

being treated with recombinant human  $\alpha$ -glucosidase.<sup>9</sup> The prolonged survival expected with this form of treatment may lead to the development of neurologic symptoms. Consequently, the problem of the transport of enzyme across the blood–brain barrier into the CNS may arise, as it has been experimented in the neuronopathic forms of Gaucher's disease.<sup>10</sup>

### Acknowledgment

The authors thank Dr. Afred Slonim and Dr. Eloisa Arbustini for helpful discussions concerning the clinical course of our patients and the neuropathologic results.

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## CME Treatable neurotransmitter deficiency in mild phenylketonuria

**Article abstract**—The authors describe a case of neurologic involvement in mild hyperphenylalaninemia (HPA), not due to tetrahydrobiopterin (BH<sub>4</sub>) deficiency, with low levels of monoamine neurotransmitter metabolites in CSF. The combined BH<sub>4</sub>-Phe loading test suggested a BH<sub>4</sub> response, confirmed by clinical improvement after BH<sub>4</sub> therapy. Molecular study revealed a compound heterozygosity of the phenylalanine hydroxylase alleles: a mild HPA-associated mutation (T380M) and the new mutation D151E. This case demonstrates that even mild HPA, generally considered a benign disorder, may present neurologic impairment.

NEUROLOGY 2001;57:908–911

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Hyperphenylalaninemia (HPA) can be caused either by a deficiency of phenylalanine hydroxylase (PAH) or by a defect of the metabolism of its cofactor tetrahydrobiopterin (BH<sub>4</sub>). Classic phenylketonuria (PKU) is characterized by mental retardation and severe neurologic symptoms, which can be prevented by early dietary treatment after detection of HPA in newborn screening. BH<sub>4</sub> deficiencies are characterized by neurologic symptoms related to monoamine

neurotransmitter deficiency, as BH<sub>4</sub> is the cofactor also of dopamine and serotonin biosynthetic enzymes. Mild forms of PAH deficiency are considered to be benign disorders that do not require any dietary treatment when plasma Phe concentrations are below 600  $\mu$ mol/L, and so far no neurologic complications have been described in subjects with these variants.<sup>1</sup>

**Case report.** A 13-year-old girl, born from nonconsanguineous parents after uneventful pregnancy and delivery, was considered normal at the neonatal metabolic screening because of the high cut-off for blood phenylalanine (240  $\mu$ mol/L) at that time. During infancy, psychomotor delay, motor difficulties, hypersalivation, and aggressivity were noticed. From the age of 5 years, episodes of gait disturbance (ataxia) and limb rigidity were reported. At the age of 11 years she was investigated in our unit: uncertain gait, hypersalivation, and mental retardation were present. Head circumference was 54 cm (90th percentile), consistent with body weight and height. Her hair color was

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Supported in part by the Swiss National Science Foundation, grant no. 31-54183.98 (N.B.), and by the Regione Veneto, Ricerca Sanitaria Finalizzata, grant no. 80803/98 (A.B.B.).

Received March 1, 2001. Accepted in final form April 27, 2001.

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**Table** Biochemical investigations in a patient with mild hyperphenylalaninemia

Investigation	Patient	Normal values
<b>Plasma</b>		
Phe ( $\mu\text{mol/L}$ )	22	26–98
Tyr ( $\mu\text{mol/L}$ )	47	19–119
<b>Urine</b>		
Neopterin (mmol/mol creat)	0.15	0.2–1.7
Biopterin (mmol/mol creat)	0.95	0.5–2.7
<b>CSF</b>		
HVA (nmol/L)	56	133–551
5-HIAA (nmol/L)	31	74–163
Neopterin (nmol/L)	11	9–20
Biopterin (nmol/L)	25	10–30
Phe ( $\mu\text{mol/L}$ )	43	<20
<b>Erythrocytes</b>		
DHPR activity (mU/gHb)	3.2	1.8–3.8
<b>Fibroblasts</b>		
Neopterin (pmol/mg)	82	18–98
Biopterin (pmol/mg)	208	154–303
GTPCH activity ( $\mu\text{U/mg}$ )	2.1	1.4–6.5
PTPS activity ( $\mu\text{U/mg}$ )	0.6	0.4–1.6
SR activity ( $\mu\text{U/mg}$ )	138	99–185
DHPR activity (mU/mg)	5	4.5–8.3

HVA = homovanillic acid; 5-HIAA = 5-hydroxy-indoleacetic acid; DHPR = dihydropteridine reductase; GTPCH = GTP cyclohydrolase; PTPS = 6-pyruvoyl-tetrahydropterin synthase.

brown, but was reported to be blond until the age of 6 years. Brain MRI and electrophysiologic studies (EEG, visual and auditory evoked potentials) were normal. Metabolic investigations, including plasma lactate/pyruvate, ammonia, urinary organic acids, plasma very long-chain

fatty acids, and urinary oligosaccharides, were normal. Mild HPA (table) was detected, with plasma Phe between 180 and 240  $\mu\text{mol/L}$  in several fasting measurements without any dietary restriction. The screening for  $\text{BH}_4$  defects (urinary pterins and erythrocyte dihydropteridine reductase activity) was normal (see the table). The oral Phe loading test (100  $\text{mg/kg}$ ) showed a clear increase of Phe (figure 1A), a slight increase of Tyr (figure 1B), and a marked increase of the Phe/Tyr ratio (figure 1C) and biopterin (figure 1D) in plasma. The combined  $\text{BH}_4$ -Phe loading test with 20  $\text{mg/kg}$  of  $\text{BH}_4$  orally 1 hour before Phe showed a marked increase in plasma Tyr (figure 2B) and a normal Phe/Tyr ratio (figure 2C). CSF investigation revealed low concentrations of homovanillic acid (HVA) and 5-hydroxy-indoleacetic acid (5-HIAA), elevated Phe, and normal pterins (see the table). Treatment with L-dopa (10  $\text{mg/kg/day}$ )/10% carbidopa, 5-OH-tryptophan (5  $\text{mg/kg/day}$ ), and tyrosine (20  $\text{mg/kg/day}$ ) resulted in improvement of the neurologic picture (ataxia and rigidity disappeared, hypersalivation was reduced, and attention improved); CSF HVA and 5-HIAA concentrations normalized. Subsequently,  $\text{BH}_4$  (3.5  $\text{mg/kg/day}$ ) was added to the therapy, resulting in further improvement of the motor skills and cognitive functions.

Neopterin and biopterin production and the activities of the  $\text{BH}_4$ -metabolizing enzymes (GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, sepiapterin reductase, and dihydropteridine reductase) in fibroblasts, measured as previously described,<sup>2</sup> were all in the normal range (see the table). DNA analysis, performed by denaturing gradient gel electrophoresis and direct sequencing, detected two mutations in the PAH gene: the previously known T380M, a mutation associated with mild HPA,<sup>3</sup> and a new mutation, D151E. No changes were found in the genes coding for GTP cyclohydrolase I and 6-pyruvoyl-tetrahydropterin synthase.

**Discussion.** Untreated classic PKU and  $\text{BH}_4$  deficiencies lead to severe neurologic impairment due to monoamine neurotransmitter deficiency. In  $\text{BH}_4$  de-

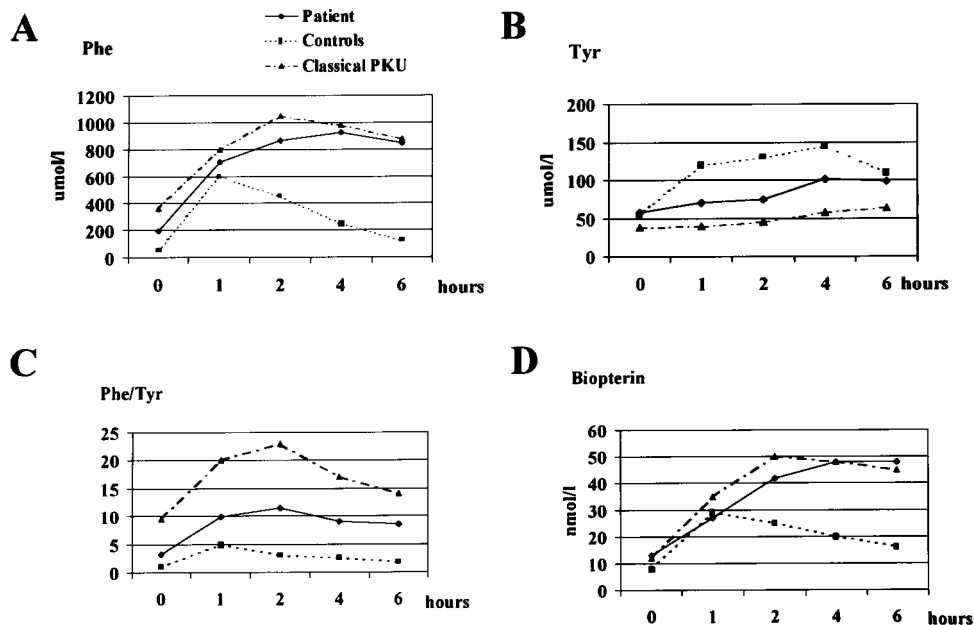


Figure 1. Plasma Phe (A), Tyr (B), Phe/Tyr ratio (C), and biopterin (D) before and after oral challenge with Phe (100  $\text{mg/kg}$ ).

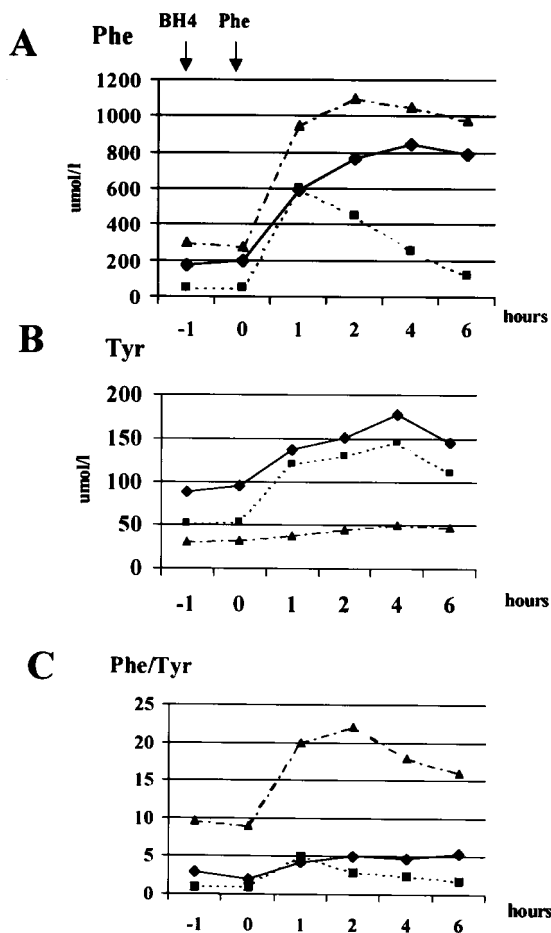


Figure 2. Plasma Phe (A), Tyr (B), Phe/Tyr ratio (C) before and after combined oral challenge with BH<sub>4</sub> (20 mg/kg) and Phe (100 mg/kg). ♦, Patient; ■, controls; ▲, classic phenylketonuria.

ficiencies the pathophysiology of the neurologic damage is well understood, owing to the function of BH<sub>4</sub> as an essential cofactor of tyrosine and tryptophan hydroxylases. In untreated PKU Phe accumulation is responsible for the neurologic syndrome. Different mechanisms can contribute to brain damage: large-neutral amino acids imbalance at the blood–brain barrier, competitive inhibition of monoamine neurotransmitter biosynthetic enzymes, decrease in brain protein synthesis, and increased myelin turnover.<sup>4</sup> Neurotransmitter deficiency has also been demonstrated in patients with long-term PKU treated with diet.<sup>5</sup> However, the relative contribution of these mechanisms to the development of neurologic disturbances as well as the interindividual variability of the neurologic consequences of similar Phe levels are unclear.<sup>4</sup>

Our patient showed plasma Phe concentrations in the range of benign HPA, with psychomotor delay, mental retardation, episodic gait and movement disorders, hypersalivation, and low CSF concentrations of monoamine neurotransmitter metabolites. BH<sub>4</sub> defects were ruled out by enzymatic analysis, and HPA was associated with compound heterozygous

mutations in the PAH gene. Because monoamine neurotransmitter deficiency is typical of BH<sub>4</sub> defects, and because cases of BH<sub>4</sub>-responsive PAH deficiency have been reported,<sup>6</sup> we performed Phe and BH<sub>4</sub> loading tests. The Phe loading test showed the pattern typical for PAH deficiency, with persistently high Phe (see figure 1A), low Tyr concentrations (see figure 1B), and high Phe/Tyr ratios (see figure 1C). Biopterin concentrations overlapped the profile of PKU patients (see figure 1D), showing a physiologic feed-back stimulation of BH<sub>4</sub> synthesis by Phe, through the GTP cyclohydrolase I feed-back regulatory protein.<sup>4</sup> The combined BH<sub>4</sub>-Phe loading test revealed a partial response to BH<sub>4</sub>: Tyr levels increased markedly (see figure 2B), Phe/Tyr ratios were similar to controls (see figure 2C) and lower than those observed during loading with Phe only (see figure 1C). The clinical improvement with BH<sub>4</sub> therapy confirmed the response observed after the combined loading test. Although Phe levels did not decrease during BH<sub>4</sub> loading, normalization of the Phe/Tyr ratio may have reduced the effects of HPA at the blood–brain barrier by restoring the physiologic transport of large-neutral amino acids.<sup>7</sup> It has previously been shown that a plasma Phe concentration of 200 μmol/L, which is ninefold higher than the *K<sub>m</sub>* of Phe transport at the human blood–brain barrier, can saturate neutral amino acid transport sites and cause disturbance of amino acid availability in the human brain.<sup>7</sup> A reduced availability of large-neutral amino acids, precursors of dopamine and serotonin, is responsible for reduced neurotransmitter synthesis.<sup>4</sup> A large variability in the blood–brain Phe concentrations, which is probably responsible for the different clinical expressions of brain damage in patients with PKU, was demonstrated by MRS measurements.<sup>8</sup> Moreover, elevated brain levels of Phe competitively inhibit tyrosine and tryptophan hydroxylases; CSF Phe concentrations above 40 μmol/L, similar to those in our patient, were shown to inhibit rat tyrosine hydroxylase.<sup>4</sup> Interindividual variations in the rate of brain Phe metabolism through these alternative enzymes were also thought to be responsible for the different responses of patients with PKU to HPA and thus for different clinical outcomes.<sup>8</sup> Indeed, the full activity of tyrosine and tryptophan hydroxylases might be protective in patients with HPA against the neurotoxic consequences of permanently elevated Phe.<sup>8</sup> Therefore, in this patient mild HPA and a particular sensitivity to high Phe levels may have led to the monoamine neurotransmitter deficiency. The monoamine precursor therapy may have improved the intracerebral synthesis of monoamines after aromatic amino acid hydroxylation, which is competitively inhibited by Phe. The administration of BH<sub>4</sub> may have contributed to protect the activities of the BH<sub>4</sub>-dependent enzymes, tyrosine, and tryptophan hydroxylases. However, the reason for the clinical response to the effect of BH<sub>4</sub> in reducing Phe/Tyr ratios in this patient remains unclear, as the new PAH mutation, D151E, does not

involve the residues believed to be responsible for BH<sub>4</sub>-PAH binding.<sup>9</sup>

We believe that, in the past, neonates with this form of HPA could have been missed at the newborn screening for PKU because of the higher cut-off value, which is currently 150 μmol/L in Europe. Moreover, patients with mild HPA and normal BH<sub>4</sub> metabolism who show neurologic symptoms need CSF investigation for monoamine neurotransmitter deficiency and subsequent testing for BH<sub>4</sub> responsiveness.

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## Clinical and molecular findings in hyperornithinemia-hyperammonemia-homocitrullinuria syndrome

**Article abstract**—The authors report the clinical and molecular findings in eight patients with hyperornithinemia, hyperammonemia, and homocitrullinuria (HHH) syndrome. The most consistent neurologic finding was spastic paraparesis, seen in five of the eight patients. However, all showed signs of pyramidal tract involvement. A broad spectrum of pathogenetic mutations (including missense, nonsense, splice site, insertion, and deletions) were identified in the *ORNT1* gene.

NEUROLOGY 2001;57:911–914

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Hyperornithinemia, hyperammonemia, and homocitrullinuria (HHH) syndrome is an autosomal recessive disease characterized by impaired ornithine transport across the inner mitochondrial membrane. Patients usually present a characteristic neurologic picture with spastic gait, pyramidal tract signs, myoclonic seizures, and ataxia.<sup>1</sup> Furthermore, the abnormal intramitochondrial ornithine transport causes a functional impairment of the urea cycle responsible

for recurrent hyperammonemia with loss of consciousness, lethargy, and coma.

HHH syndrome is a relatively rare inborn error of metabolism, with approximately 50 patients reported.<sup>2</sup> Two recent studies showed that HHH syndrome is caused by mutations in the *ORNT1* gene,<sup>3,4</sup> a member of the mitochondrial carrier family proteins that catalyzes the electroneutral exchange of ornithine for H<sup>+</sup>. We report the clinical and molecular characteristics of eight patients with HHH syndrome.

**Patients and methods.** We collected retrospective clinical information and biologic samples from eight unrelated patients with HHH syndrome followed up by Italian referring centers for inborn metabolic diseases. The table summarizes the major clinical and molecular features of the patients. The diagnosis was based on the measurement of plasma and urine amino acids, blood ammonia, and urinary orotate. The early course of patients H2, H4, and H7 has been previously reported.<sup>6,7</sup>

Neuroimaging was performed in all patients, four of whom underwent brain MR spectroscopy. Motor and so-

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Supported by the Italian Ministry of Health (Ricerca Finalizzata to C.D.-V. and F.M.S.).

Received January 8, 2001. Accepted in final form April 13, 2001.

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