

Two mutations of dihydropteridine reductase deficiency

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SUMMARY Two patients with dihydropteridine reductase (DHPR) deficiency, in one case due to the absence of any enzyme protein (DHPR⁻ cross reactive material (CRM)⁻) and in the other case due to the production of a mutant type devoid of catalytic activity (DHPR⁻ CRM⁺) were examined. This latter form of malignant phenylketonuria, whose relative frequency seems to be higher in the Italian population, possibly has a worse prognosis. The earlier onset and the greater severity of clinical symptoms are associated with a more pronounced hydroxylation defect, as shown by higher degree of neonatal hyperphenylalaninaemia, unresponsiveness to an oral tetrahydrobiopterin load, lower concentrations of neurotransmitter metabolites, and reduced tyrosine production after an oral phenylalanine load.

The first observation that concerns the lack of response of phenylketonuric children to dietary treatment is that of Mary Efron in 1965 reported by McKusick.¹ Dr Efron pointed out that phenylketonuria (PKU) seldom affects people of southern Italian origin, but when it does, it is unresponsive to diet and causes death in the first years of life. The observation may have been the result of some type of tetrahydrobiopterin (BH₄) deficiency, which was not recognised at that time, as a possible cause of primary hyperphenylalaninaemia. This hypothesis is supported by the recent finding that among people of southern Italian origin the frequency of BH₄ deficiency seems higher than in other populations, mainly due to the higher prevalence, and clustering in Sicily, of dihydropteridine reductase (DHPR) deficiency.² As this form of atypical PKU is known to be heterogeneous with respect to the molecular defect³ and the response of an oral BH₄ load,⁴ two patients deficient in DHPR, originating from the south of Italy and with different clinical presentations, were evaluated in an attempt to determine a correlation with their enzyme state and BH₄ responsiveness.

Case reports

A girl (case 1), born on 20 February 1983, was the

second child of second cousin parents originally from Calabria, a region of southern Italy. She had 7 mg% plasma phenylalanine at 4 days of age on Guthrie mass screening, and had confirmed diagnosis of hyperphenylalaninaemia (1268 μmol/l) at the age of 20 days when a phenylalanine restricted diet was started. Physical and neurological development was judged to be normal up to the age of 7 months, although head circumference seemed to be reduced from the sixth month. Her phenylalanine tolerance, unlike that in PKU, was stable at the high value of about 60 mg/kg/day. Over the next few months it was evident that she was slow in reaching developmental milestones and there was subsequent regression with progression of the typical neurological picture of BH₄ deficiency. An electroencephalogram gave repeatedly normal results, and no episodes of hyperthermia occurred. The diagnosis of DHPR deficiency was made at the age of 10 months and treatment with levodopa, 5-hydroxytryptophan, and carbidopa was added to the phenylalanine restricted diet. After several adjustments,⁵ the clinical response to the treatment was good. The girl is now attending a kindergarten, has no gross motor or speech disabilities, and her intelligence quotient was 95 at the age of 3 years and 9 months.

A boy (case 2), born on 4 April 1983, was the third child of first cousin parents who originated

from Sicily. A neonatal Guthrie test was not done and he was on a free diet until the age of 10 months when the hyperphenylalaninaemia was ascertained (1320 $\mu\text{mol/l}$). Difficulty in feeding, extreme truncal hypotonia, and eye deviation had appeared in the first two months of life, and by the fifth month psychomotor deterioration, microcephaly (head circumference below the third centile), repeated daily myoclonic convulsions and hypsarrhythmic activity shown on electroencephalography were present. A phenylalanine restricted diet was started at 10 months, and neurotransmitter treatment was added at 13 months after the diagnosis of DHPR deficiency. Despite the severity of the clinical picture and the delay in beginning treatment, the patient slowly improved. Convulsions stopped within six months with electroencephalography giving normal results. Motor disabilities and behavioural abnormalities diminished and head circumference increased (it is now over the 10th centile). At 2 years 6 months of age the patient started walking and speaking some simple words; his intelligence quotient, however, was only 45 at the age of 3 years and 5 months.

Methods and results

The activity of DHPR was measured by assay of dried blood spots⁶ and on cultured fibroblasts.⁷ Both methods showed no activity in the patient's cells. All four parents exhibited intermediate values (table 1).

Immunoprecipitation of DHPR was carried out after labelling of fibroblasts with ³⁵S methionine.^{8,9} Immunoprecipitates were then separated by sodium dodecyl sulphate polyacrylamide electrophoresis according to Laemmli¹⁰ and detected by fluorography. The method was sensitive enough to detect 5% of normal enzyme concentrations. Cross reactive

material (CRM) was present at near normal concentrations in the boy (case 2) and was absent in the girl (case 1); this has already been reported by us.⁹

Serum folate measurements were performed by means of a commercial radioassay kit (Ciba Corning MAGIC Vitamin B₁₂/Folate NB) at diagnosis and after 10 days of oral administration of folic acid at 15 mg/day (table 1). Only the girl was found deficient in folate, but both patients failed to show any metabolic or clinical improvement with this treatment.

An oral BH₄ loading test was given to both patients.¹¹ As the boy showed no reduction in plasma phenylalanine concentrations he was given 2 mg/kg BH₄ intravenously, to which he responded partially (table 2).

Urine and cerebrospinal fluid (CSF) pterins, CSF neurotransmitter metabolites, homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) were measured by high performance liquid chromatography with electrochemical detection at high and low plasma concentrations of phenylalanine (table 3).¹² The urinary and CSF pterin patterns were similar in the two patients, in both of whom the pterin pattern reverted towards normal under dietary treatment. At diagnosis the boy showed

Table 2 Plasma phenylalanine response to loading with tetrahydrobiopterin (BH₄) in the two patients

Case No and route of administration of BH ₄	Dose of BH ₄ (mg/kg)	Plasma phenylalanine concentration ($\mu\text{mol/l}$)		
		0	4 hours	8 hours
Case 1: oral	7.5	478	208	110
Case 2: oral	7.5	1386	1380	1344
intravenous	2.0	960	934	764

Table 1 Dihydropteridine reductase (DHPR) activity in dried blood spots and in cultured fibroblasts of the two families, and serum folate values in the patients before and after folic acid treatment

	DHPR activity		Serum folate (ng/ml)	
	Erythrocytes	Fibroblasts	Day 0	Day 10
Case 1	None detected	None detected‡	2.7	20
Father	1.14	Not determined		
Mother	1.82	Not determined		
Case 2	None detected	None detected	20	43
Father	1.37	Not determined		
Mother	0.92	Not determined		
Controls	3.34 (0.88)* (n=20)	49.6 (2.8)† (n=11)	3-17	

*Value is mean (SD) (nmol cytochrome C reduced/minute/5 mm diameter, filter paper disc).

†Value is mean (SD) (nmol NADH oxidised/minute/mg protein).

‡1% of normal activity could be confidently assayed.

Table 3 Pattern of pterins in urine and cerebrospinal fluid; concentrations of homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) recorded in the two patients in basal conditions and during phenylalanine restricted diet with substitutive neurotransmitter treatment

Case No	Age (months)	Treatment	Plasma phenylalanine concentrations (mg%)	Urine				Cerebrospinal fluid			
				Biopterin	Neopterin	%B*	BNCR†	Biopterin	Neopterin	HVA	5-HIAA
Case 1	14	‡	6	6.51	1.02	86	563	57.3	11.4	81.0	23.0
	24	Restricted diet, levodopa, 5-hydroxytryptophan, carbidopa	3	4.03	2.80	59	237	50.9	23.7	681	334
Case 2	13	‡	24	13.8	2.86	83	1143	63.1	10.5	50.8	6.9
	23	Restricted diet, levodopa, 5-hydroxytryptophan, carbidopa	3	1.95	1.29	60	117	38.6	15.5	474	173
Healthy controls				1.10	0.47	44	33	10	9	250	110
				4.00	3.00	77	139	34	20	880	360

*%B=100×biopterin/biopterin+neopterin.

†BNCR (biopterin neopterin creatinine ratio)=biopterin/biopterin+neopterin·biopterin/creatinine·10⁵.

‡In both cases the treatment was discontinued for more than a week: in case 1 because the drugs were not available and because of the high phenylalanine tolerance; in case 2 the parents were initially not fully convinced of the benefit of the treatment.

lower HVA and 5-HIAA concentrations in the CSF than the girl, but he also had higher phenylalanine concentrations. Correction was achieved in both patients with similar doses of hydroxylated neurotransmitter precursors.

An oral load of phenylalanine (0.6 mmol/kg) was given,¹³ and amino acids were measured chromatographically with the Kontron Chromakon 500 automatic analyser. The impairment of hydroxylating activity was possibly more pronounced in the boy, as

indicated by a somewhat slower fall in plasma concentrations of phenylalanine and lesser rise in tyrosine (figure).

Discussion

Clinical variation in patients with inborn errors of metabolism is common due to quantitative as well as qualitative differences in underlying mutations and additional factors. Three different enzyme defects

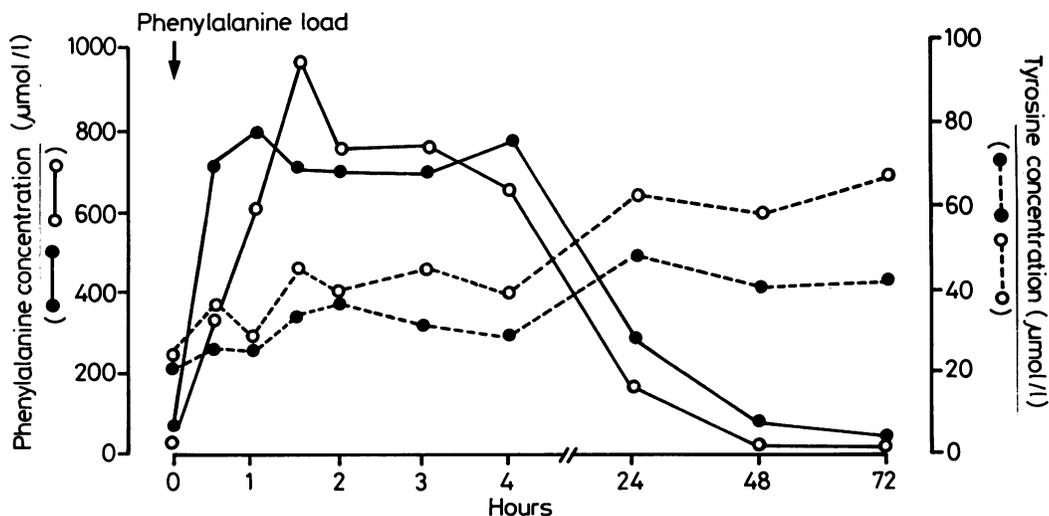


Figure Plasma phenylalanine (—) and tyrosine (---) responses to an oral phenylalanine load in the girl (○) who did respond to the load with tetrahydrobiopterin (BH₄) and the boy (●) who did not respond.

cause BH₄ deficiency, guanosine triphosphate cyclohydrolase I (GTP-CH I) and 6-pyruvoyl tetrahydropterin synthase (PTPS) deficiency causing impaired synthesis and DHPR deficiency causing impaired recycling of the cofactor.⁴ Further heterogeneity has been described within these defects.¹⁴ The use of a BH₄ oral load as a diagnostic tool has shown that a minority of patients deficient in DHPR fail to lower their plasma phenylalanine after loading,⁴ and in addition it has been shown that some patients have no mutant protein (DHPR⁻ CRM⁻) in their cells, whereas others have (DHPR⁻ CRM⁺).^{3, 15} It has been reported that CRM⁻ cases are BH₄ responders and CRM⁺ are non-responders and that the latter have a worse prognosis.⁹

The present study strengthens the hypothesis that the presence of the mutant DHPR is worse clinically: shown by the earlier onset of symptoms and the relatively poor clinical response to neurotransmitter treatment in the patient with CRM⁺, although the delay in starting a phenylalanine restricted diet could also have contributed.

Contrary to the opinion that the unresponsiveness to exogenous BH₄ could be due to the total deficiency of DHPR catalytic activity,¹⁶ this was present in both our patients and only the child who was CRM⁺ gave a negative response after oral loading. Unresponsiveness to exogenous BH₄ could be due to the binding of cofactor to the mutant enzyme, making BH₄ unavailable for hydroxylation. If so, a decrease of plasma phenylalanine might be achieved by loading these patients with doses of BH₄ large enough to exceed the CRM⁺ binding capacity; the CRM⁺ patient described here did respond to intravenous BH₄, and together with another non-responding patient also responded to higher oral doses.¹⁷ Alternative hypotheses might be the production of a reactive pterin, which inactivates hydroxylases, or the inhibition of the hydroxylases by the mutant enzyme. In any of these cases, the endogenous biosynthetic BH₄ would be less available in CRM⁺ patients, leading to a worse outcome. The CRM⁺ patient described here may have had a more pronounced hydroxylation defect than the child who was CRM⁻ in view of the lower basal concentrations of HVA and 5-HIAA, and the reduced tyrosine production after oral phenylalanine load. Furthermore neonatal hyperphenylalaninaemia has been reported to be higher in patients who were CRM⁺ than in patients who were CRM⁻.⁹

Within the DHPR deficiency the relative frequency of non-responding and responding cases to the oral load of BH₄ has been reported in worldwide surveys as 1:3–1:4,^{4, 14} although this may not be true for the Italian population, as two out of four

patients, both originally from Sicily, did not respond to the load.² A continuum of conditions is to be expected, depending on differences in the mutations. Cases with residual reductase activity¹⁸ or partial CRM⁺⁹ are known to have a milder course.

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