

## LETTER TO THE EDITOR

## Tetrahydrobiopterin-Responsive Phenylalanine Hydroxylase Deficiency: Possible Regulation of Gene Expression in a Patient with the Homozygous L48S Mutation

To the Editor:

Tetrahydrobiopterin (BH<sub>4</sub>)-responsive phenylalanine hydroxylase (PAH) deficiency is a recently recognized variant of hyperphenylalaninemia (HPA) characterized by a positive response to a BH<sub>4</sub>-loading test (1). Up to the present time, 11 different mutations have been described which are associated with mild HPA (Table 1) and all patients have been shown to be compound heterozygotes (1–6). They respond to an oral challenge with BH<sub>4</sub> (10–20 mg/kg) in that plasma phenylalanine concentrations normalize within 4–8 h after administration and they can be treated with BH<sub>4</sub> monotherapy instead of a low-phenylalanine diet. It has been speculated that some of mutations in the *PAH* gene result in a *Km* variant of the PAH enzyme in which enhancement of the residual activity can be achieved by supplementation of BH<sub>4</sub>. These mutations, located in the DNA region encoding for the catalytic domain (exons 6 to 12) of the enzyme, may potentially alter the tertiary structure. Most of these mutations, when expressed recombinantly in eukaryotic cells, showed at least 25% residual activity (7). However, Lindner *et al.* (4) showed that BH<sub>4</sub>-responsiveness may differ between patients with the same genotype and suggested that the difference must be due to other as yet unknown factors. Only one out of three patients with the common R408W/Y414C mutations responded to the loading with 20 mg BH<sub>4</sub>/kg body weight.

Recently, a patient with a BH<sub>4</sub>-responsive PAH deficiency, whose plasma phenylalanine normalized within 8 h from 726 to 120 μmol/L after oral administration of BH<sub>4</sub> (20 mg/kg/day), was diagnosed at the Children's Hospital of Reutlingen. Surprisingly, he was found to be a homozygote for the Leu to Ser

substitution at codon 48 (L48S/L48S). This mutation is located in the regulatory domain (exons 1–5) of the *PAH* gene and thus differs from other previously described mutations in patients with BH<sub>4</sub>-responsive HPA. Lässker *et al.* (5) compared *PAH* mutations with their phenotypes and found that some missense mutations detected in BH<sub>4</sub>-responsive patients have been associated with inconsistent phenotypes. Thus, in addition to the possible *Km* vari-

**TABLE 1**  
Mutations in the *PAH* Gene Identified in Patients with BH<sub>4</sub>-Responsive PAH Deficiency

Allele 1	PAH enzyme activity <sup>a</sup>	Allele 2	PAH enzyme activity <sup>a</sup>	Reference
<u>L48S</u>	39	<u>L48S</u>	39	This study
<u>V190A</u>	nd	R243X	0	(3)
<u>A313T</u>	nd	L367fsinsC	0	(3)
<u>A300C</u>	nd	<u>A403V</u>	32	(3)
<u>R241C</u>	25	<u>A403V</u>	32	(3)
<u>E390G</u>	70	IVS10 <sup>-11g&gt;a</sup>	0	(2)
<u>R241C</u>	25	R111X	0	(1)
<u>P407S</u>	nd	R252W	<1	(1)
<u>P407S</u>	nd	R111X	0	(1)
<u>A373T</u>	nd	IVS4 <sup>-1g&gt;a</sup>	0	(1)
<u>R241C</u>	25	R413P	<3	(1)
<u>Y414C</u>	28	R408W	<3	(4)
<u>Y414C</u>	28	delI194	0	(6)
<u>A395P</u>	nd	IVS12 <sup>-11g&gt;a</sup>	0	(5)
<u>R261Q</u>	27	<u>I65T</u>	26	(5)

*Note.* Underlined mutations may be associated with BH<sub>4</sub>-responsive phenotype. Association with BH<sub>4</sub>-responsiveness was defined on the basis of the residual activity when expressed recombinantly and/or when the second allele was a nonsense mutation with the presumed 0 activity.

<sup>a</sup> In cell lysates (as % of wild type); nd, not done.

ant, additional factors may be responsible for the PAH activation by BH<sub>4</sub>.

We propose that BH<sub>4</sub> regulates PAH gene expression in patients with BH<sub>4</sub>-responsive PAH deficiency. The mutant L48S/L48S homotetramere enzyme may be activated by the excess of the BH<sub>4</sub> cofactor, similarly and as demonstrated in the GTP cyclohydrolase/BH<sub>4</sub>-deficient *hph-1* mouse model (8). BH<sub>4</sub> (200 mg/kg ip) increased the *hph-1* mouse PAH mRNA, enzyme activity, and protein levels by 50% within 30 min. Thus, BH<sub>4</sub> can regulate PAH gene expression and this new function may have significance in regard to the treatment of patients with mild PAH mutations.

These data also demonstrate the importance of a BH<sub>4</sub>-loading test in all patients with even mild HPA. Based upon our experience, tests should be performed with 20 mg BH<sub>4</sub>/kg body weight and plasma phenylalanine and tyrosine should be monitored at 0, 4, and 8 h. Because the response is rather slow in some patients, an additional blood sample at 12 h after or even 24 h post BH<sub>4</sub> administration is recommended. Treatment with BH<sub>4</sub> is a practical, but not inexpensive, alternative to the low-phenylalanine diet. One of our first patients with BH<sub>4</sub>-responsive PAH deficiency, now on 10 mg BH<sub>4</sub>/kg per day, is doing perfectly well (plasma phenylalanine 84–222 μmol/L), almost 2 years post diagnosis.

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