

## BRIEF COMMUNICATION

## Tetrahydrobiopterin Responsiveness in Phenylketonuria Differs between Patients with the Same Genotype

**Recently, BH<sub>4</sub>-responsive phenylalanine hydroxylase (PAH) deficiency was reported in patients with specific mutations in the PAH gene, and it was suggested that BH<sub>4</sub> responsiveness may be determined by the respective genotypes. We now report on three patients with PAH deficiency and the same genotype but different responses to standardized BH<sub>4</sub> loading. Our results suggest that BH<sub>4</sub> responsiveness in PAH deficiency is at least partly independent from PAH genotype.** © 2001 Academic Press

Hyperphenylalaninemia (HPA) is detected by newborn screening from dried blood spots in most western countries. About 1 in 5000 newborns in Germany are affected by this condition. Most patients with HPA suffer from the autosomal recessive disorder phenylketonuria (PKU, OMIM 261600), i.e., severe deficiency of the enzyme phenylalanine hydroxylase (PAH, EC 1.14.16.1). Wild-type PAH gene product is assembled to a tetramer of four identical subunits; more than 400 mutations in the PAH gene have been identified (phenylalanine hydroxylase locus knowledgebase, <http://www.mcgill.ca/pahdb>).

Treatment of phenylketonuria-PKU (phenylalanine (phe) > 600  $\mu\text{mol/L}$  on a normal diet) consists of a special diet poor in natural protein supplemented by synthetic amino acid mixtures free of phe. Mild forms of PAH deficiency with blood phe levels constantly <600  $\mu\text{mol/L}$  do not require dietary treatment (1).

The PAH reaction requires tetrahydrobiopterin (BH<sub>4</sub>) as a cofactor, and 1–3% of HPAs are caused by defects in the enzymes for synthesis or recycling of BH<sub>4</sub>. These conditions are treated with a different therapeutic regimen because BH<sub>4</sub> is an essential cofactor for other hydroxylases, notably in biogenic neurotransmitter biosynthesis (2).

To differentiate between PAH and cofactor deficiency an oral loading test with 20 mg BH<sub>4</sub>/kg body weight (bw) is recommended in all hyperphenylalaninemic newborns (3). No decline of phe levels is expected in PAH deficiency whereas in cofactor deficiency phe levels normalize within 4–8 h (3).

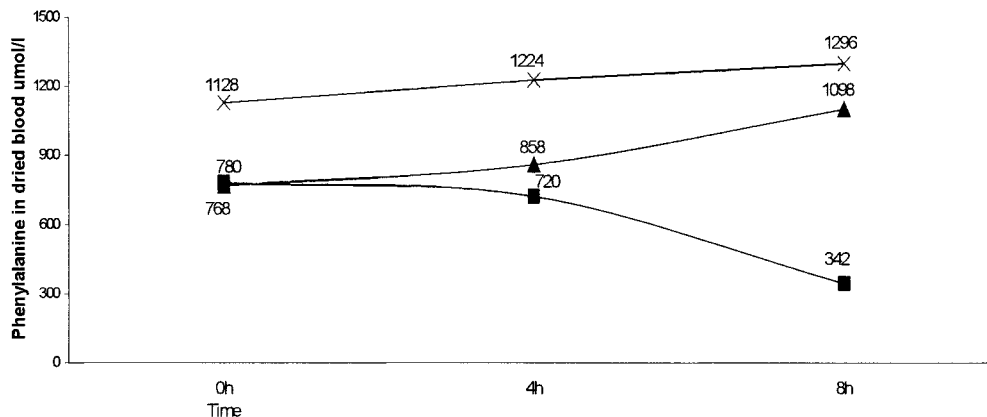
In 1999 Kure *et al.* (4) reported for the first time BH<sub>4</sub> responsiveness in four Japanese patients who were compound heterozygous for mutations in the PAH gene and did not have evidence of cofactor deficiency. A therapeutic effect was obtained with BH<sub>4</sub> in a dose of 10 mg/kg bw/24 h (4). Similar observations were reported by independent groups at the recent International Congress of Inborn Errors of Metabolism (5,6). It was suggested that BH<sub>4</sub> responsiveness was directly linked to specific mutations in the PAH gene. We now provide evidence that BH<sub>4</sub> responsiveness cannot be fully explained by specific PAH gene mutations but requires other, as yet unexplained, factors.

## PATIENTS AND METHODS

Three patients with HPA were identified by newborn screening from dried blood spots. All children were born to nonconsanguineous German parents and were not related to each other.

Oral BH<sub>4</sub>-loading test with 20 mg BH<sub>4</sub>/kg bw was performed at the age of 10–20 days after a 4-h fast. No dietary restrictions were made during the test and all patients were breastfed. Phe levels in dried capillary blood spots were measured immediately before and 4 and 8 h after the loading. Urine was collected for two 6-h intervals before and after the test, respectively.

Pterine and biogenic amine neurotransmitter analysis in CSF was performed as previously described (7,8).



**FIG. 1.** BH<sub>4</sub> loading with 20 mg/kg bw in patient 1 (■), 2 (▲), and 3 (×), all genotype Y414C/R408W. Detailed information is given in the text.

Mutations of the PAH gene were determined by DGGE and subsequent sequencing as previously described (9).

## RESULTS

**PAH genotype.** All patients are compound heterozygous for the same two common European mutations R408W and Y414C. Monoallelic double heterozygosity was excluded by analysis of parental DNA in all cases.

**BH<sub>4</sub> loading.** Phenylalanine levels before and after BH<sub>4</sub> loading are shown in Fig. 1. Patient 1 shows a marked decline in phe levels whereas phe increased in patients 2 and 3.

**BH<sub>4</sub> treatment.** Breast milk feeding without BH<sub>4</sub> supplementation in patient 1 led to a constant rise of blood phe levels to 720–960 μmol/L. This was reversed again by reintroduction of BH<sub>4</sub> at a dose of 12–15 mg/kg bw/day divided in three doses. Blood phe levels remained constant between 240 and 360 μmol/L under that treatment for several weeks.

**Pterin and biogenic amine neurotransmitter studies.** The analyses of pterines in urine and DHPR activity in erythrocytes gave normal results in all patients. Pterines and homovanillic and 5-hydroxyindolacetic acid in CSF were normal in patient 1 (data not shown).

## DISCUSSION

BH<sub>4</sub> responsiveness is a new observation in PAH deficiency. Patients previously reported carried uncommon mutations in the PAH gene, and in one

study, two patients with the same genotype both showed BH<sub>4</sub> responsiveness (4). It was concluded that BH<sub>4</sub> responsiveness is a novel subtype of PAH deficiency associated with distinct mutations in the PAH gene and explained either by stabilization of the specific mutant PAH tetramer (4) or by BH<sub>4</sub>-mediated counteraction of decreased cofactor affinity of specific mutant proteins (5).

We now report discordant BH<sub>4</sub> responsiveness in three patients compound heterozygous for the same, common mutations Y414C and R408W. Patient 1 responded to an oral load with 20 mg BH<sub>4</sub>/kg bw with a marked decline of blood phe concentrations to therapeutic levels. This was reproduced in a therapeutic trial with oral BH<sub>4</sub> over a period of several weeks. Patients 2 and 3, in contrast, did not show a decline of phe values in the BH<sub>4</sub> loading test despite normal BH<sub>4</sub> reabsorption and urinary excretion of pterin metabolites (data not shown). As all three patients share the same genotype, BH<sub>4</sub> responsiveness in PAH deficiency cannot be exclusively determined by specific PAH gene mutations and therefore the difference between our patients must be due to other as yet unknown factors. These may comprise intragenic differences (e.g., polymorphisms), modifier genes or nongenetic factors (10,11).

It is important to note that our patients as well as all previously described patients showed a mild PKU or MHP phenotype, i.e., considerable residual PAH enzyme activity. It is not likely that BH<sub>4</sub> responsiveness is mediated through an alternative pathway of phe metabolism but acts on mutant PAH enzymes with residual activity. In our patients the mild PKU phenotype is due to the presence of Y414C, the most common mild PKU mutation in

Northern and Western Europe (12). R408W, the second mutation in our patients, is a known null mutation that completely removes enzyme activity and that is associated with minimal amounts of PAH immunoreactive protein in *in vitro* expression studies (13).

In line with that explanation, BH<sub>4</sub> responsiveness in our patient 1 may probably be mediated through the production of a Y414C homotetramer that is stabilized by BH<sub>4</sub>. However, other factors that are present in some but not all patients are required to explain the discordant BH<sub>4</sub> responsiveness in our patients with identical genotype.

Cofactor substitution may be an alternative treatment strategy for a subgroup of patients with PKU, provided that long-term effectiveness can be shown and that BH<sub>4</sub> will be available at a reasonable price.

### ACKNOWLEDGMENTS

We are grateful to PD Dr. N. Blau, University Children's Hospital, Zürich, Switzerland, for the analysis of DHPR activity in dried blood and pterine analysis in Urin. Parts of this study are results of a project by Zschocke and Burgard funded by the German Research Foundation, ZS 17/2-1 und 2-2.

### REFERENCES

1. Burgard P, Bremer HJ, Bührdel P, Clemens PC, Mönch E, Przyrembel H, Trefz FK, Ullrich K. Rationale for the German recommendations for phenylalanine level control in phenylketonuria 1997. *Eur J Pediatr* **158**:46–54, 1999.
2. Kaufman S. Metabolism of the phenylalanine hydroxylation cofactor. *J Biol Chem* **242**:3934–3943, 1967.
3. Blau N, Thöny B, Heizmann CW, Dhondt JL. Tetrahydrobiopterin deficiency: From genotype to phenotype. *Pteridines* **4**:1–10, 1994.
4. Kure S, Hou D-C, Ohura T, Iwamoto H, Suzuki S, Sugiyama N, Sakamoto O, Fujii K, Matsubara Y, Narisawa K. Tetrahydrobiopterin-responsive phenylalanine hydroxylase deficiency. *J Pediatr* **135**:375–378, 1999.
5. Spaapen LJM, Bakker JA, Velter C, Loots W, Rubio ME, Forget PP, Duran M, Dorland L, Poll-The BT, van Amstel HK, Bekhof J, Blau N. Tetrahydrobiopterin-responsive hy-

perphenylalaninemia (HPA) in dutch neonates. *J Inherit Metab Dis* **23**(Suppl 1):45, 2000.

6. Trefz F, Blau N, Aulehla-Scholz C, Korall H, Frauendienst-Egger G. Treatment of mild phenylketonuria (PKU) by tetrahydrobiopterin (BH<sub>4</sub>). *J Inherit Metab Dis* **23**(Suppl 1):47, 2000.
7. Hyland K. Estimation of tetrahydro-, dihydro- and fully oxidised pterins by high-performance liquid chromatography using sequential electrochemical and fluorescence detection. *J Chromatogr* **343**:35–41, 1985.
8. Bräutigam C, Wevers RA, Jansen RJT, Smeitink JAM, Rijkvan Andel JF de, Gabrëels FJM, Hoffmann GF. Biochemical hallmarks of tyrosine deficiency. *Clin Chem* **44**:1897–1904, 1998.
9. Zschocke J, Hoffmann GF. Phenylketonuria mutations in Germany. *Hum Genet* **104**:390–98, 1999.
10. Scriver CR, Waters PJ. Monogenic traits are not simple: Lessons from phenylketonuria. *Trends Genet* **15**:267–72, 1999.
11. Dipple KM, McCabe ER. Phenotypes of patients with simple mendelian disorders are complex traits: Thresholds, modifiers and system dynamics. *Am J Hum Genet* **66**:1729–35, 2000.
12. Okano Y, Eisensmith RC, Dasovich M, Wang T, Güttler F, Woo SLC. A prevalent missense mutation in Northern Europe associated with hyperphenylalaninaemia. *Eur J Pediatr* **150**:347–335, 1991.
13. DiLella AG, Marvit J, Brayton K, Woo SLC. An amino-acid substitution involved in phenylketonuria is in linkage disequilibrium with DNA haplotype 2. *Nature* **327**:333–338, 1987.

M. Lindner<sup>1</sup>  
D. Haas  
J. Zschocke  
P. Burgard

*Division of Metabolic and Endocrine Diseases  
University Children's Hospital  
Heidelberg, Germany*

*Received November 30, 2000; published online April 24, 2001*

<sup>1</sup> To whom correspondence should be addressed at Division of Metabolic and Endocrine Diseases, University-Children's Hospital, Im Neuenheimer Feld 150, 69120 Heidelberg, Germany. Fax: +49-6221-56-4069. E-mail: martin\_lindner@med.uni-heidelberg.de.