

Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency: Diagnosis, treatment, genetics, and international BIOPKU database

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Introduction

Tetrahydrobiopterin (BH₄)-responsive phenylalanine hydroxylase (PAH) deficiency is a recently recognized variant of hyperphenylalaninemia (HPA) caused by specific mutations in the *PAH* gene and characterized by a positive BH₄ loading test. HPAs can be divided into two groups: 1) those due to a defective apoenzyme PAH (1), and 2) those due to a deficient cofactor BH₄ (BH₄ deficiencies) (2). The spectrum of HPAs caused by PAH deficiency ranges from the mild HPA (MHP), to mild phenylketonuria (PKU), and classical PKU. Patients with BH₄-responsive PAH deficiency belong mainly to the groups of MHP and mild PKU.

Historically, patients in which whom BH₄ deficiency was excluded, but who responded to the oral loading test with BH₄ by lowering their plasma phenylalanine (Phe) levels were have been observed for many years and Niederwieser and Curtius suggested that this their responses may be due to the *Km* mutant in the *PAH* gene (3). However, until the publication by Kure et al. (4) not much attention was paid to these phenomena. They investigated four patients with HPA who responded to the loading test with 10 mg BH₄/kg body weight. In all of them BH₄ defects were excluded and mutations detected in the *PAH* gene suggested a novel subtype of PAH deficiency. Subsequently Trefz et al. (5) introduced BH₄ therapy in a similar patient with PAH deficiency (E390G/IVS10nt-11g>a) who responded to 20 mg BH₄/kg by lowering plasma Phe concentrations from initially 885 μmol/L to 67 μmol/L after 8 hours after loading. Under normal diet and 10 mg/kg of BH₄ blood Phe levels remained in this patient remained between 84 and 222 μmol/L. Further patients characterised as BH₄-responsiveness were described by Spaapen et al. (6) and Lindner et al. (7). In these patients, the response to BH₄ was variable and one patient with the same genotype (R408W/Y4414C) did not respond at all. This inconsistency within the same genotype group was most probably due to a non-standardized BH₄ loading test. Some of these patients were tested with the older BH₄ product containing 1/3 of the inactive isomer, thus with ~30% lower amount of the active BH₄. During the last two years a number of additional cases were published (8-17), and different mechanisms for the phenotype were proposed. Based on the location and proximity of particular mutations to the BH₄-binding site in the three-dimensional structure of PAH, a hypothesis of *Km* variants (with a lower binding affinity for BH₄) was proposed (18). However, some mutations found in patients with BH₄-responsive HPA/PKU are not located within the BH₄-binding site, and mechanisms such as stabilization of the protein or introduction of 3D structural changes in the PAH protein were suggested. BH₄ may also act as a chaperon and in some patients with

homozygous mutations (L48S/L48S) it may increase enzyme activity by inducing the gene expression (19, 20). The fact that the BH₄-responsive phenotype is quite common among patients with HPA, as demonstrated by our group (21), and that more than 60% of patients with MPH or mild PKU may benefit from BH₄ substitution (22) warrants further careful investigations of the etiology of BH₄-responsiveness.

Laboratory diagnosis

Diagnosis is straight forward and starts with the newborn screening for HPA (Guthrie test or tandem mass-spectrometry, TMS). All positive newborns (plasma Phe >120 μmol/L) as well as older HPA/PKU children, previously not tested, need to be investigated for BH₄ defects (urinary pterins and dihydropteridine reductase activity in blood spots) (23). For many years the BH₄ loading test was considered to be an practical additional tool to discriminate between cofactor defects and PKU (24). Now we know that this test discriminates only between BH₄-responders and non-responders, but can not distinguish between BH₄ deficiency and PAH deficiency alone (Figure 1). The following protocol proposed at the European Metabolic Group Workshop in Zürich (25) is recommended to detect patients with BH₄-responsive HPA/PKU:

Loading test with BH₄ (20 mg/kg body weight)

Only 6R-BH₄ should be used. 6R-BH₄ is available as tablets (Schircks Laboratories, Jona, Switzerland, 10 or 50 mg) with ascorbic acid (10 or 50 mg) plus N-acetyl-cysteine (5 or 25 mg) or as a granules (0.4 g, Daiichi Suntory, Japan) with ascorbic acid plus cysteine.

Initial plasma Phe levels should be > 400 μmol/L. Food (Phe) intake should be continuous during the test. Procedure: Dissolve BH₄ tablets in 20 ml water, orange juice, or infantile formula by gently mixing in dim light (BH₄ is light- and oxygen- sensitive). Administer the suspension (20 mg/kg) within 30 minutes and after at least 3 hours of fasting. Older children and adults may swallow the tablets un-dissolved.

The Phe and tyrosine should be measured in plasma or blood before, 4, 8, and 24 hours after administration of BH₄. Urine should be collected before and 4-8 hours after BH₄ administration in order to control intestinal BH₄ adsorption. The same urine sample is used to exclude BH₄ deficiency.

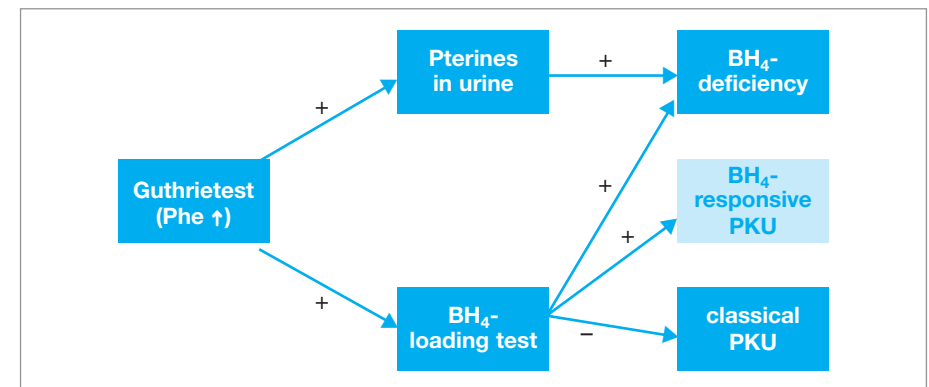


Figure 1: The diagnostic flow-chart for differentiation of HPA variants.

The BH₄ loading test is considered positive when initial plasma Phe concentrations decrease by at least 30% after 8 hours. Another criterion to differentiate between responders and non-responders is to calculate the "hydroxylation rate" as suggested by Bernegger and Blau (21). They calculated the percentage of Phe elimination between 0 and 4 h and between 4 and 8 h after loading and calculated the slope. Thus "hydroxylation rates" S of at least 30% between 0 and 4 h and between 4 and 8 h were considered to be significantly positive (S ≥ 3.75). When using the above protocol for the oral loading test, 60-70% of patients with MPH and mild PKU responded significantly (Figure 2).

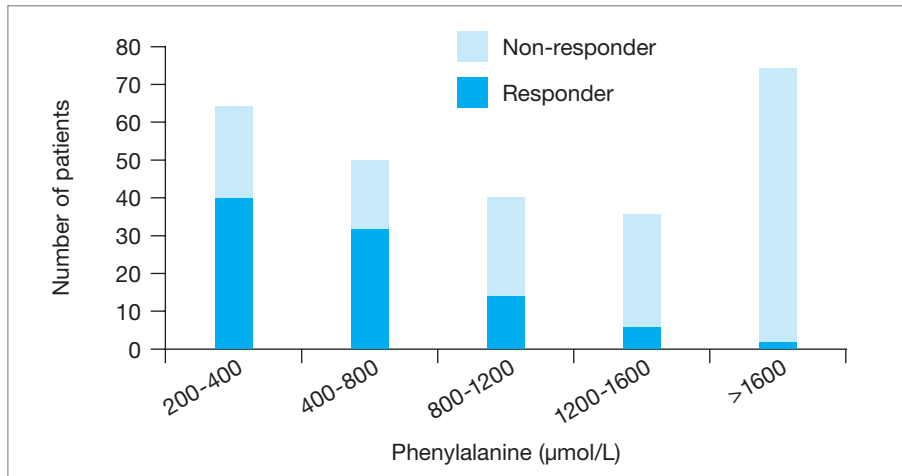


Figure 2: Patients with different degree of HPA who responded to BH₄ loading test (20 mg/kg body weight) by lowering blood phenylalanine levels by at least 30% within 8 hours. Modified from Bernegger et al. (21).

Figure 3 shows, that the efficiency of BH₄ to reduce plasma Phe levels down to the therapeutic range (360 μmol/L) depends on the initial plasma levels and the amount of BH₄ administered. Patients, who were found to be slow responders with "hydroxylation rates" < 3.75 need to be investigated for more than 24 hours with repeated administration of 10 mg BH₄/kg/day. Using this extended protocol H. Shintaku. (personal communication) was able to detect and treat additional patients with mild or moderate PKU (slow responders).

When interpreting the BH₄ loading test one should consider a few important facts. BH₄ is adsorbed mostly in the duodenum and the jejunum and less in the stomach and adsorption may differ with the age. Furthermore, great inter- and intra-individual variations occurs with regard to the maximal BH₄ plasma levels and the half-life time (26). For some patients the 8-hours protocol may be optimal, for others with a shorter BH₄ half-life time longer protocols (24 h) or higher BH₄ doses (2 x 10-20 mg/kg) may be necessary. A typical BH₄ and total biopterin plasma BH₄ after administration of 10 mg BH₄ per kg body weight are shown in Figure 4.

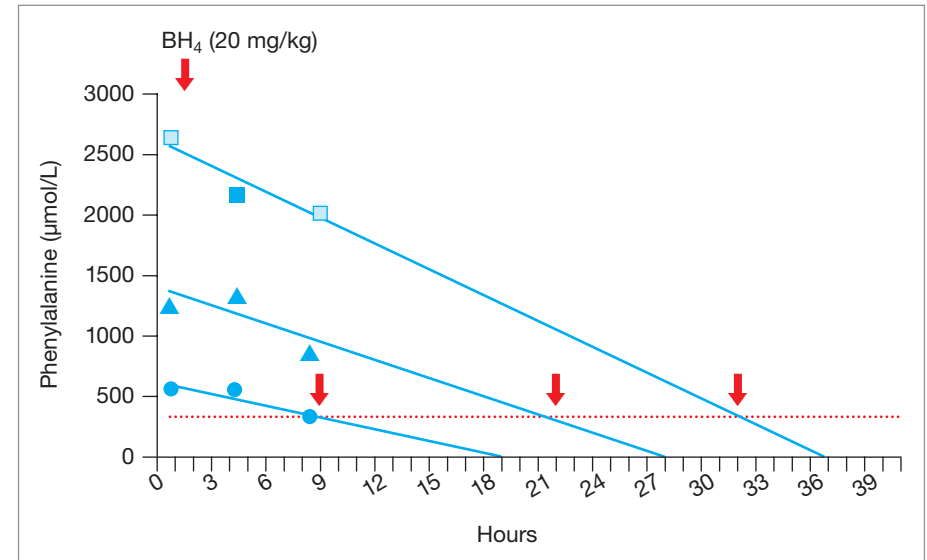


Figure 3: BH₄ loading test in three patients with different degree of HPA but with similar slopes (S). Arrows indicates the time needed to reach the phenylalanine levels of 360 μmol/L. Modified from Bernegger et al. (21).

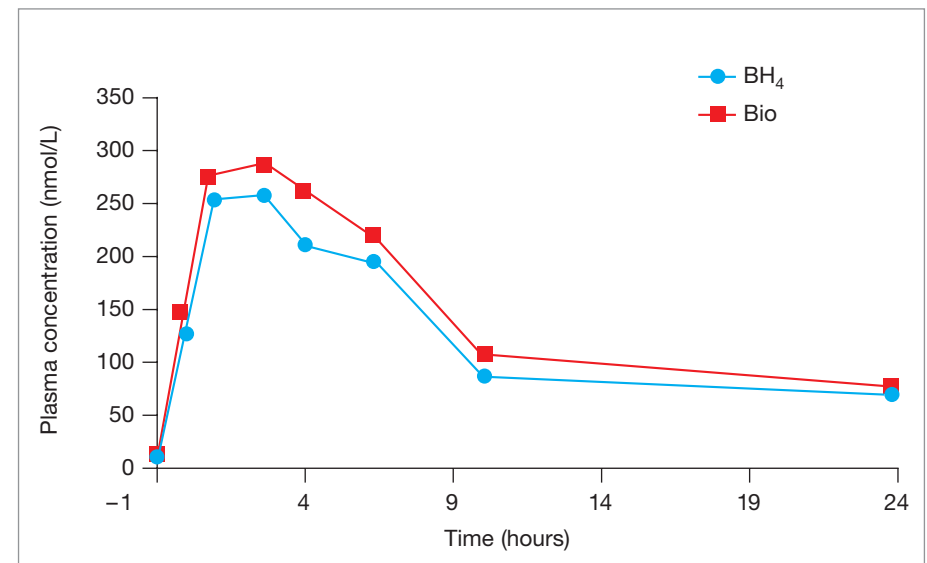


Figure 4: BH₄ and total biopterin profile in plasma of a person loaded with BH₄ (10 mg/kg).

Combined Phe (100 mg/kg) and BH₄ (20 mg/kg) loading test

This protocol is used in patients with plasma Phe levels <400 μmol/L or in children, who are on the diet. The procedure is the same as described for the single BH₄ loading test except that Phe (100 mg/kg) is administered 3 hours before BH₄ and there is one additional blood sampling (25). Thus blood sampling is done at 0, 3, 7, 11, and 27 hours.

Muntau et al. (12) modified this test by administration of BH₄ one hour after Phe loading. Blood Phe and tyrosine were measured before Phe loading and 4, 8, and 15 h after BH₄ loading. Although this test is useful, one should take into account, that plasma Phe peaks 3 hours after the challenge and that a portion of the administered Phe is not metabolized in the liver, but rather eliminated via other routes. Under ideal conditions one would need to perform both the combined Phe/BH₄ as well as a single Phe (100 mg/kg) loading test.

More accurate is the *in vivo* analysis of Phe hydroxylation with and without BH₄ (10 mg/kg) after oral administration of L-[¹³C]-Phe (6 mg/kg) (12). The recovery of carbon-13 in breath was measured and calculated for residual Phe hydroxylation. Using this method 87% of patients with MHP or mild PKU were found to respond to BH₄ by lowering Phe levels by at least 30% after 15 hours. Furthermore, this test confirms the hypothesis, that impaired Phe hydroxylation can be corrected by BH₄.

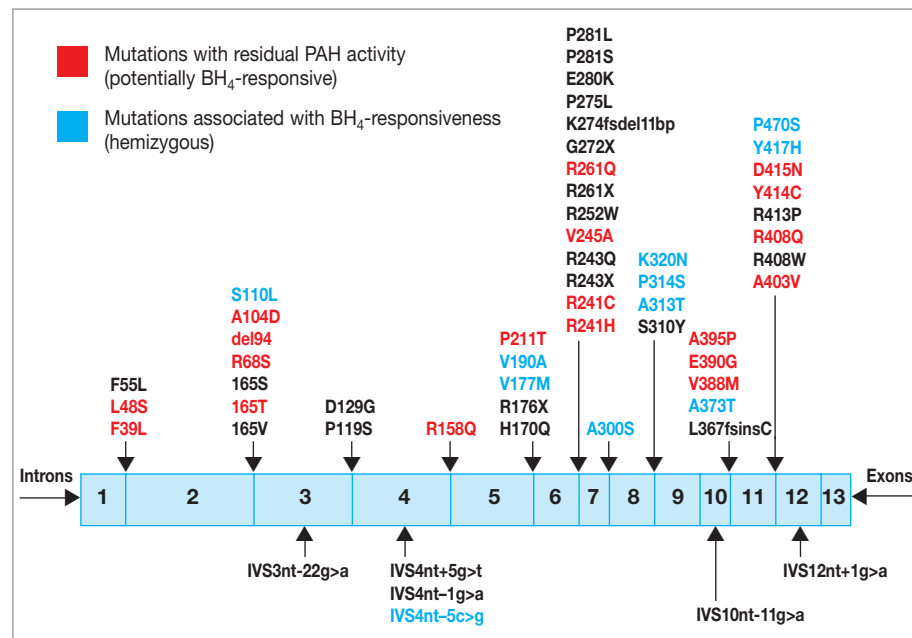


Figure 5: PAH mutations map. Mutations detected in patients with BH₄-responsive PAH deficiency. Source: BIOPKU database (www.bh4.org).

Genotypes

Figure 5 summarizes mutations detected in patients with BH₄-responsive HPA/PKU.

A total of 56 mutations, most of them in the compound heterozygous state, were described in 75 patients and about 50% of them were detected in more than one allele (27). The Y414C mutation is the most common one (19 alleles), followed by R408W (10 alleles), R261Q (9 alleles), A300S and A403V (7 alleles each), and IVS12nt+1g>a (6 alleles). The complete list of mutations is available from the BIOPKU database (27). Within the mutations described, some were expressed recombinantly in eukaryotic cell systems or *E. coli* and found to have substantial residual activity (Figure 6). About 67% of all mutations are located in the catalytic domain of PAH, 25% in the regulatory domain, and 8% (4 mutations) are located in the tetramerization domain. Only very few of the described mutations are located within the two cofactor-binding regions CBR1 (V245A, R252W, R261X, R261Q) and CBR2 (P281S, P281L). Based on the present knowledge about the regulative properties of the cofactor BH₄ and substrate phenylalanine, the following mechanisms were suggested as a possible causes for BH₄-responsiveness: 1) *K_m* mutants with reduced affinity for BH₄, 2) stabilization of the PAH dimer by the chaperon activity of BH₄, 3) 3-dimensional structural changes, and 4) induction of PAH expression by BH₄ (12, 18, 19).

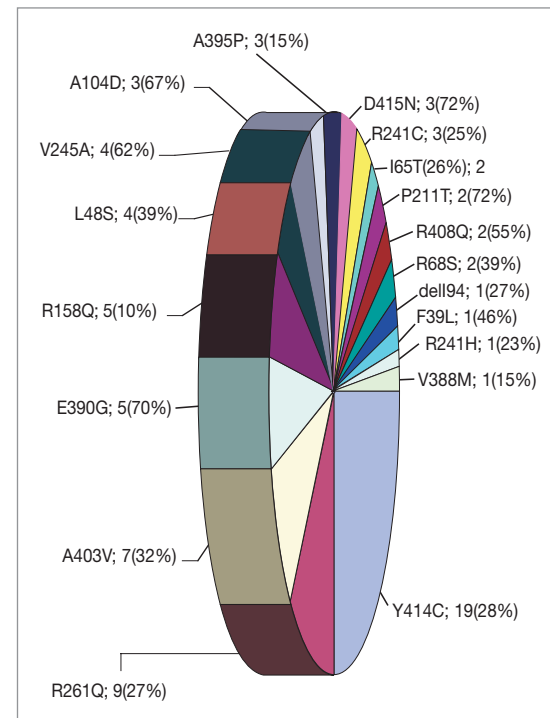


Figure 6: Frequency of mutations detected in patients with BH₄-responsive PAH deficiency. Mutation designation; number of alleles (residual activity when expressed recombinantly in cell systems).

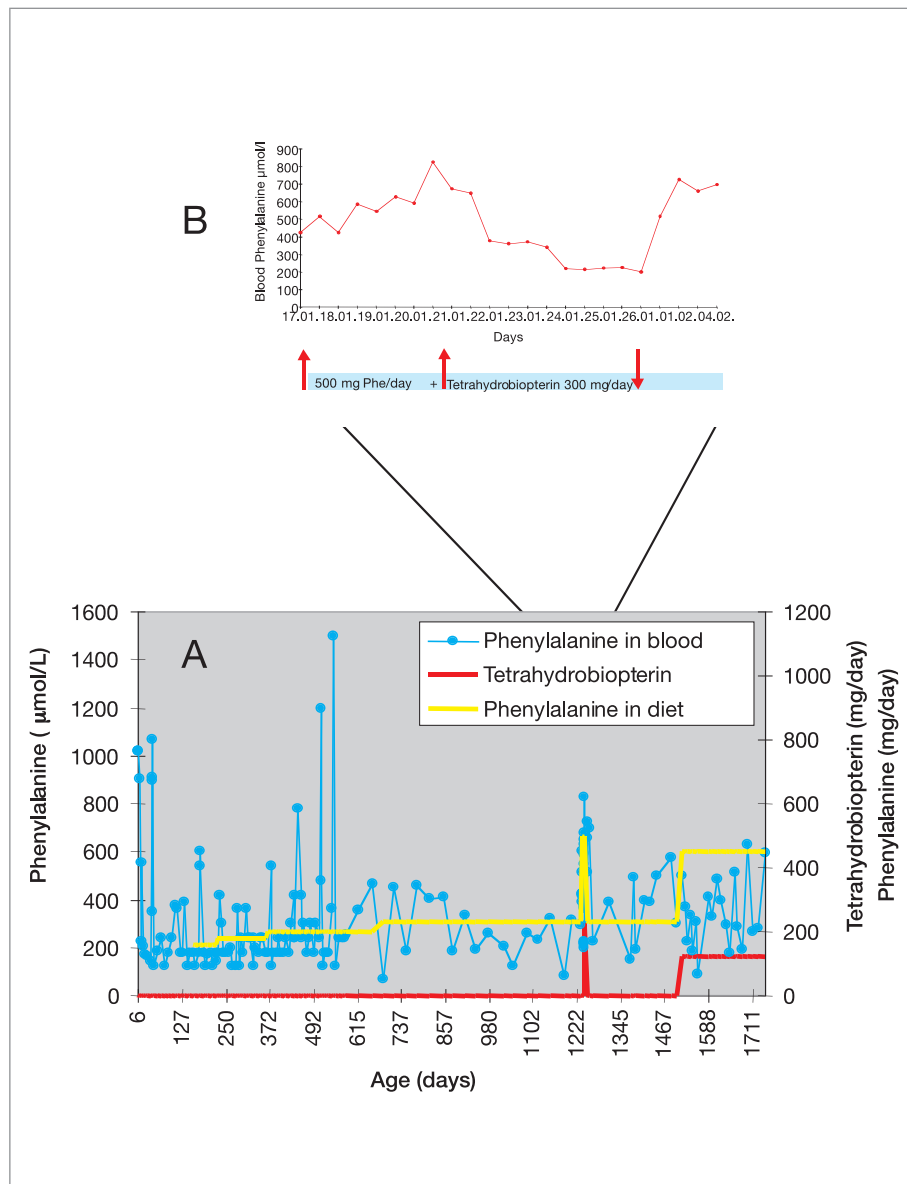


Figure 7: Combined BH₄-low-Phe treatment in a patient with mild PKU. Plasma phenylalanine, BH₄-dosage, and daily phenylalanine intake during the long-term follow-up (A). Change in phenylalanine tolerance at the age of 3.5 years (B).

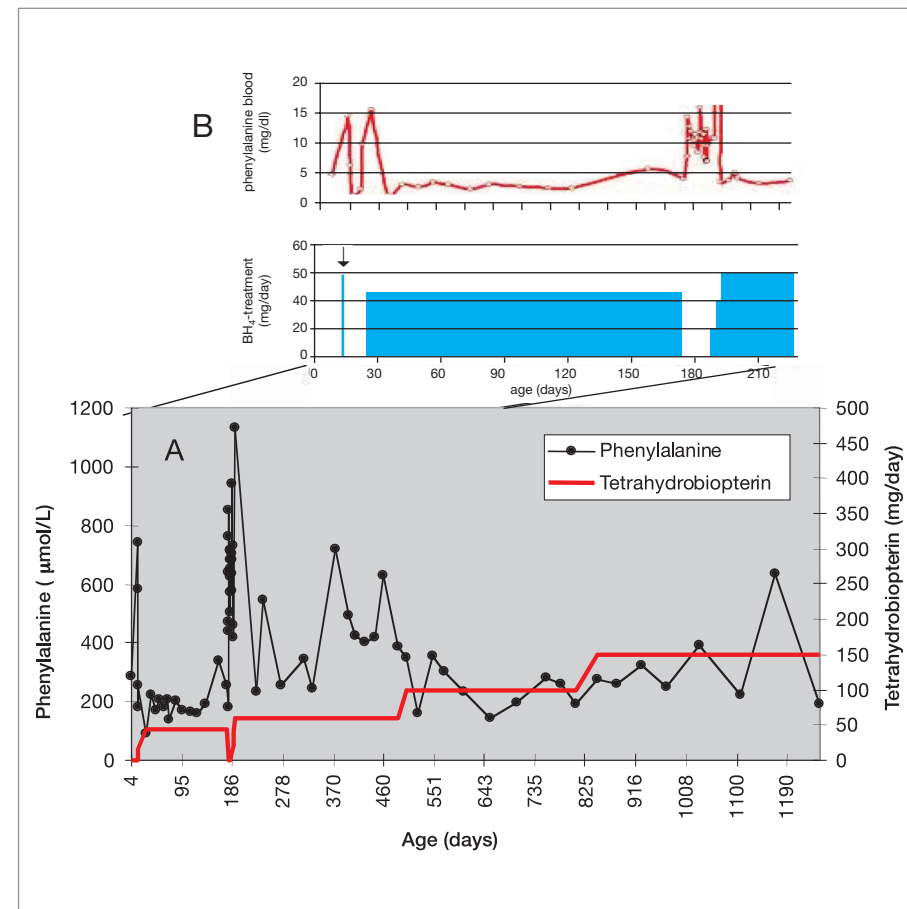


Figure 8: Long-term therapy with BH₄ in a patient with mild HPA (A). Plasma phenylalanine and BH₄ dosage during the first year of life (B).

Treatment

There are only few reports on the long-term follow-up of HPA/PKU patients on treatment with BH₄ (5, 28). According to unpublished communications a number of patients with BH₄-responsive PAH deficiency are presently on different BH₄ treatment protocols, either as a monotherapy or in a combination with the low-phenylalanine or low-protein diet. The following two cases demonstrate the benefit of BH₄ substitution in patients with different forms of BH₄-responsive PAH deficiency:

Patient 1 (mild PKU, R408W/Y414C genotype) was initially investigated using an older, less active BH₄ product and found to be a non-responder. At the age of 3.5 years this patient was reinvestigated using more active 6R-BH₄ (20 mg/kg) and showed a clear decrease of plasma phenylalanine concentrations. Figure 7 shows the effect of combined BH₄ and diet therapy on the plasma phenylalanine levels. The patient was first allowed to relax the phenylalanine-restricted diet to an intake of 500 mg Phe/day, resulting in increased plasma phenylalanine levels. Administration of BH₄ (300 mg/day) for 5 days reduced the phenylalanine levels to less than 300 μmol/L. Therapy with BH₄ was initiated at the age of 4 years, resulting in increased phenylalanine tolerance (from 220 to 500 mg/day) and much better compliance.

Patient 2 (mild HPA, E390G/IVS10nt-11g>a genotype) was treated from the birth with BH₄ (Figure 8) (5). At the age of 6 months BH₄ supplementation was stopped resulting in an increase of higher blood phenylalanine levels. Since then the patient is on BH₄ (10 mg/kg/day), divided to three doses. The plasma phenylalanine levels were below 300 μmol/L and no adverse effects were observed. The patient is now 3 years old and developing normally.

Potentially, one third of mild HPA/PKU patients can be treated with BH₄. In addition, some pregnant PKU women may also benefit from BH₄ administration (22). Unfortunately, BH₄ is expensive and not available for all patients at low or no costs. Lack of well designed long-term studies with patients with BH₄-responsive HPA/PKU makes the registration of BH₄ as an orphan drug even more difficult. However, several long-term cross-over or double-blind studies are currently running in different countries and there is a hope, that BH₄ will be available for pharmacological therapy of the mild variant of PAH deficiency in the next 3-5 years.

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