

Minireview

Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency, state of the art

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Abstract

Since 1999 an increasing number of patients with phenylalanine hydroxylase (PAH) deficiency are reported to be able to decrease their plasma phenylalanine (Phe) concentrations after a 6R-tetrahydrobiopterin (BH₄) challenge. The majority of these patients have mild PKU or MHP (mild hyperphenylalaninemia) and harbour at least one missense mutation in the PAH gene associated with this phenotype. The rate of decrease and the lowest achieved Phe level vary between patients with different genotypes but appears to be similar in patients with the same genotype. A number of the mutations associated with BH₄-responsiveness have been studied in an 'in vitro' eukaryotic cell expression system leading to biosynthesis of a mutant PAH enzyme with some residual activity. Patients bearing mutations that cause severe structural distortion in the expressed protein (loss of function mutations), leading to undetectable PAH activity, are not responsive to BH₄. These observations suggest that residual PAH activity (in vitro) is a prerequisite for BH₄-responsiveness. However, an in vitro residual PAH activity is not a guarantee for in vivo BH₄-responsiveness. Mechanisms behind this responsiveness could be relieve of decreased binding affinity for BH₄, BH₄-mediated increase of PAH gene expression or stabilization of the mutant enzyme protein by BH₄. BH₄-responsive PAH-deficient patients have only been reported since 1999. For the western countries this is explained by the fact that the manufacturer changed the diastereoisomeric purity of the BH₄ preparation from 69% of the natural 6R-BH₄ (31% of 6S-BH₄) to 99.5% 6R-BH₄. The new findings on BH₄-responsiveness may be of clinical relevance because these patients can be treated with BH₄ with concomitant relief or withdrawal of the burdensome PKU diet. These observations warrant further clinical studies to assess efficacy, optimal dosage, and safety of BH₄ treatment in this group. The data strongly emphasize the necessity of the BH₄ loading test in patients detected in the newborn PKU screening. © 2002 Elsevier Science (USA). All rights reserved.

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Introduction

Phenylalanine hydroxylase (PAH, EC 1.14.16.1) deficiency leading to mild or severe phenylketonuria (PKU) is one of the most common autosomal recessive inborn errors of metabolism with an average incidence of 1/10.000 [1]. PAH is the rate-controlling enzyme of phenylalanine homeostasis. Phenylalanine, an essential amino acid, is converted to tyrosine in the liver by PAH requiring 6(R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄) as cofactor. Untreated PKU leads to mental retardation whilst early dietary treatment (phenylala-

nine restriction) prevents development of mental retardation. PAH deficiency (McKusick 261600) is associated with a wide range of phenotypes for which various classifications are used. Recently, Guldberg and co-workers [2] proposed a classification system for PKU patients based on the data of a European multi-centre study and assigned 105 pathologic mutations in the PAH gene to four arbitrary phenotype categories (Table 1). Otherwise, PKU can be classified on basis of serum Phe levels on unrestricted nutrition: classic PKU (Phe > 1200 μmol/L), mild PKU (Phe 600–1200 μmol/L), and mild HPA (Phe < 600 μmol/L) [3].

Detection of hyperphenylalaninemia (HPA) is included in the newborn screening programs of most western countries. Primary HPA can be either due to a

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Table 1
Classification of PKU phenotypes [2]

PKU phenotype	Phe tolerance (mg Phe/kg body weight/day)	mg Phe/day
Classic PKU	<20	250–350
Moderate PKU	20–25	350–400
Mild PKU	25–50	400–600
MPH	Normal diet	

diminished activity of PAH or deficiency of its cofactor BH₄. The latter group comprises defects in either the biosynthesis or in the regeneration of BH₄. Screening for BH₄ deficiency is generally performed by analysis of pterins in urine and measurement of dihydropteridine reductase (DHPR) activity in erythrocytes or skin fibroblasts [4]. Additionally, in several countries, either a BH₄ loading test or a combined Phe/BH₄ loading test is included in the work-up of HPA [4,5]. So far, BH₄-responsiveness suggested a defect in the biosynthesis or regeneration of BH₄ and prompted further investigations. However, recently, several groups have reported BH₄-responsiveness in patients with defects in PAH, confirmed by detection of pathologic mutations in the PAH gene, and normal BH₄ biosynthesis and regeneration ([6–16], http://data.mch.mcgill.ca/pahdb_new/, Curators' Page).

These findings have established the existence of a new sub-group of PAH-deficient patients who potentially can be treated with BH₄.

BH₄-responsive patient's characteristics

At the EMG workshop in Zurich, June 1st, 2002, BH₄-responsiveness in PAH deficiency is arbitrarily defined as a decrease in plasma Phe of more than 30% of the value before the BH₄ challenge (20 mg/kg body weight) within maximally 24 h post-load [17]. BH₄ deficiency should be ruled out and pathologic mutations in the PAH gene should be established.

BH₄-responsiveness reported in the literature has been assessed in different ways. Newborns have usually been tested using the standard BH₄ loading test [4], however, different doses of BH₄ have been used (10 or 20 mg/kg). In some cases a treatment trial with BH₄ has been carried out. Table 2 lists the phenotype, genotype, way of testing, and percentage of decrease in Phe level in blood after BH₄ loading of BH₄-sensitive PAH-deficient patients known to us (our centre, personal communications and literature). With regard to the rate of Phe decrease and the lowest Phe level achieved, BH₄-responsiveness appears to vary between patients with different genotypes. Fig. 1 shows the time course of the plasma Phe concentration in combined Phe/BH₄ loading tests in 4 MPH patients (own data). These patients can be classified as complete responders because their Phe

levels decreased rapidly to quite normal levels within 8–21 h after BH₄ administration. In some of the reported BH₄-responsive patients Phe levels appear to decrease significantly, but do not reach normal concentrations [6,9]. They can be classified as partial responders.

In combined Phe/BH₄ loading tests the possibility of spontaneous elimination of plasma Phe has to be considered. It was reported that after a Phe load of 100 mg/kg in two patients with non-PKU HPA plasma Phe levels returned to pre-load values within 24–48 h [18]. MPH patients who were investigated in our department by a combined Phe/BH₄ (69% 6R-BH₄, 31% 6S-BH₄) loading test in the years before 1999 did not show any or only a slight decrease of plasma Phe within 8 h after the BH₄ challenge. Furthermore, in a Phe challenge test (100 mg/kg) in our first BH₄-responsive patient no spontaneous decrease of plasma Phe was noticed within 6 h post-load [7]. Because these tests did not last more than 8 h, decreases of Phe in 24 h lasting combined Phe/BH₄ tests may be partly due to spontaneous Phe elimination.

Data on BH₄-responsiveness in genotypically identical patients are limited. Three patients with the genotype A300S; A403V (Fig. 1, 30), two patients with the genotype R241C; R413P [6] and two with E390G; IVS12nt^{1g→a} ([29], Scriver, personal communication) showed however, a similar BH₄ response. A different response in mild PKU patients with the same genotype has been published [9] but the patients appeared to be tested with different BH₄ preparations (see below). Another pitfall in interpretation of results may be caused by inadequate intestinal resorption of the administered BH₄. Most patients have mild or moderate phenotype and harbour at least one missense mutation associated with MPH, mild PKU or moderate PKU, though some classic PKU patients have been reported to be BH₄-responsive [15,16,19]. A number of PAH mutations have been studied by in vitro expression analysis, resulting in expression of mutant PAH protein with more or less enzymatic activity ([21]; http://data.mch.mcgill.ca/pahdb_new/).

BH₄-responsiveness has not been found in any patient harbouring two null mutations in either homoallelic or heteroallelic state (Fig. 2). The observations suggest that some residual PAH activity (in vivo) is a prerequisite for BH₄-responsiveness and that mutations causing severe structural distortion (truncation) in the expressed protein, leading to undetectable PAH activity, are not likely to be stimulated by BH₄. However, in vitro expression of mutant PAH enzyme with residual activity does not guarantee BH₄-responsiveness. Two patients with moderate PKU and homozygous for the I65T mutation appeared to be non-responsive to BH₄ (Fig. 2; patient from our centre and patient from Dr. M.A. Vilaseca, Spain, personal communication). The I65T missense mutation, associated with an inconsistent

Table 2

BH₄-responsive PAH-deficient patients: phenotype, genotype, way of testing (treatment), and BH₄ effect on plasma Phe level

Phenotype ^b	Genotype	Test/treatment ^a		Effect of BH ₄ decrease Phe (%) T8 (24) h	Ref
		Phe mg/kg	BH ₄ mg/kg		
MHP	A300S; A403V	100	20	92 (87)	[7]
MHP	R241C; A403V	100	20	94 (87)	[7]
MHP	A300S; A403V	100	20	87	A
MHP	V245A; G272X	100	20	75	A
MHP	V245A; R261Q	100	20	90 (93)	A
MHP	P407S; R252W		10	58	[6]
MHP	A373T; IVS4 ^{-1g→a}		10	60	[6]
MHP	R241C; R413P		10	38	[6]
MHP	R241C; R413P		10	34	[6]
MHP	A403V; A395P		n.s.	n.s.	[11]
MHP	T380M; D151E		3.5 ^a	n.s.	[14]
MHP	P211T; P211T		5–10 ^a	n.s.	[15]
MHP	S110L; P281L		10 ^a	n.s.	[15]
MHP	S110L; P281L		10 ^a	n.s.	[15]
MHP	A403V; G346fs		20	83	[30]
MHP	A403V; A300S		20	80	[30]
MHP	A403V; IVS4 ^{+5g→t}	100	20	89 (70)	[30]
Mild PKU	A313T; L367fsinsC		20	n.s. (90)	[7]
Mild PKU	V190A; R243X		20	44	[7]
Mild PKU	R158Q; A300S	100	20	57 (77)	A
Mild PKU	R68S; R68S		20	79	B
Mild PKU	Y414C; E280K		20	64	C
Mild PKU	R408Q; R408Q	100	20	41	C
Mild PKU	Y414C; Del 194		20	88	[10]
Mild PKU	E390G; IVS10 ^{-11g→a}		20	92	[8]
Mild PKU	Y414C; R408W		20	55	[9]
Mild PKU	L48S; L48S		20	83	[12]
Mild PKU	E390G; IVS12nt ^{1g→a}		0.7 ^a	n.s.	[29]
Mild PKU	A104D; K320N		5–10 ^a	n.s.	[11]
Mild PKU	Y414C; Y414C		10–20 ^a	n.s.	[11]
Mild PKU	A395P; IVS12nt ^{1g→a}		10 ^a	n.s.	[13]
Mild PKU	D129G; R408W		10 ^a	n.s.	[15]
Mild PKU	E390G; IVS12nt ^{1g→a}		20	n.s. (70)	E
Mild PKU	Y414C; IVS3nt ^{-22g→a}		20	71	[27]
Mild PKU	Y414C; A104D		20	58	[27]
Mild PKU	R243Q; Y414C		20	36	[30]
Mild PKU	R261Q; E390G		20	69	[30]
Mild PKU	A403V; IVS10 ^{-11g→a}		20	87	[30]
Mild PKU	A403V; IVS10 ^{-11g→a}	100	20	64 (85)	[30]
Mild PKU	R261Q; I65T		10 ^a	n.s.	[13]
Mild PKU	R261Q; R243X		10	69	D
Classic PKU	R261Q; R158Q		10	39	D
Classic PKU	R261Q; V388M		10	41	D
Classic PKU	P281S; P281S		10	48	D
Classic PKU	Y414C; R408W		20 ^a	n.s.	[15]
Classic PKU	Y414C; R252W		20 ^a	n.s.	[15]

MHP, mild hyperphenylalaninemia not requiring diet; PKU, phenylketonuria; n.s., not stated.

Sources: A, own patients; B, C, D, E, personal communications, respectively: R.C.A. Sengers, T.J. de Koning, M.A. Vilaseca, C.R. Scriver.

^a Daily dose of BH₄ in treatment trial.

^b The phenotype is either adopted from the reference or based on the pre-treatment plasma Phe concentration [3].

phenotype [2], leads to a PAH enzyme with considerable residual activity (http://data.mch.mcgill.ca/pahdb_new/). Recently, the I65T mutation has been reported to be associated with BH₄-sensitivity ([13], quoted in [12,19]). However, the BH₄-responsive patient in this report was heterozygous for this mutation, the other being R261Q which most probably is associated with BH₄-respon-

siveness (Tables 2 and 3). Other patients with mild PKU are reported to be non-sensitive [17,20]; one of them with the genotype: R176P; P281L.

Missense mutations found in homozygous state or in heterozygous state in combination with a functionally hemizygous null mutation can be assigned being associated with BH₄-responsiveness. So far 20 mutations can

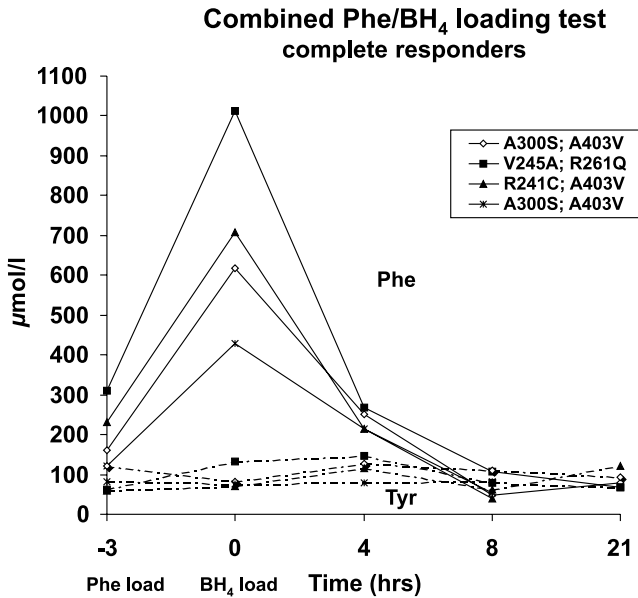


Fig. 1. Courses of plasma Phe and Tyr concentrations in combined Phe/BH₄ loading tests in four complete responsive MHP patients with the indicated genotypes. At T0 100 mg/kg Phe was administered followed by 20 mg/kg 6R-BH₄ 3 h later. During the test, patients maintained an unrestricted diet.

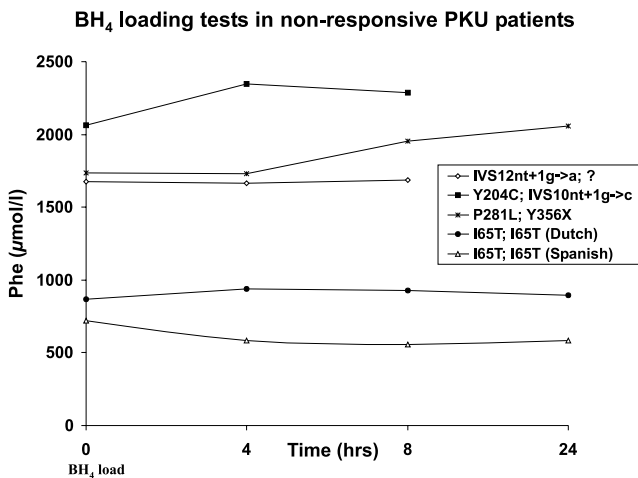


Fig. 2. Courses of plasma Phe concentrations during a challenge with 6R-BH₄ (20 mg/kg) in non-responsive PKU patients with the indicated genotypes. During the test, patients maintained an unrestricted diet.

be positively classified as BH₄-sensitive. The other 10 missense mutations have been found in heterozygous combinations (Table 3). These observations strongly suggest that BH₄-responsiveness is primarily dependent on the nature of the PAH mutations but other factors may be involved as well.

BH₄-responsiveness: possible mechanisms

The underlying mechanisms of BH₄-sensitivity in PAH-deficient patients remain to be elucidated. Three possible mechanisms are proposed:

Table 3

Identified BH₄-sensitive mutations in the PAH gene

Mutation ^a	PAHact % wildtype ^d	Positive identification (H or N)	Mutation ^b	PAHact % wildtype ^d
F39L ^c	46	N	del I94 ^c	n.d.
L48S	39	H	A104D	18–16
R68S	18–39	H	D151E	n.d.
S110L	n.d.	N	R158Q	10
D129G	n.d.	N	E280K	0–12
E178G ^c	n.d.	N	A300S	n.d.
V190A	n.d.	N	L308F ^c	n.d.
P211T	72	H	K320N	n.d.
R241C	25	N	T380M	n.d.
V245A	39–63	N	V388M	23–43
R261Q	24–72	N		
P281S	n.d.	H		
A313T	n.d.	N		
A373T	n.d.	N		
E390G	70	N		
A395P	16	N		
P407S	n.d.	N		
R408Q	55	H		
Y414C	28	H		
A403V	32	N		

n.d., not determined.

^aPositively identified to be associated with BH₄-responsiveness because of presence in homozygous state (H) or in combination with a functionally hemizygous null mutation (N).

^bMild mutations present in hemizygous state in combination with another missense mutation in BH₄-responsive PAH-deficient patients; association with BH₄-responsiveness to be confirmed.

^cData from [16].

^dPhenylalanine hydroxylase database (http://data.mch.mcgill.ca/phaedb_new/).

Alleviation of a disturbance in BH₄ binding to the PAH enzyme

A diminished binding affinity for BH₄ in the mutant enzymes as suggested by several authors [6–8] may play a role if mutations are located in or near the cofactor-binding regions (CBR). In their structural hypothesis for BH₄-responsiveness Erlandsen and Stevens [22] suggested that BH₄-sensitive mutations mapped onto the catalytic domain of the PAH gene may be located either in the CBRs or in regions that interact with secondary structures in the protein involved in cofactor binding. The mutations V245A, R261Q, E280K, and P281S are located in the reported CBR #1 and CBR #2. Mutation A300S is supposed to change the shape of the cofactor-binding site (CBR #1) probably leading to decreased binding affinity for BH₄ [22]. These mutations would result in mutant enzymes that are K_m variants that are still able to bind the cofactor to some extent. BH₄ supplementation in these cases may augment the L-Phe hydroxylation reaction by the mutant PAH enzyme.

Regulation of PAH gene expression

Blau and Trefz [12] suggested that the BH₄-responsiveness in their patient, harbouring mutations (L48S;

L48S) in the N-terminal regulatory domain of the gene, might be caused by a BH₄-mediated increase of PAH gene expression. It has been reported, recently, that BH₄ regulates tyrosine hydroxylase and phenylalanine hydroxylase gene-expression in a GTP-cyclohydrolase/BH₄-deficient *hph-1* mouse [23].

Stabilization of the PAH enzyme

BH₄ may enhance the stability of mutated homo- and heteropolymeric PAH enzyme molecules in cases of homozygous or hemizygous missense mutations of which one or both are located in the C-terminal tetramerization domain (e.g., Y414C). In patients with functionally hemizygous heteroallelic genotypes (missense/null) the null mutation may result in a severely truncated PAH monomer which has lost capacity of formation of homo- and heteropolymeric enzyme molecules. In this case only homopolymeric PAH molecules in which one amino acid is substituted will be formed. The stability and/or the activity of the latter mutated enzyme may be enhanced by BH₄.

BH₄-responsiveness: new 6R-BH₄ versus old 6R,S-BH₄ preparation

The recent finding of BH₄-responsive PAH-deficient patients has raised an important question: why are BH₄-responsive PAH-deficient patients detected only since 1999?

For the European patients the answer is most probably found in the purity of the BH₄ preparation. Before 1999 the BH₄ tablets of Dr. Schircks Laboratories (Jona, Switzerland) were composed of 69% of the natural 6R-BH₄ and 31% of 6S-BH₄. Since 1999 the diastereoisomeric purity of the BH₄ preparation has been improved to 99.5% 6R-BH₄. The (6R)-L-erythro-dihydroxypropyl side chain of the natural cofactor is critical for many aspects of the regulation of the PAH enzyme. The affinity of 6R-BH₄ to rat liver PAH appears to be 2–3 times higher than of the unnatural 6S-BH₄ [24]. It is reported however, that 6S-BH₄ causes an irreversible inactivation of rat liver PAH [25,26]. Extrapolating these findings to the human PAH makes it quite conceivable that the mixture of the 6R- and 6S-epimer (69:31) would not have enhanced in vivo PAH activity in potentially 6R-BH₄-responsive PAH-deficient patients.

This change in preparation might be the reason why Lindner et al. [9] found a different BH₄-response in patients with the same genotype (two out of three patients were tested before 1999, personal communication).

Evaluating BH₄-responsiveness retrospectively in a large series of PKU patients can lead to an underestimation of the number of BH₄-responsive patients if this change in preparation is not taken into account and patients tested before 1999 with the less pure BH₄ are

included [27]. Already in 1992, 6R-BH₄ (~100%, Sun-
tary) was introduced in Japan and the first HPA-patient with a slow and incomplete response was reported at the annual meeting of the Japan Society of Inborn Errors of Metabolism (1992). No abnormalities in biopterin metabolism were found. Mutations in the PAH gene were only detected in 1998 (Prof. Y. Matsubara, personal communication; no abstract in English). The dose of 6R-BH₄ used (7.5 mg/kg) may have been too small to detect most of the responsive patients (Dr. M. Yoshino, personal communication). The mentioned effect of the change in purity of the BH₄ preparation should lead to consideration of (re-)testing patients diagnosed before 1999.

BH₄-responsiveness and clinical relevance

The clinical implication of the discovery of BH₄-sensitive PAH deficiency is the opening to a new treatment strategy for a sub-group of PKU patients. In principle this PKU variant is treatable with BH₄ with concomitant relief or withdrawal of the burdensome PKU diet. Such a simplification of treatment will improve the quality of life for a considerable number of PKU patients. The group of BH₄-responsive PKU patients seems to be quite large. Searching our database with genotyped MHP/PKU patients (partly published in [28]) revealed that 42 out of 85 PAH-deficient patients bear one or two of the mutations associated with BH₄-sensitivity from Table 3. In a retrospective study of 1730 MHP/PKU patients 278 were tested with 6R-BH₄, the others with 6R,S-BH₄. Of the patients tested with 6R-BH₄ responsiveness was noticed in approximately 70% of the MHP/mild PKU patients, 25% of moderate PKU patients and 10% of classic PKU patients. Among the others tested with 6R,S-BH₄ the number of responsive patients appeared to be low [19]. The authors considered a decrease in plasma Phe of 5% between 0 and 4 h and between 4 and 8 h post-BH₄ load to be positive and a decrease of at least 30% to be significant positive. The possibility that a 5% decrease in Phe concentration may well be within the analytical imprecision of the Phe measurement was not discussed by the authors.

With the present insights the clinical relevance of BH₄-responsiveness is limited to PKU patients who require dietary treatment.

There is limited expertise about treatment of these patients with BH₄. One of the first BH₄-responsive patients has been treated with 5 mg BH₄/kg/day for approximately 3 years now, without Phe restriction ([7], T.J. de Koning, personal communication). Her plasma Phe levels are continuously around 130 μmol/L. No side effects have been noted. Reports on successful treatment of mild PKU patients appeared from Trefz et al. [8] who supplemented a mild PKU patient with 10 mg BH₄/kg/day. Under this regimen the Phe levels dropped from

934 $\mu\text{mol/L}$ to values between 84 and 222 $\mu\text{mol/L}$. Their patient is developing normally. Bonafé et al. [14] reported on a mild PKU patient who presented with neurologic impairment. This patient showed both a clinical and a biochemical response on supplementation with 3.5 mg $\text{BH}_4/\text{kg/day}$. Lindner et al. [9] succeeded in treating a mild PKU patient with 12–15 mg $\text{BH}_4/\text{kg/day}$ divided in three doses, resulting in blood Phe levels constantly between 240 and 360 $\mu\text{mol/L}$. Another German group has reported on two infants with mild PKU who were treated with 10 mg $\text{BH}_4/\text{kg/day}$ without protein restriction or PKU formulas. This treatment appeared sufficient to keep plasma Phe concentrations below 343 $\mu\text{mol/L}$. Both infants developed normally [11]. A Swiss patient with mild PKU is supplemented with 50 mg BH_4/day leading to Phe levels remaining below 200 $\mu\text{mol/L}$ [10]. Finally, a 25-year-old American woman with mild HPA, presenting with disabling depression and panic attacks appeared to be responsive to BH_4 supplementation (100 mg/day). Without protein restriction her blood Phe levels decreased from 630 $\mu\text{mol/L}$ to values between 360 and 480 $\mu\text{mol/L}$. Maintenance BH_4 dosage of 100 mg/day has resulted in significant improvement of depression and panic attacks, with discontinuation of psychotropic medication [29]. However, Weglage et al. [27] reported three patients who were responsive after a loading test with BH_4 but who did not respond on ongoing BH_4 treatment.

In order to assess BH_4 -responsiveness the data strongly emphasize the necessity of a 24-h lasting BH_4 -loading test in patients detected in the newborn PKU screening. However, if a combined Phe/ BH_4 test is performed and found positive, an additional Phe-loading test (100 mg/kg) is strongly recommended in order to assess the spontaneous elimination of plasma Phe during 24 h after administration of Phe. The presented observations compel to further clinical studies to assess efficacy, optimal dosage and safety of BH_4 supplementation in this group of patients.

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