum of GTPCH variants.

A diagnosis of HPA is usually based upon the confirmation of an elevated blood phenylalanine level obtained on a normal diet, following a positive newborn screening test. Normal breast milk or formula feeding for only 24 h is sufficient to raise the baby's blood phenylalanine sufficiently to trigger a positive test level (>120 µmol/l). In general, an infant will be found to have a positive screening test 12 h postnatal. The tandem mass spectrometry (TMS) is today the method of choice for newborns screening. A detection as early as possible is essential in order to introduce appropriate treatment to prevent effects on mental development.

In PAH and BH4 deficiencies, factors like a relatively high phenylalanine intake or catabolic situations may be responsible for high phenylalanine concentrations in blood. Once HPA has been detected, a sequence of quantitative tests (see Sect. 1.8) enables the differentiation between variants, i.e., BH4-non-responsive PKU (usually the patients with the most severe PAH deficiency), BH4-responsive PKU (Heintz et al. 2013), and BH4 deficiencies. Because the BH4 deficiencies are actually a group of diseases which may be detected because of HPA, but not simply and routinely identified by neonatal mass screening, selective screening for a BH4 deficiency is essential in every newborn with even slightly elevated phenylalanine levels. Differential testing for BH4 deficiencies should be done in all newborns with plasma phenylalanine levels greater than 120 µmol/l (2 mg/dl), as well as in older infants and children with neurological signs and symptoms.

BH4 deficiencies presenting *without* HPA are detectable only by investigations for neurotransmitter metabolites and pterins in CSF or by clinical signs and symptoms. In DRD, a phenylalanine loading test, a trial with L-dopa, and enzyme activity measurement in cytokine-stimulated fibroblasts and molecular testing are confirmatory for the diagnosis. SR deficiency can be definitely diagnosed by an enzyme assay of cultured fibroblasts or DNA testing, but phenylalanine loading test is also positive.

The goals of treatment are to control HPA in PAH and BH4 deficiencies and to restore CNS neurotransmitter homeostasis in BH4 deficiencies (Blau et al. 2010). To that aim, dietary restriction in phenylalanine intake, supplementation with BH4, and oral administration of dopamine and serotonin precursors (L-dopa/carbidopa and 5-hydroxytryptophan, respectively), as well as some other drugs are available (Opladen et al. 2012). In this respect it should be taken into account that some patients with PAH deficiency, historically only treated by diet, can be treated with BH4 (sapropterin dihydrochloride). At the same time, in patients with DPHR deficiency, in whom historically the HPA was not treated with BH4, the diet restricting phenylalanine intake is the treatment of choice. Only about 20 % of DHPR-deficient patients are on BH4 treatment (Opladen et al. 2012).

Late detection of PAH or BH4 deficiencies and late introduction of treatment lead to irreversible brain damage. In contrast to early and continuously treated patients with PAH deficiency, some patients with BH4 deficiencies show progressive neurological deterioration despite treatment. Patients with PCD deficiency are at risk for developing early-onset diabetes in puberty.

1.2 Nomenclature

No.	Disorder	Alternative Name	Abbreviation	Gene Symbol	Chromosomal Localization	Affected Protein	OMIM No.	Sub Type
1.1	Phenylalanine hydroxylase deficiency	Classic phenylketonuria	Classic PKU	*PAH*	12q22-24.1	Phenylalanine hydroxylase	261600	Mild to severe
1.2	GTP cyclohydrolase deficiency		arGTPCH	*GCH1*	14q22.1-22.2	GTP cyclohydrolase I	233910	Autosomal recessive
1.3	6-Pyruvoyl-tetrahydropterin synthase deficiency		PTPS	*PTS*	11q22.3-23.3	6-Pyruvoyl-tetrahydropterin synthase	261640	
1.4	Dihydropteridine reductase deficiency		DHPR	*QDPR*	4p15.3	Dihydropteridine reductase	261630	Moderate and severe
1.5	Pterin-4a-carbinolamine dehydratase deficiency	Primapterinuria	PCD	*PCBD1*	10q22	Pterin-4a-carbinolamine dehydratase	264070	Benign HPA Early-onset diabetes
1.6	Dopa-responsive dystonia	Segawa disease	adGTPCH, DRD	*GCH1*	14q22.1-22.2	GTP cyclohydrolase I	600225	
1.7	Sepiapterin reductase deficiency		SR	*SPR*	2p14-p12	Sepiapterin reductase	182125	

1.3 Metabolic Pathway

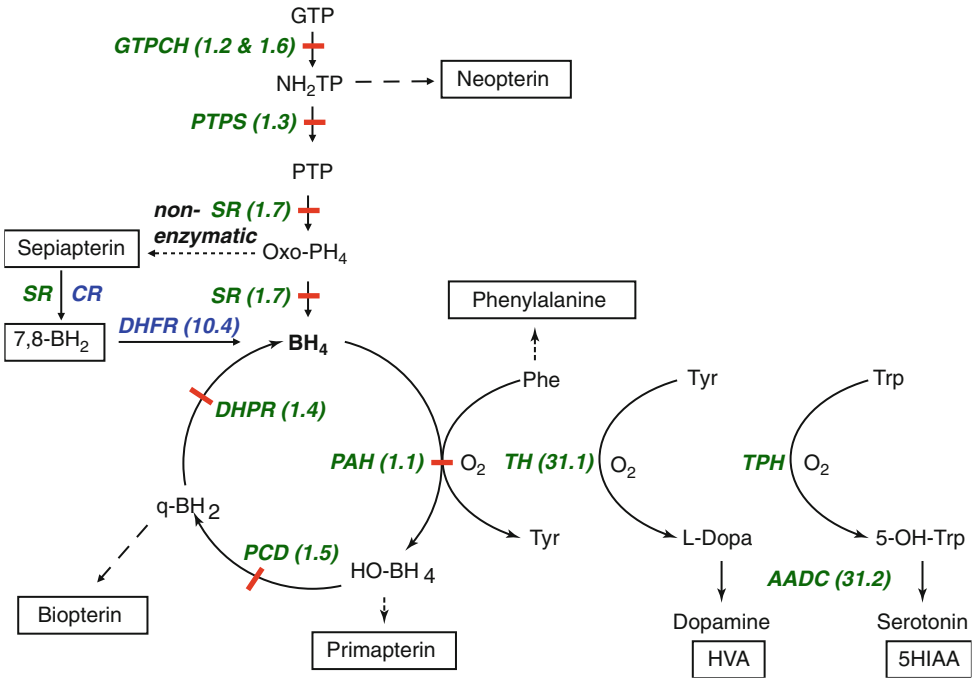


Fig. 1.1 Biosynthesis and regeneration of tetrahydrobiopterin (BH4) including possible metabolic defects in hyperphenylalaninemia (*HPA*) and catabolism of phenylalanine. **1.1** phenylalanine-4-hydroxylase (*PAH*), **1.2/1.6** GTP cyclohydrolase I, **1.3** 6-pyruvoyl-tetrahydropterin synthase (*PTPS*), **1.4** dihydropteridine reductase (*DHPR*), **1.5** pterin-4a-carbinolamine dehydratase (*PCD*), **1.7** sepiapterin reductase, carbonyl reductase (*CR*), aldose reductase (*AR*), dihydrofolate reductase (*DHFR*), aromatic amino acid decarboxylase (*AADC*), tyrosine hydroxylase (*TH*), and tryptophan hydroxylase (*TPH*). 7,8-BH2: 7,8-dihydrobiopterin; PTP: 6-pyruvoyl-tetrahydropterin; HVA: homovanillic acid; 5HIAA: 5-hydroxyindoleacetic acid. Pathological metabolites used as specific markers in the differential diagnosis are marked in squares

1.4 Signs and Symptoms

Table 1.1 Phenylalanine hydroxylase deficiency, classic PKU

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Autism			±	±	±
	Hypertonia		±	±	±	
	Irritability		±	±	±	±
	Mental retardation	±	+	+	+	+
	Seizures			±	±	±
Dermatological	**Hypopigmentation**	+	+	+	+	+
	Skin rash	±	±	±	±	±
Digestive	Vomiting	±	±			
Musculoskeletal	Head circumference	↓-n	↓-n			
	Height	↓-n	↓-n	↓-n		
	Microcephaly		+	+	+	+
Other	**Odor (urine and body)**	±	+	+	+	+
	Birth weight	↓-n				
Special laboratory	5-Hydroxyindoleacetic acid, 5HIAA (CSF)		↓-n	↓-n	↓	↓
	BH4 test	n	n	n	n	n
	Homovanillic acid, HVA (CSF)		↓-n	↓-n	↓	↓
	MRI: brain		±	±	±	±
	Phenylalanine (P, U, CSF)	↑↑↑	↑↑↑	↑↑↑	↑↑↑	↑↑↑
	Phenylpyruvic acid (U)	n-↑	↑	↑	↑	↑

Table 1.2 GTP cyclohydrolase deficiency

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Autonomic system	Temperature instability	+	+	+		
CNS	Hypertonia, extremities	+	+	+	+	
	Hypotonia, axial	+	+	+	+	
	Mental retardation		+	+	+	
	Seizures, myoclonic		+	+		
Digestive	Drooling	+	+	+		
	Feeding difficulties	+	+	+		
Musculoskeletal	Microcephaly	+	+	+	+	
Special laboratory	5-Hydroxyindoleacetic acid, 5HIAA (CSF)	↓	↓	↓	↓	
	Biopterin (U, CSF, DBS)	↓↓	↓↓	↓↓	↓↓	
	GTPCH activity, cytokine-stimulated (FB)	↓	↓	↓	↓	↓
	Homovanillic acid, HVA (CSF)	↓↓	↓↓	↓↓	↓↓	
	Neopterin (U, CSF, DBS)	↓↓	↓↓	↓↓	↓↓	
	Phenylalanine (P, U, CSF)	n-↑	↑	↑	↑	
	Tetrahydrobiopterin (BH4) loading test	+++	+++	+++	+++	

Table 1.3 6-Pyruvoyl-tetrahydropterin synthase deficiency

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Autonomic system	Temperature instability	+	+	+		
CNS	Choreoathetosis		+	+	+	
	Hypotonia, axial	+	+	+	+	
	Mental retardation	±	+	+	+	+
	Retardation, psychomotor		+	+		
	Seizures, myoclonic		+	+		
Dermatological	Hypopigmented hair		+	+		
	Rash, eczematous		+	+	+	
Digestive	Drooling	+	+	+	+	
Musculoskeletal	Microcephaly	+	+	+		
Respiratory	Pneumonia		+	+		
Other	**Birth weight**	↓-n				
	Sudden death		±	±		
Routine laboratory	EEG: abnormal	+	+	+	+	+
Special laboratory	5-Hydroxyindoleacetic acid, 5HIAA (CSF)	↓↓	↓↓	↓↓	↓	↓
	Biopterin (U, CSF, DBS)	↓↓↓	↓↓↓	↓↓	↓↓	↓↓
	Homovanillic acid, HVA (CSF)	↓↓	↓↓	↓↓	↓↓	↓
	MRI: cortical and subcortical atrophy	+	+	+	+	+
	Neopterin (U, CSF, DBS)	↑↑↑	↑↑↑	↑↑	↑↑	↑↑
	Phenylalanine (P, U, CSF)	↑	↑	↑	↑	↑
	Prolactin (P)	↑	↑	↑	↑	↑
	PTPS activity (RBC, FB)	↓	↓	↓	↓	↓
	Tetrahydrobiopterin (BH4) loading test	++	++	++	++	++

Table 1.4 Dihydropteridine reductase deficiency

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Autonomic system	Temperature instability	+	+	+		
CNS	Choreoathetosis		+	+	+	
	Hypotonia, axial	+	+	+	+	
	Hypotonia, axial		+	+	+	
	Mental retardation	±	+	+	+	+
	Retardation, psychomotor		+	+		
	Seizures, myoclonic		+	+		

Table 1.4 (continued)

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Dermatological	Hypopigmented hair		+	+		
	Rash, eczematous		+	+	+	
Digestive	Drooling	+	+	+	+	
Musculoskeletal	Microcephaly	+	+	+		
Respiratory	Pneumonia		+	+		
Other	Sudden death		+	+		
Routine laboratory	**CT scan: basal ganglia calcifications**		+	+	+	
	EEG: spike wave discharges and generalized slowing, abnormal	+	+	+	+	+
Special laboratory	5-Hydroxyindoleacetic acid, 5HIAA (CSF)	↓↓	↓↓	↓↓	↓	↓
	Biopterin (U, CSF, DBS)	n-↑	n-↑	n-↑	↑	↑
	Dihydrobiopterin (CSF)	↑↑	↑↑	↑↑	↑↑	↑↑
	Dihydropteridine reductase (DBS)	↓↓	↓↓	↓↓	↓↓	↓↓
	Homovanillic acid, HVA (CSF)	↓↓	↓↓	↓↓	↓↓	↓
	MRI: cortical and subcortical atrophy	(+)	+	+	+	+
	Neopterin (U, CSF, DBS)	n	n	n	n	n
	Phenylalanine (P, U, CSF)	↑	↑	↑	↑	↑
	Prolactin (P)	↑	↑	↑	↑	↑
	Tetrahydrobiopterin (BH4) loading test	+	+	+	+	+

Table 1.5 Pterin-4a-carbinolamine dehydratase deficiency

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Hypotonia, mild	+				
	Transient alteration in tone	+				
Endocrine	Diabetes MODY3-like				±	±
Routine laboratory	Glucose (P)				n-↑	n-↑
	Magnesium (P)				↓-n	↓-n
Special laboratory	Neopterin (U)	↑↑	↑			
	Phenylalanine (P)	↑	(↑)	(↑)	n	n
	Primapterin (U)	↑↑	↑↑	↑		
	BH4 loading test	+	+	+	+	+

Table 1.6 Dopa-responsive dystonia

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Bradykinesia		±	±	±	±
	Diurnal fluctuation of symptoms		±	+	+	±
	Dyskinesia				±	±
	Hypertonia	±	±	±	±	±
	Hypokinesia		+	++	++	++
	Hypotonia		±	±	±	±
	Parkinsonism			±	±	±
	Spasticity	±	±	±	±	±
	Tendon reflexes, increased	±	±	±	±	±
	Tremor				+	+
Digestive	Dysphagia		±	±	±	±
Musculoskeletal	Pes equinovarus			±	±	
	Rigidity	±	+	+	+	+
	Scoliosis			±	±	
Special laboratory	5-Hydroxyindoleacetic acid, 5HIAA (CSF)	↓-n	↓-n	↓-n	↓-n	↓-n
	Biopterin (CSF)	↓	↓	↓	↓	↓
	Homovanillic acid, HVA (CSF)	↓	↓	↓	↓	↓
	Neopterin (CSF)	↓	↓	↓	↓	↓
	Phe loading test	+	+	+	+	+
	Phenylalanine (P)	n	n	n	n	n

Table 1.7 Sepiapterin reductase deficiency

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
CNS	Behavior, psychotic		±	±	±	±
	Cerebral palsy			±	±	±
	Diurnal fluctuation of symptoms		+	+	±	±
	Dysarthria	±	±	±	±	
	Hypo or hypertonia	±	±	±	±	
	Hypokinesia	+	++	±	±	±
	Hypotonia, axial	++	++	++	+	
	Language difficulties		++	++	+	
	Parkinsonism		±	±	±	
	Retardation, psychomotor		++	++		
	Tendon reflexes, increased	±	±	±		
Digestive	Gastrointestinal dysmotility	±	±	±		
Eye	Eye movements, abnormal, oculogyric crisis	±	±	±		
Musculoskeletal	Muscle weakness	+	±	±	±	
Special laboratory	5-Hydroxyindoleacetic acid, 5HIAA (CSF)	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓
	Biopterin (CSF)	↑	↑	↑	↑	↑
	Biopterin (U)	n	n	n	n	n
	Dihydrobiopterin (CSF)	↑↑	↑↑	↑↑	↑↑	↑↑
	Homovanillic acid, HVA (CSF)	↓↓↓	↓↓↓	↓↓↓	↓↓↓	↓↓↓
	Neopterin (U, CSF, DBS)	n	n	n	n	n
	Phe loading test		+	+	+	+
	Phenylalanine (P)	n	n	n	n	n
	Prolactin (P)	↑	↑	↑	↑	↑
	Sepiapterin (CSF)	↑↑	↑↑	↑↑	↑↑	↑↑

1.5 Reference Values

Serum, urine, and dried blood spots

Age	Phe (S) µmol/l	Neo (S) nmol/l	Bio (S) nmol/l	Neo (U) mmol/mol Creat	Bio (U) mmol/mol Creat	Neo (DBS) nmol/g Hb	Bio (DBS) nmol/g Hb
Newborns	<120	3–11	4–18	1.1–4.0	0.5–3.0	0.19–2.93	0.08–1.20
0–1 years	<80	3–11	4–18	1.1–4.0	0.5–3.0	0.19–2.93	0.08–1.20
2–4 years	<80	3–11	4–18	1.1–4.0	0.5–3.0	0.19–2.93	0.08–1.20
5–10 years	<80	3–11	4–18	1.1–4.0	0.5–3.0	0.19–2.93	0.08–1.20
11–16 years	<70	3–11	4–18	0.2–1.7	0.5–2.7	0.19–2.93	0.08–1.20
>16 years	<70	3–11	4–18	0.2–1.7	0.5–2.7	0.19–2.93	0.08–1.20

CSF

Age	BH4 (CSF) nmol/l	BH2 (CSF) nmol/l	Neo (CSF)[a] nmol/l	Bio (CSF)[a] nmol/l	5HIAA (CSF) nmol/l	HVA (CSF) nmol/l	5MTHF (CSF) nmol/l
Newborns	25–121	4–18	15–35	20–70	144–800	300–1,000	64–182
0–1 years	24–59	4–18	12–30	15–40	114–336	295–932	64–182
2–4 years	20–61	4–18	9–20	10–30	105–299	211–871	63–111
5–10 years	20–49	4–18	9–20	10–30	88–178	144–801	41–117
11–16 years	20–49	4–18	9–20	10–30	74–163	133–551	41–117
>16 years	18–53	4–18	9–20	10–30	66–141	115–488	41–117

[a]Total neopterin or biopterin

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	Fetus		<14[b]	<115[b]		
Fibroblasts[a]	Infants (0–12 m)		11–73[b]	183–303[b]		
	Children and adults		18–98[b]	154–303[b]		

[a]In cytokine-stimulated cells
[b]pmol/mg

Enzymes

Age	DHPR (RBC) mU/mg Hb	PTPS (RBC) µU/g Hb	GTPCH (FB)[a] µU/mg protein	PTPS (FB) µU/mg protein	DHPR (FB) mU/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 months)	1.8–4.8	34–64				
Infants (0–12 months)	1.8–4.8		1.7–4.9	0.5–1.7	6.3–8.7	97–185
Children and adults	1.8–4.8	11–29	1.4–6.5	0.4–1.6	4.5–8.3	99–185

[a]In cytokine-stimulated cells

1.6 Pathological Values/Differential Diagnosis

Plasma

Actual Phe[a] µmol/l	Neo (S) nmol/l	Bio (S) nmol/l
<200	3–11	4–18
200–600	2–32	12–46
600–1,200	9–27	24–39

[a]Plasma neopterin and biopterin values depend strongly upon the actual blood Phe concentrations

Plasma, urine, DBS, and CSF

Variant	Phe (S)	Neo (U)	Bio (U)	%Bio[c]	Neo (DBS)	Bio (DBS)	Neo (CSF)	Bio (CSF)	5HIAA (CSF)	HVA (CSF)	5-MTHF (CSF)
	µmol/l	mmol/mol Creat			nmol/g Hb		nmol/l				
1.1 PAH def. (classical)	>1,200	1.1–16.9	1.2–8.1	~50	0.15–4.62	0.08–1.68	9–118	15–143	14–471	47–1,174	n
1.1 PAH def. (variant)	600–1,200	1.1–16.9	1.2–8.1	~50	0.15–4.62	0.08–1.68	9–118	15–143	n	n	n
1.1 PHA def. (benign)	120–600	1.1–16.9	1.2–8.1	~50	0.15–4.62	0.08–1.68	n	n	n	n	n
1.2 GTPCH def.	120–1,200[a]	<0.2	<0.2	~50	<0.15	<0.08	0.05–3.0	1.5–7.5	61–183	15–48	n
1.3 PTPS def. (severe)	250–2,500	5.0–51.2	<0.5	<5	2.2–6.3	0	47–402	1.0–16.0	5–113	5–223	n
1.3 PTPS def. (mild)	240–2,200	5.0–51.2	<0.5	<5	2.2–6.3	0	25–230	13–56	93–420	249–998	n
1.4 DHPR def. (severe)	180–2,500	0.5–23.2	3.8–25.6	>80	0.47–2.1	0.67–1.5	11–70	43–117[d]	4–75	19–204	↓
1.4 DHPR def. (mild)	280–600	0.5–23.2	3.8–25.6	>80	0.47–2.1	0.67–1.5	11–70	43–117[d]	21–66	n	↓-n
1.5 PCD def. (benign)	180–1,200	4.1–22.5	0.7–1.5[b]	<50	–	–	43–117	16–96	n	n	n
1.6 DRD	<120	n	n	~50	n	n	1.1–6.2	3.1–7.6	48–97	120–239	n
1.7 SR def.	<120	n	n	~50	n	n	14–51	72–102[d]	3–15	49–111	n

[a]Several patients were missed in the newborn screening due to the negative Guthrie test
[b]Primapterin (7-Bio) ↑
[c]%Bio = 100 * Bio/(Neo + Bio)
[d]7,8-Dihydrobiopterin ↑

1.7 Loading Tests

Loading test with BH4

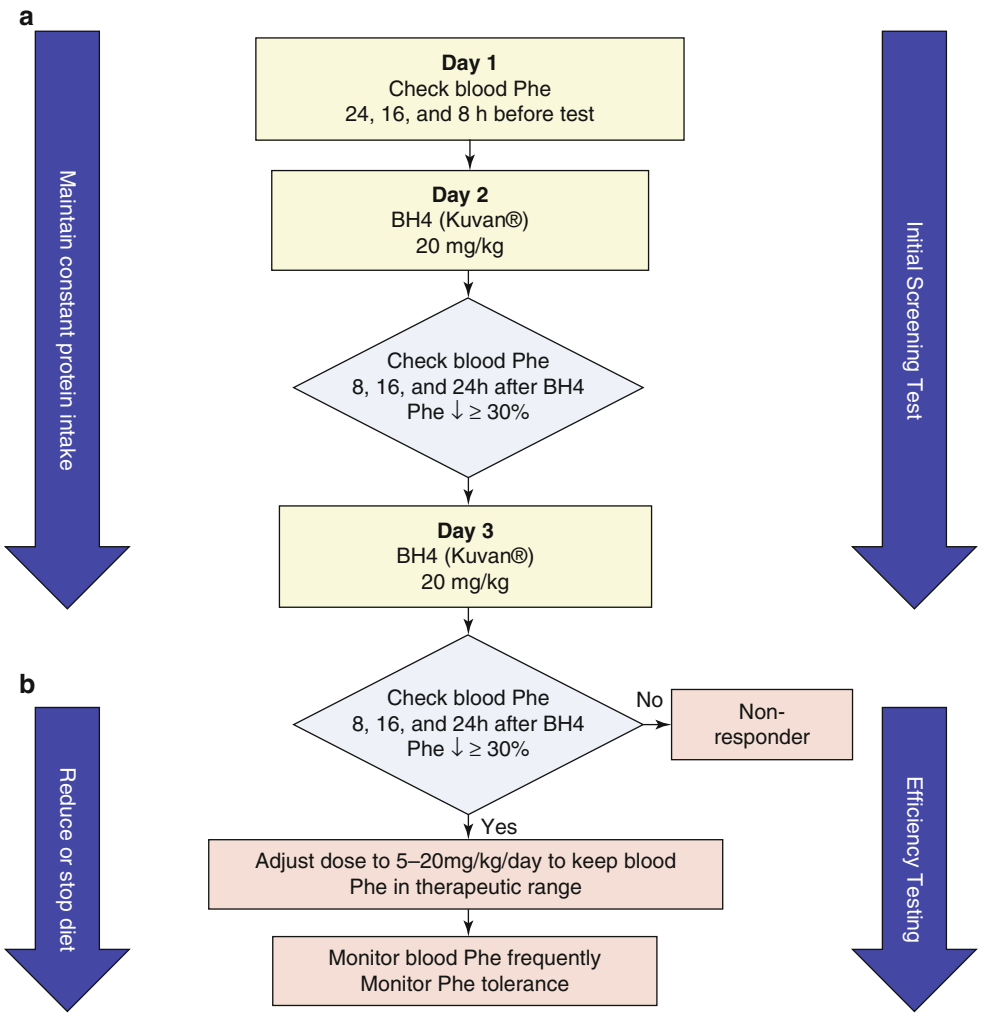


Fig. 1.2 Proposed algorithms for the BH4 (sapropterin dihydrochloride; Kuvan) challenge, screening, and initiating treatment in BH4-responsive PKU patients. (**a**) Initial screening test with blood Phe monitoring on the first day and BH4 (sapropterin dihydrochloride) administration (20 mg/kg) on two following days. (**b**) Efficiency testing in BH4-reponsive patients over several weeks with BH4 doses adjusted individually according to Phe tolerance and therapeutic blood Phe levels. Combined Phe (100 mg/kg) and BH4 (20 mg/kg) loading test is sometimes difficult to interpret and is therefore not recommended (Blau et al. 2011)

Loading tests with Phe

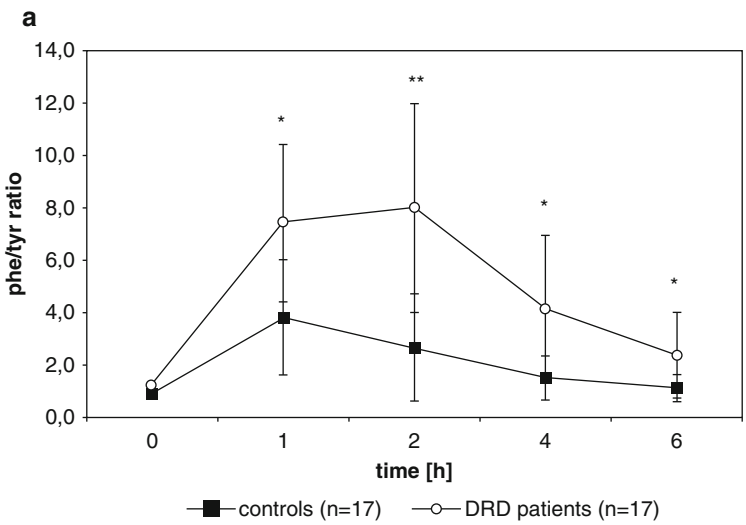
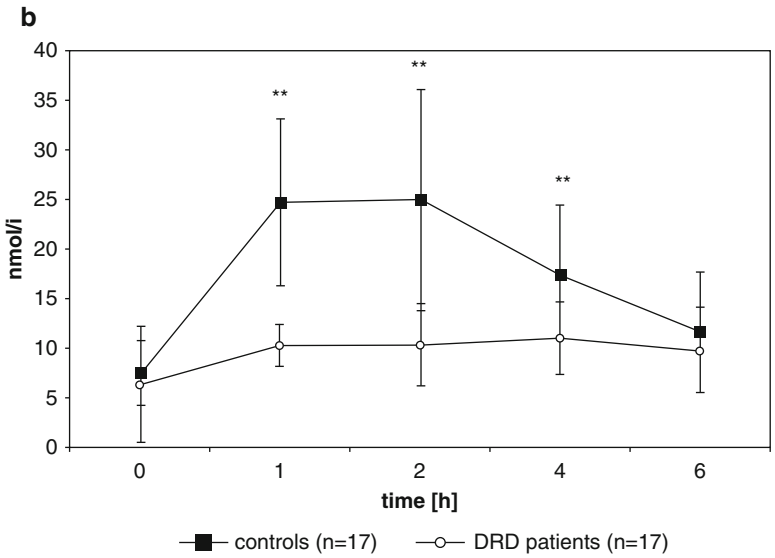


Fig. 1.3 Oral phenylalanine loading with 100 mg Phe/kg body weight is performed, as previously described by Hyland et al. (1997). Samples were collected at time T-0, T-1h, T-2h, T-4h, and T-6h. Plasma was frozen immediately and kept in the dark; DBS were kept in dry conditions at 4 °C. The clearest differentiation measured by the standardized mean difference between the patients and controls was seen 2 h after Phe administration (Opladen et al. 2010). See also the flowchart with cut-off values. (**a**) Means and standard deviations (SDs) of phenylalanine/tyrosine (Phe/Tyr) ratios in pediatric patients after Phe loading [*open circles* dopa-responsive dystonia (DRD) patients; *black squares* controls; * $p<0.05$; ** $p<0.01$] (**b**) Means and standard deviations (SDs) of biopterin concentration in pediatric patients after phenylalanine loading [*open circles* dopa-responsive dystonia (DRD) patients; *black squares* controls; ** $p<0.01$] (Opladen et al. 2010)

Fig. 1.3 (continued)



1.8 Diagnostic Flowcharts

Differential diagnosis of HPAs

Screening for a BH_4 deficiency should be done in all newborns and children with even slight HPA (plasma Phe >120 µmol/l) as well as in older children without HPA 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 BH_4 challenge
4. DNA testing

The first two tests are essential and will allow the differentiation between all variants with BH_4 deficiencies. With some limitations (DHPR def.), the BH_4 loading test is an additional useful diagnostic tool for the rapid discrimination between classical PKU and biopterin variants. This test is also useful for identifying the recently described BH_4-responsive PAH deficiency. For the interpretation and determination of the various disorders based upon loading tests, see Sect. 1.6.

Biochemical and loading test data are summarized in Fig. 1.5 (Blau et al. 2010):

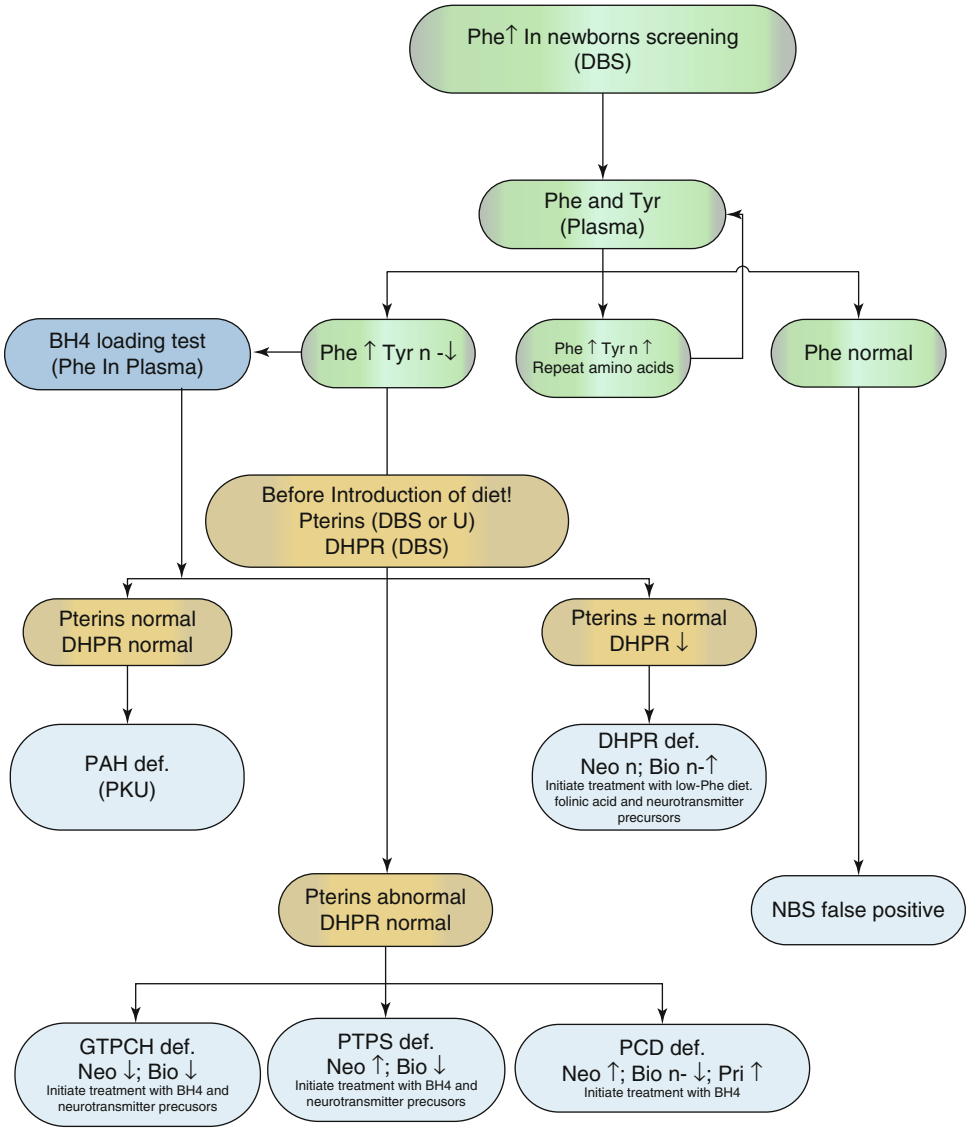


Fig. 1.4 Diagnostic flowchart for the laboratory diagnosis of PKU and BH4 deficiencies (Blau et al. 2011). Dried blood spots (DBS) or random urine (U) can be used for the differential diagnosis and depending on the profile of neopterin (*Neo*), biopterin (*Bio*), and primapterin (*Pri*) and dihydropteridine reductase (*DHPR*) activity in DBS, diagnosis of following BH4 deficiencies can be established: GTP cyclohydrolase I (*GTPCH*) deficiency (low or no detectable neopterin and biopterin), 6-pyruvoyl-tetrahydropterin synthase (*PTPS*) deficiency (high neopterin and low or no detectable biopterin), dihydropteridine reductase (*DHPR*) deficiency (normal neopterin and normal or elevated biopterin and no DHPR activity), and pterins-4acarbinolamine dehydratase (*PCD*) deficiency (elevated neopterin, low-normal biopterin, and elevated primapterin). *N* normal (Blau et al. 2011)

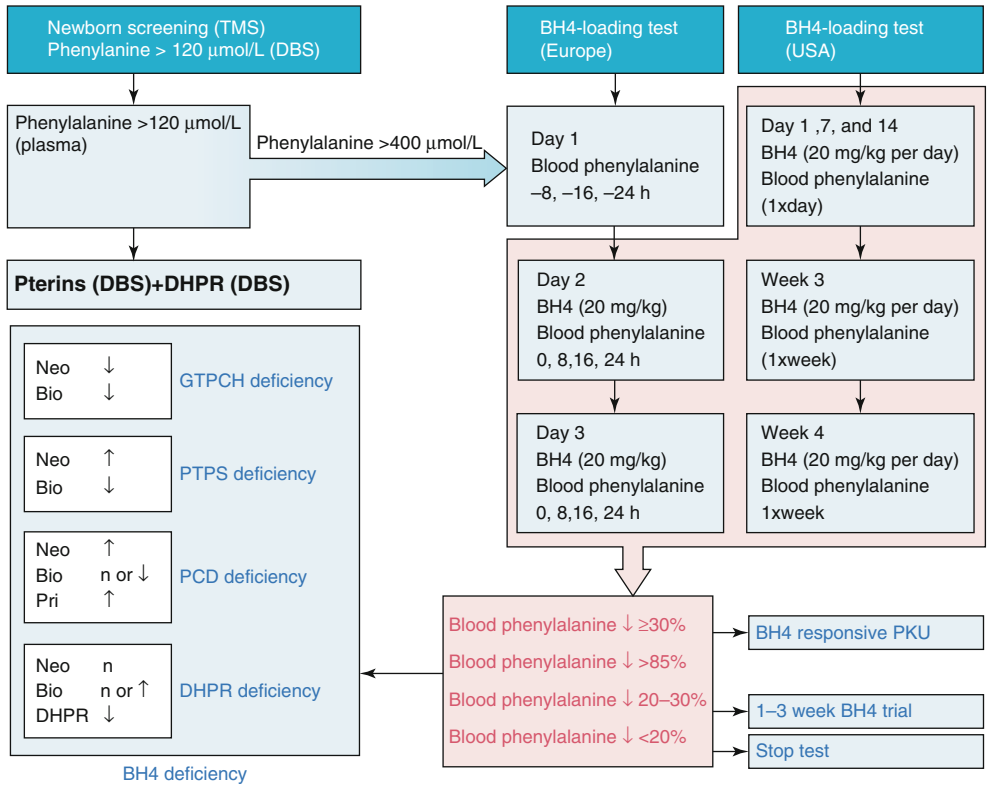


Fig. 1.5 Flow-chart for the differential diagnosis of BH4 responsive PKU and BH4 deficiencies. *Phe* phenylalanine, *DBS* dried blood spots, *DHPR* dihydropteridine reductase, *Neo* neopterin, *Bio* biopterin, *Pri* primapterin, *GTPCH* GTP-cyclohydrase I, *PTPS* 6-pyruvoyl-tetrahydropterin synthase, *PCD* pterin-4a-carbinolamine dehydratase, *BH4* tetrahydrobiopterin. (Blau et al. 2010)

Interpretation of the Phe loading test

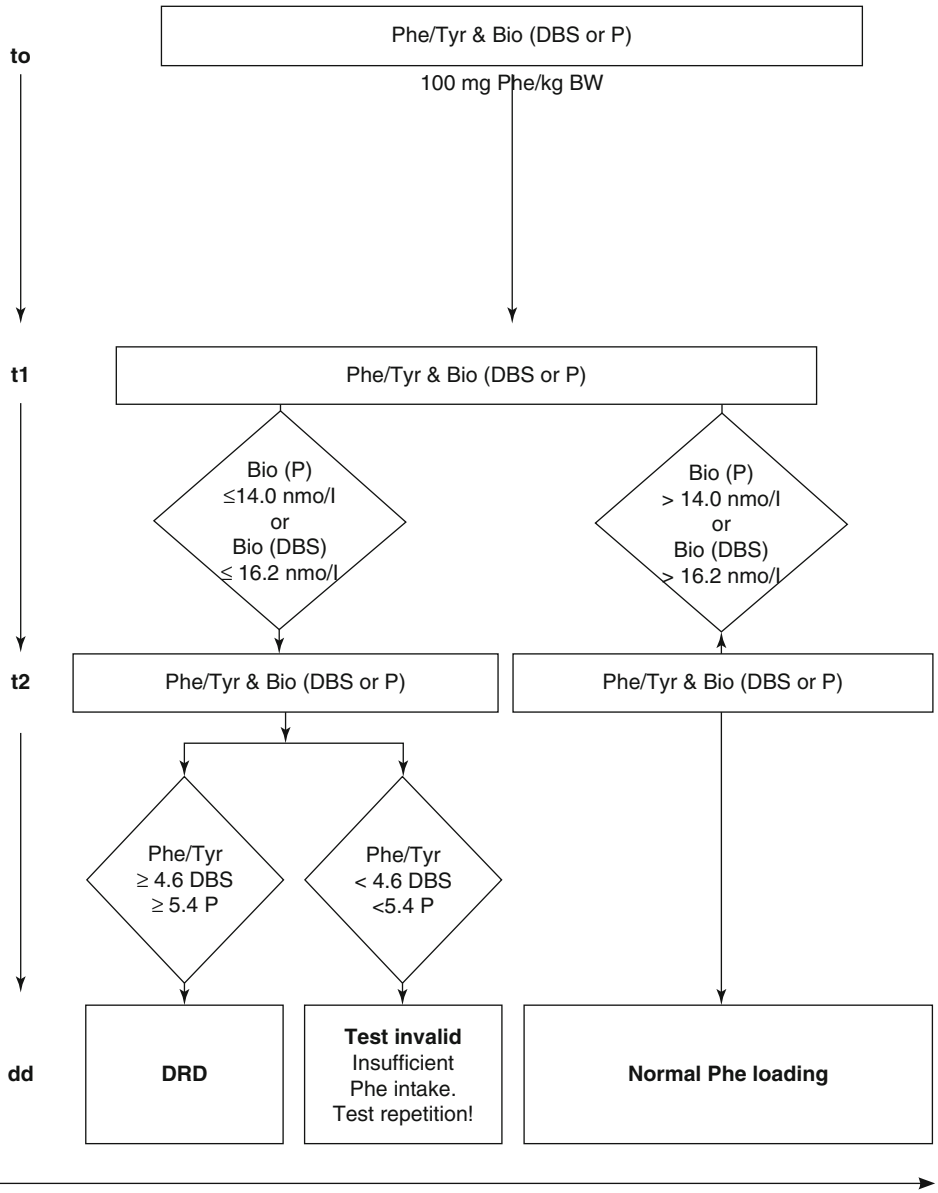
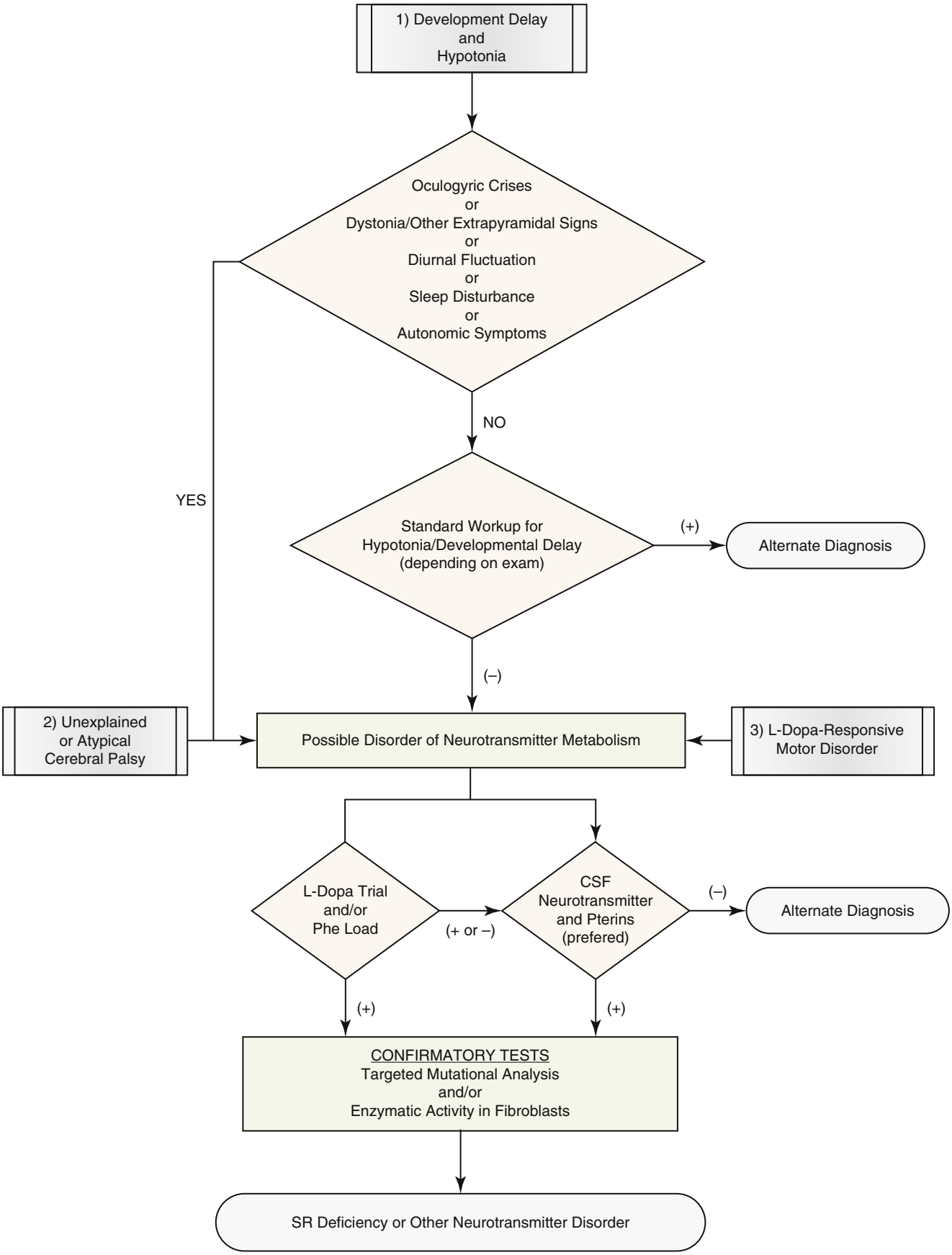


Fig. 1.6 Phenylalanine (*Phe*) loading in children: revised shortened test procedure and test interpretation (*Bio* biopterin, *Phe/Tyr* Phe/tyrosine ratio, *DBS* dried blood spot, *P* plasma, *BW* body weight, *dd* diagnostic decision) (Opladen et al. 2010)

Fig. 1.7 Diagnostic algorithm for patients with a possible disorder of neurotransmitter metabolism. Sepiapterin reductase (SR) deficiency and other disorders of neurotransmitter metabolism should be considered in patients with (1) developmental delay with hypotonia, (2) suspect but unexplained cerebral palsy (CP) or CP with atypical features, and (3) uncharacterized L-dopa-responsive motor disorders. (1) In a patient with developmental delay and hypotonia, if oculogyric crises, diurnal fluctuation, sleep disturbance, or extrapyramidal or autonomic signs exist, a disorder of neurotransmitter biosynthesis is likely and cerebrospinal fluid (CSF) analysis should be done. If no other signs are present, CSF analysis should be considered if standard workup for hypotonia is unrevealing. If CSF analysis is abnormal, then mutational screening and/or measurement of enzymatic activity can be targeted to confirm the specific disorder suggested by the pattern of CSF abnormalities. If CSF evaluation is impractical, alternative evaluation may include L-dopa trial and/or phenylalanine load. If negative, CSF analysis must still be done to exclude a disorder of neurotransmitter metabolism. If L-dopa trial and/or phenylalanine loading are positive, CSF analysis will allow targeted mutational screening; however, one should keep in mind that phenylalanine (Phe) load can be positive in heterozygote carriers for phenylketonuria. Alternatively, CSF analysis may be skipped and broad mutational screening undertaken. Mutational and gene dosage screening may be time-consuming and costly, and false negatives may still occur. Therefore, this alternative evaluation route should be reserved for cases in which CSF analysis is not available or is declined, or in which other clinical features lead to suspicion of a specific diagnosis. (2) In a patient with unexplained CP or CP with atypical features, a disorder of neurotransmitter metabolism should be considered and diagnostic algorithm, as outlined above, should be followed. Atypical or unexplained features suggesting need for further metabolic investigation in a child with possible CP include lack of adequate antecedent, nondiagnostic magnetic resonance imaging, progressive symptoms, familial occurrence, episodic encephalopathy, and features not expected in the CPs such as diurnal variation, sleep disturbance, autonomic symptoms, or oculogyric crises. (3) All patients with an L-dopa-responsive motor disorder should be evaluated for a disorder of neurotransmitter metabolism. CSF analysis (after discontinuation of L-dopa therapy for at least 10 days) is the recommended first step. If L-dopa withdrawal is impractical, the results of CSF analyses may still be informative if either pterins or 5-hydroxyindoleacetic acid levels are abnormal. Alternatively, molecular investigations can be done, guided either by results of phenylalanine loading test or clinical symptoms with caveats as noted above.1 CSF analysis should consist of homovanillic acid, 5-hydroxyindoleacetic acid (5HIAA), pterins (neopterin, biopterin, and sepiapterin), and 5-methyltetrahydrofolate (Blau et al. 2010)

1.9 Diagnostic Flowchart in the Differential Diagnosis of Non-HPA Variants



1.10 Specimen Collection

Test	Preconditions	Material	Handling	Pitfalls
Phe	Free diet	Guthrie card, serum/plasma, CSF	Keep cool (−20 °C) for serum or plasma Guthrie card at RT	
Neo	Free diet, Phe in plasma high enough	Random urine, spotted urine	Keep cool, dark (−20 °C)	Infections (Neo ↑)
Bio			Oxidized sample, dark, RT	
		Serum/plasma	Keep cool, dark (−20 °C)	
		CSF	EDTA tube (−20 °C)	
BH_4, BH_2		CSF	DTE/DETAPAC tube (−80 °C)	
HVA	1 h before medication, withdraw first 0.5 ml	CSF	(−80 °C)	
5HIAA				
5-CH_3-THF				
DHPR		Erythrocytes from heparinized blood	Frozen (−20 °C)	
		Guthrie card	RT	
		Fibroblasts	RT	
	Min. 50 mg	Chorionic villi	Frozen (−80 °C)	
PTPS	Before medication, no BH_4	Erythrocytes from heparinized blood	frozen (−20 °C)	
	Min. 50 mg	Chorionic villi	Frozen (−80 °C)	
GTPCH		Fibroblasts	RT	
SR		Fibroblasts	RT	

RT room temperature

1.11 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
	CV	I
1.4	Amniotic fluid, liver, erythrocytes, amniocytes, DNA	II
	CV	I

1.12 DNA Analysis

DNA analysis is possible for PAH and all BH_4 deficiencies

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

1.13 Treatment

PAH or BH4 deficiency present either with or without HPA. In those presenting with HPA (**1.1**–**1.5**; see below), the main goal of treatment is to reduce or normalize blood phenylalanine levels without causing deficiencies of other amino acids and other nutrients that are usually received by intake of natural intake (Belanger-Quintana et al. 2011). This can be done by introduction of the low-phenylalanine (or low-natural protein) diet; in some patients administration of the synthetic cofactor BH4 can relax or even replace the diet (Keil et al. 2013). In such cases it is important to be sure that patients use normal amounts of natural protein and not causing deficiencies of amino acids.

In PAH deficiency, one of the areas that need consensus is whether to start treatment at blood phenylalanine levels above 360–600 µmol/l (van Spronsen et al. 2011). Treatment targets have not reached consensus yet, but for the first decade, more or less every center will try to keep phenylalanine below 360 µmol/l or even lower (Blau et al. 2010). Very recent data even show that outcome is even better when phenylalanine is kept below 240 µmol/l (Jahja R et al., J Pediatr, in press). The next issue to decide on is the search for BH4-responsive patients. To this end we refer to paragraph **1.7**. All three existing methods (i.e., DNA, 7–24 days BH4

loading test (Levy 2007), and the 48-h BH4 loading test (Blau et al. 2011; Anjema et al. 2013)) have their own considerations. For all tests, long-term data are necessary to proof the predictive correctness of the method. Other issues that need attention are the amount of total protein (natural protein plus amino acids), giving extra tyrosine in case of low tyrosine concentrations (especially in case of pregnancy), and the question how strict treatment should be during adulthood (especially in males and in females after reproductive age).

One should be aware that there are individuals with "severe" PKU mutations that have escaped severe mental retardation despite high blood phenylalanine levels and very poor dietary control. One explanation for this phenomenon is that they have near normal brain phenylalanine levels, despite high blood phenylalanine levels. A number of studies have now demonstrated considerably variability in blood versus brain phenylalanine levels in PKU patients. Outcome in PKU appears to be related to both blood and brain phenylalanine levels. This, in all probability, will assume greater importance in making decisions about the strictness and duration of dietary control in the future.

In BH4 deficiencies, the mode of treatment depends on the type of disease, may differ with the patient's age, and the policies in various countries and centers (Opladen et al. 2012). In addition, patients with HPA due to a cofactor defect need more strict blood phenylalanine control and additional supplementations with neurotransmitter precursors L-dopa and 5-hydroxytryptophan in a combination with the peripheral decarboxylase inhibitor carbidopa. Patients with dihydropteridine reductase deficiency (DHPR, **1.4**) need additional folinic acid substitution. In patients revealing levodopa-induced peak-dose dyskinesia, slow-release forms of drugs can be used, and reaching the upper therapeutic limits of L-dopa may be an indication for the use of MAO and/or COMT inhibitors.

Patients with dopa-responsive dystonia (DRD, dominant GTPCH cyclohydrolase I deficiency, **1.6**) and sepiapterin reductase deficiency (SR, **1.7**) respond to low dosage L-dopa/carbidopa therapy and patients with SR deficiency need additional supplementation with 5-hydroxytryptophan and probably also BH4 (Friedman et al. 2012).

Prognosis and outcome strongly depend on the age when the diagnosis is made and treatment introduced but also on the type of mutation (Jäggi et al. 2008).

Recommendations for treatment and monitoring are not completely uniform worldwide. Therefore, where possible and necessary, recommendations have been combined and ranges of values indicating lower and upper limits are reported.

1.1 Phenylalanine hydroxylase deficiency (PKU)

Age	Protein requirement (g/kg BW/day)[a]	*Phe tolerance* (mg/day)	Target blood Phe (µmol/l)				*Phe-free AAM*	
			Germany	Netherlands	UK	USA	Type	g/day[b]
0–3 months	2.1–2.7	~130–400	40–240	120–360	120–360	120–360	1	3–10
4–12 months	2.1–2.0	~130–400	40–240	120–360	120–360	120–360	1	3–10
1–2 years	1.7	~130–400	40–240	120–360	120–360	120–360	2	20–50
2–3 years	1.7	~200–400	40–240	120–360	120–360	120–360	2	20–50
4–6 years	1.6	~200–400	40–240	120–360	120–360	120–360	2	20–50
7–9 years	1.4	~200–400	40–240	120–360	120–480	120–360	2	20–50
10–12 years	1.1	~350–800	40–900	120–360	120–480	120–360	2	50–90
13–15 years	1.0	~350–800	40–900	120–600	120–700	120–600	2	50–90
>16 years	0.9	~450–1,000	40–1,200	120–600	120–700	120–900	3	60–150

Protein requirement for PKU diet is assigned higher than recommendations for healthy people since bioavailability of amino acid mixtures is equivalent to natural protein

[a]DGE 1985; RDA; WHO

[b]Spread as evenly as possible through the 24 h

Non-PKU Hyperphenylalaninemia

Treatment only necessary for pregnant women with blood Phe levels >250–360 µmol/l (see below). Clinical monitoring is advised for all patients with Phe >360 µmol/l

Tetrahydrobiopterin (BH4)-Responsive PKU/HPA

There are no specific recommendations for the treatment of this group of HPA. The following table summarizes the current knowledge based on several experimental trials.

Age	Protein requirement g/kg BW/day	Phe tolerance mg/day	Target blood Phe µmol/l	mg BH4/kg BW[a]
All ages	See 1.1	Near normal	See 1.1	5–20

[a]This applies to BH4 responsive PKU, but there it is not necessary to distribute in >1 time daily (usually); no long-term clinical experience; BH_4 tablets contain (per 100 mg BH_4) 100 mg ascorbic acid

Maternal PKU/HPA

Trimester	Protein requirement g/kg BW/day	Phe tolerance mg/day	Target blood Phe µmol/l	Phe-free AAM	
				Type	g/day[a]
1	1.1	~180–1,600	120–360	3	60–150
2–3	1.3–1.5	~180–1,600	120–360	3	60–150

[a]Spread as evenly as possible through the 24 h

1.2 GTP cyclohydrolase I deficiency and 1.3 6-Pyruvoyl-tetrahydropterin synthase deficiency (severe form)

No.	Symbol	Age	Medication/diet	Dosage (mg/kg/day)	Dosages per day
1.2	GTPCH	Newborn	L-Dopa	1–3	3–6
1.3	PTPS (severe)		Carbidopa	10–25 %[a]	3–6
			5-Hydroxytryptophan	1–2	3–6
			Tetrahydrobiopterin (BH4)	5–10	3
		<1–2 years	L-Dopa	4–7	3–6
			Carbidopa	10–25 %[a]	3–6
			5-Hydroxytryptophan	3–5	3–6
			Tetrahydrobiopterin (BH4)	5–10	2
		>1–2 years	L-Dopa	8–15	3–6
			Carbidopa	10–25 %[a]	3–6
			5-Hydroxytryptophan	6–9	3–6
			Tetrahydrobiopterin (BH4)	5–10	2

[a]Percentage compared to L-dopa

1.3 6-Pyruvoyl-tetrahydropterin synthase deficiency (mild form)

No.	Symbol	Age	Medication/diet	Dosage (mg/kg/day)	Dosages per day
1.3	PTPS (mild)	All ages	Tetrahydrobiopterin (BH4)[a]	5–10	2

[a]BH_4 tablets contain (per 100 mg BH_4) 100 mg ascorbic acid

Beware/Pitfalls

1. Patients are on an unrestricted (i.e., protein-rich) diet.
2. BH_4 may significantly reduce plasma and CSF tyrosine levels. Consider nutrition and tyrosine supplementation.
3. L-Dopa/carbidopa/5-hydroxytryptophan therapy should be introduced slowly but continuously increased according to the clinical picture in steps of 1 mg/kg/day per week. 5-Hydroxytryptophan may not be tolerated due to gastrointestinal side effect. In these cases monotherapy with L-dopa/carbidopa may be sufficient.
4. L-Dopa/carbidopa/5-hydroxytryptophan therapy may reduce CSF folates (CH_3-group trapping by L-dopa to 3-*O*-methyl-dopa). Determine 5-methyltetrahydrofolate in CSF. Consider folinic acid (5-formyltetrahydrofolate, leucovorin) substitution (10–20 mg/day).
5. Drugs like trimethoprim-sulfamethoxazoles or methotrexate may induce hyperphenylalaninemia by inhibiting DHPR.

1.4 Dihydropteridine reductase deficiency

No.	Symbol	Age	Medication/diet	Dosage (mg/kg/day)	Dosages per day
1.4	DHPR	Newborn	L-Dopa	1–3	3–6
			Carbidopa	10–25 %[a]	3–6
			5-Hydroxytryptophan	1–2	3–6
			Folinic acid	15–20 mg/day	1–2
			Diet (see **1.1** PKU)		
		<1–2 years	L-Dopa	4–7	3–6
			Carbidopa	10–25 %[a]	3–6
			5-Hydroxytryptophan	3–5	3–6
			Folinic acid	15–20 mg/day	1–2
			Diet (see **1.1** PKU)		
		>1–2 years	L-Dopa	8–15	3–6
			Carbidopa	10–25 %[a]	3–6
			5-Hydroxytryptophan	6–9	3–6
			Folinic acid	15–20 mg/day	1–2
			Diet (see **1.1** PKU)		

[a]Percentage compared to L-dopa

Beware/Pitfalls

1. Patients are on low-Phe diet (see Table 1.1); however, blood Phe levels should be close to normal. These patients are more sensitive to high Phe levels than other PKU.
2. L-Dopa/carbidopa/5-hydroxytryptophan therapy should be introduced slowly but continuously increased according to the clinical picture in steps of 1 mg/kg/day per week. 5-Hydroxytryptophan may not be tolerated due to gastrointestinal side effect. In these cases monotherapy with L-dopa/carbidopa may be sufficient.
3. Drugs like trimethoprim-sulfamethoxazoles or methotrexate may induce hyperphenylalaninemia by inhibiting DHPR.

1.5 Pterin-4a-carbinolamine dehydratase deficiency

No.	Symbol	Age	Medication/diet	Dosage (mg/kg/day)	Dosages per day
1.5	PCD	Newborn	Tetrahydrobiopterin (BH4)[a]	5–10	2
		>1 years	No treatment[b]		

[a]BH_4 tablets contain (per 100 mg BH_4) 100 mg ascorbic acid
[b]Due to the possible development of diabetes type MODY3-like, periodic control necessary

Beware/Pitfalls

1. Patients are on an unrestricted (i.e., protein-rich) diet.
2. BH4 may significantly reduce plasma and CSF tyrosine levels. Consider tyrosine supplementation.
3. Drugs like trimethoprim-sulfamethoxazoles or methotrexate may induce hyperphenylalaninemia by inhibiting DHPR.

1.6 Dopa-responsive dystonia/autosomal dominant GTPCH deficiency

No.	Symbol	Age	Medication/diet	Dosage (mg/kg/day)	Dosages per day
1.6	DRD	Newborn	L-Dopa	1–3	3–4
			Carbidopa	10–25 %[a]	3–4
		>1 years	L-Dopa	4–12	3–4
			Carbidopa	10–25 %[a]	3–4

[a]Percentage compared to L-dopa

Beware/Pitfalls

1. L-Dopa/carbidopa therapy should be introduced slowly but continuously increased according to the clinical picture in steps of 1 mg/kg/day per week.

1.7 Sepiapterin reductase deficiency

No.	Symbol	Age	Medication/diet	Dosage (mg/kg/day)	Dosages per day
1.7	SR	Newborn	L-Dopa	1–3	3–4
			Carbidopa	10–25 %[a]	3–4
			5-Hydroxytryptophan	1–2	3–4
		>1 years	L-Dopa	4–10	3–4
			Carbidopa	10–25 %[a]	3–4
			5-Hydroxytryptophan	3–9	3–4

[a]Percentage compared to L-dopa

Beware/Pitfalls

1. L-Dopa/carbidopa/5-hydroxytryptophan therapy should be introduced slowly and increased in steps of not more than 1 mg/kg over days/weeks.

Alternative therapies/experimental trials

No.	Deficiency symbol	Age	Medication	Dosage (mg/kg/day)	Dosages per day
1.1	BH4-responsive PKU	>4 years	BH4[a]	10–20	1–2
1.3–1.5	GTPCH, PTPS, DHPR	All ages	Deprenyl[b]	0.1–0.3	3–4
			Entacapone[c]	~30	1–2
1.7	SR	All ages	Deprenyl[b]	0.07–0.14	3–4
			Sertraline[d]	0.71–2.14	2–3
			Melatonin[e]	0.01–0.03	1–2
			Bromocriptine	Not reported	Not reported

[a]Tetrahydrobiopterin (BH_4; sapropterin dihydrochloride, Kuvan®) treatment has been recently introduced as a standard therapy for children with phenylalanine hydroxylase deficiency that showed a decrease of Phe levels after BH_4 loading (see above)
[b]MAO-B inhibitor (Selegiline)
[c]COMT inhibitor
[d]Serotonin reuptake inhibitor
[e]Product of serotonin metabolism

Beware/Pitfalls

1. Administration of MAO-B or COMT inhibitors allows a 30 % reduction of the daily dosage of neurotransmitter precursors.

1.14 Follow-Up/Monitoring

1.1. PAH deficiency

Age	Biochemical monitoring Phe and Tyr	Clinical monitoring[a]	Intellectual and personality development
0–3 months	Weekly	1–3 monthly	
4–12 months	Weekly	1–3 monthly	X
1–2 years	Weekly	2–6 monthly	
2–3 years	Weekly	2–6 monthly	X
4–6 years	Fortnightly	3–6 monthly	X
7–9 years	Fortnightly	6 monthly	
10–12 years	Monthly	6 monthly	X
13–15 years	Monthly	6 monthly	X
Adolescents/adults	Monthly	6–12 monthly	X
Maternal PKU	Weekly[b]	Bimonthly[c]	

[a]Nutrient intake, body growth, general health, as well as laboratory tests: blood count, calcium, phosphate, magnesium, iron, liver and kidney function tests, alkaline phosphatase, total protein, albumin, prealbumin, cholesterol, triglycerides, vitamins
[b]Plasma AA, albumin, cholesterol, ferritin, folate, vitamin B12
[c]Nutrient intake including micronutrients, body growth, general health

Standard protocol for intercurrent illness

Aim the best possible intake of fluid, energy, and Phe-free AAM, with special attention for higher need of energy, while taking AAM in these periods may be a real.

1.2–1.7. BH_4 deficiencies

Plasma Phe and Tyr are monitored in all forms of HPA, CSF investigations only in disorders affecting BH_4 metabolism with and without HPA (**1.2**–**1.7**).

Test	Age	Frequency	Comments
Phe and Tyr (blood)	1–3 years	Weekly to fortnightly	Phe levels: 40–240 (360) µmol/l[a]
	4–10 years	Fortnightly to monthly	
	11–16 years	Monthly	Phe levels: 40–900 µmol/l[a]
	>16 years	Every 2–3 months	Phe levels: 40–1,200 µmol/l[a]
Neopterin Biopterin 5HIAA HVA Folates (CSF)[b]	<1 month	Fortnightly	Close to normal range
	1 month–1 years	Every 4–8 weeks	Close to normal range
	<1 years	Monthly to yearly	Close to normal range
Glucose (P)	>5 years	Yearly	Only in PCD-deficient patients

[a]In DHPR-deficient patients, Phe levels should be close to 240–360 µmol/l at all ages
[b]Lumbar puncture in the morning before medication. Discard first 0.5 ml and collect the next 1–2 ml (−80 °C)

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