References

- ¹ Komrower G M, Sardharwalla I B, Coutts J M J, Ingham D. Management of maternal phenylketonuria: an emerging clinical problem. *Br Med J* 1979; **i**: 1383-7.
- ² Buist N R M, Lis E W, Tuerck J M, Murphey W H. Letter: Maternal phenylketonuria. *Lancet* 1979; **ii**: 589.
- ³ Lenke R R, Levy H L. International survey of maternal phenylketonuria and hyperphenylalaninaemia. *N Engl J Med* 1980; in press.
- ⁴ Nielsen K B, Wamberg E, Weber J. Letter: Successful outcome of pregnancy in a phenylketonuric woman after low-phenylalanine diet introduced before conception. *Lancet* 1979; **i**: 1245.
- ⁵ Anonymous. Dietary promise for PKU patients. *Medical Research Council News* 1980; No. 8, 3.

Biopterin defect in a normal-appearing child affected by a transient phenylketonuria

FRANCOISE REY, ROBERT J LEEMING, JOHN A BLAIR, AND JEAN REY

Unité de Recherches de Génétique Médicale (INSERM), Hôpital des Enfants Malades, Paris, France, and General Hospital, Birmingham

SUMMARY A child diagnosed as having transient phenylketonuria was found to have reduced synthesis of tetrahydrobiopterin and an abnormal clearance of phenylalanine, but he remained clinically normal when on a normal diet. A small amount of 7,8-dihydrobiopterin was found in his serum; this distinguishes the case from that of malignant hyperphenylalaninaemia.

A defect in biopterin metabolism has been found in a few patients with hyperphenylalaninaemia although their hydroxylase and reductase activities have been normal.¹⁻⁴ In two of these patients the serum biopterin concentration, as assayed by *Crithidia fasciculata*, did not rise after an oral or an intravenous phenylalanine load as it does in normal subjects and in those with the usual variants of phenylketonuria (PKU), and in patients affected by dihydropteridine reductase deficiency.⁵⁻⁷ The mechanism of the biopterin response to the rise of plasma phenylalanine level is unclear, but this characteristic distinguishes without liver biopsy, among the new variants with progressive neurological illness,⁸ those who are affected by dihydropteridine reductase deficiency and those suffering from a defect in biopterin synthesis.⁵ We now report a case of so-called transient PKU⁹ in which a low biopterin serum level and an atypical zero-order kinetics of phenylalanine disappearance were present at 5 years of age in a seemingly normal child.

Methods and results

This boy was born on 9 December 1973, and was the second child of healthy unrelated parents. Birth-weight was 3.82 kg. At 5 days a Guthrie test showed a serum phenylalanine level of 0.6 mmol/l (10 mg/100 ml). The infant was bottle fed with dried cows' milk formula (protein intake 6 g/kg per day)

and one month later the phenylalanine plasma level was 3.5 mmol/l (58 mg/100 ml), but with a protein intake of 3 g/kg per day (adapted formula) it returned within 4 days to 1.1 mmol/l (18.2 mg/100 ml) (tyrosine 0.72 mg/100 ml (0.04 mmol/l)). A low phenylalanine diet (Lofenalac) was started on the 36th day of life. It was possible rapidly to increase the phenylalanine intake from 300 to 450 mg/day and at 6 months, when a normal diet was reintroduced, the phenylalanine level remained below 0.2 mmol/l (3.3 mg/100 ml). Lofenalac was stopped and until he was 1 year old he received a restricted natural protein diet (2.5 g/kg). Subsequently a regular diet was instituted and since then the phenylalanine plasma level, measured by fluorimetry, has not exceeded 0.2 mmol/l. The physical and intellectual development have been normal: at 5 years IQ is 108.

An intravenous phenylalanine load was performed at age 1 year; the plasma concentration returned from 2.7 mmol/l (44.6 mg/100 ml) to normal values within 2 days, after a very slow and atypical kinetics of disappearance; phenylpyruvic acid and o-hydroxy-phenylacetic acid were present during the load. At 3 years, very low biopterin levels were found in serum (0.4 ng/ml); at this age chromatography of serum¹ showed only 7,8-dihydrobiopterin to be present. At 5 years, after an oral load which increased the phenylalanine level to 2.1 mmol/l (34.7 mg/100 ml) after 2 hours, and to 2.8 mmol/l (46.3 mg/100 ml) after 4 hours, the initially low biopterin concentration in serum (0.2 ng/ml) rose to 0.6 ng/ml at the 3rd hour and to 0.55 ng/ml at the 6th hour. The phenylalanine plasma level was still high (0.9 mmol/l; 14.9 mg/100 ml) 18 hours later. During this time the child was unusually sleepy. The parents did not give permission for a needle biopsy.

Both parents had normal fasting phenylalanine plasma levels and their phenylalanine/tyrosine molar ratios were also normal.

Discussion

Transient hyperphenylalaninaemia or, to be precise, pseudotransient PKU is a rare condition that can be mistaken in the neonatal period for a classical or an atypical PKU; however it differs in that the phenylalanine tolerance increases with time, so that by about age 6 months the phenylalanine plasma level is no higher than 0.2 or 0.3 mmol/l on a regular diet. Furthermore, when parents are investigated, neither a raised fasting phenylalanine level, nor a phenylalanine/tyrosine molar ratio increase is found,⁹⁻¹⁶ unlike the common variants of the disease, thus showing a transient defect. It is distinct from the immaturity of the phenylalanine hydroxylating system in which the response to a phenylalanine load is clearly normal after the normalisation of the phenylalanine plasma level.¹¹⁻¹³ In so-called transient PKU a delayed clearing of phenylalanine can be found several months or years later, showing the persistency of a metabolic disorder.^{9, 14} Until now, the primary defect has been unknown but in view of the very slow phenylalanine disappearance after intravenous perfusion, which suggests the accumulation of an intermediate (the concentration of which remains constant during the hydroxylation process), a phenylalanine-hydroxylase stimulating protein deficiency has been suggested.⁹

From these criteria, the findings in our patient clearly correspond to this diagnosis. Unfortunately no enzymatic studies were performed as the parents refused to give permission for a liver biopsy in this seemingly normal 5-year-old boy. However, unlike 2 other patients who were apparently affected by the same phenotype but in whom biopterin derivatives were not depressed, this child had a very low biopterin level in his plasma and urine with only a small increase after an oral phenylalanine load. Indeed in normal subjects and in heterozygotes for PKU, as well as in classical PKU or its variants, a

4- to 5-fold increase in biopterin concentration is observed after a rise in phenylalanine plasma level, the biopterin/phenylalanine ratio being above 15 when the phenylalanine level is in the normal range, and at about 5 in untreated PKU or in phenylalanine-challenged subjects.^{3, 6} The increase in biopterin concentration is even greater in dihydropteridine reductase deficiency and in such cases large amounts of dihydrobiopterin and dihydroxanthopterin are found in urine.^{5, 15}

Malignant hyperphenylalaninaemia is the only condition in which a biopterin deficiency has been described and small amounts of crithidia factors which co-chromatographed with neopterin, biopterin, and tetrahydrobiopterin were found in urine and serum.¹ These compounds were initially taken for neopterin or biopterin⁵ but it has recently been proved by using high voltage electrophoresis followed by ultraviolet detection that the species were neopterin and 7,8-dihydroneopterin and not 7,8-dihydrobiopterin, indicating a 7,8-dihydrobiopterin-synthesis deficiency.⁴

The condition differs strikingly from malignant hyperphenylalaninaemia, since our patient is normally developed and shows no symptoms of impaired neurotransmitter synthesis. He can be distinguished also on biochemical grounds as small quantities of 7,8-dihydrobiopterin were found in serum chromatography, suggesting a partial defect of 7,8-dihydrobiopterin synthesis. The site of this defect in the synthesis of tetrahydrobiopterin may be shown by analysing urinary pterins—such as neopterin. In this case the synthesis of dihydrobiopterin, although adequate for a low serum phenylalanine level, was not sufficient to remove serum phenylalanine after challenging doses; this explains the apparent 'inhibition' of the hydroxylating system suggested by the zero-order kinetics of disappearance.

In such a partial enzymatic defect the child may be

Table *Enzymatic data and biopterin values in malignant hyperphenylalaninaemia and in our patient*

	Phenylalanine-hydroxylase activity*	DHPR activity†	In vivo phenylalanine disappearance rate‡	Fasting and maximum values after load			
				Phenylalanine (mmol/l)		Biopterin (ng/ml)	
DHPR deficiency							
Case 1 ⁵	61	0	3.2	0.07	2.10	5.5	30
Biopterin synthesis deficiency							
Case 2	49	61	2.5	0.26	2.48	0.8	1.2
Case 3	51	60	0.8	0.07	2.70	0.2	0.5
Present case			0	0.2	2.8	0.2	0.6

* μmol tyrosine/hour per g protein,

†nmol NADH oxidised/min per mg protein,

‡percent of normal ($1.37 \times 10^{-2} \mu\text{mol/ml per minute}$).

DHPR = dihydropteridine reductase.

Conversion: traditional to SI units—biopterin: 1 ng/ml $\approx 4.2 \text{ nmol/l}$

SI to traditional units—phenylalanine: 1 mmol/l $\approx 16.5 \text{ mg/100 ml}$.

heterozygote for 7,8-dihydrobiopterin synthesis deficiency as the parents of a child affected by malignant hyperphenylalaninaemia (Case 2, Table),^{1 5} have similar very low biopterin levels (1979, unpublished data).

This case shows that a low serum *C. fasciculata* activity together with high serum phenylalanine concentration does not necessarily mean that the child is affected by a malignant hyperphenylalaninaemia unless chromatographic analysis of the serum also shows lack of 7,8-dihydrobiopterin. It also demonstrates that transient hyperphenylalaninaemia is a heterogeneous condition and can be caused by reduced biosynthesis of 7,8-dihydrobiopterin.

This investigation was supported by a research grant (ATP: 37.76.69) from the Institut National de la Santé et de la Recherche Médicale (INSERM).

References

- 1 Leeming R J, Blair J A, Rey F. Letter: Biopterin derivatives in atypical phenylketonuria. *Lancet* 1976; **1**: 99-100.
- 2 Rey F, Blandin-Savoja F, Rey J. Letter: Atypical phenylketonuria with normal dihydropteridine reductase activity. *N Engl J Med* 1976; **295**: 1138-9.
- 3 Kaufman S, Berlow S, Summer G K, et al. Hyperphenylalaninemia due to a deficiency of biopterin. A variant form of phenylketonuria. *N Engl J Med* 1978; **299**: 673-9.
- 4 Niederwieser A, Curtius H-Ch, Bettoni O, et al. Atypical phenylketonuria caused by 7,8 dihydrobiopterin synthesis deficiency. *Lancet* 1979; **1**: 131-3.
- 5 Rey F, Harpey J P, Leeming R J, Blair J A, Aidardi J, Rey J. Les hyperphénylalaninémies avec activité normale de la phénylalaninehydroxylase. Le déficit en tétrahydrobiopterine et le déficit en dihydropteridine reductase. *Arch Fr Pédiatr* 1977, **34**: Supplement II, 109-20.
- 6 Leeming R J, Blair J A, Green A, Raine D N. Biopterin derivatives in normal and phenylketonuric patients after oral loads of L-phenylalanine, L-tyrosine, and L-tryptophan. *Arch Dis Child* 1976; **51**: 771-7.
- 7 Gröbe H, Bartholome K, Milstein S, Kaufman S. Hyperphenylalaninaemia due to dihydropteridine reductase deficiency. *Eur J Pediatr* 1978, **129**: 93-8.
- 8 Smith I, Clayton B E, Wolff O H. New variant of phenylketonuria with progressive neurological illness unresponsive to phenylalanine restriction. *Lancet* 1975; **i**: 1108-11.
- 9 Rey F, Blandin-Savoja F, Rey J. Kinetics of phenylalanine disappearance after intravenous load in phenylketonuria and its genetic variants. *Pediatr Res* 1979; **13**: 21-5.
- 10 Griffin R F, Elsas L J. Classic phenylketonuria: diagnosis through heterozygote detection. *J Pediatr* 1975; **86**: 512-7.
- 11 O'Flynn M E, Tillman P, Hsia D Y. Hyperphenylalaninemia without phenylketonuria. *Am J Dis Child* 1967; **113**: 22-30.
- 12 Stephenson J B, McBean M S. Diagnosis of phenylketonuria (phenylalanine hydroxylase deficiency temporary and permanent). *Br Med J* 1967; **iii**: 579-81.
- 13 Castell S, Lacamera R G, Wessel M A, Brandt I K. Transient hyperphenylalaninemia. *J Pediatr* 1968; **72**: 530-3.
- 14 Bremer H J. Transitory hyperphenylalaninaemia. In: Bickel H, Hudson F P, Woolf L I, eds. *Phenylketonuria and some other inborn errors of amino acid metabolism*. Stuttgart: Thieme, 1971: 93-7.
- 15 Schlesinger P, Watson B M, Cotton R G H, Danks D M. Urinary dihydroxanthopterin in the diagnosis of malignant hyperphenylalaninaemia and phenylketonuria. *Clin Chim Acta* 1979; **92**: 187-95.

Correspondence to Dr F Rey, Unité de Recherches de Génétique Médicale, Hôpital des Enfants-Malades, 149 rue de Sèrves, 75730 Paris, Cedex 15, France.

Familial thyroid ectopy and hemiagenesis

T ROSENBERG AND Y GILBOA

The Endocrine Service, Assaf Harofeh Hospital, Zerifin, and the Sackler School of Medicine, University of Tel-Aviv, Israel

SUMMARY Two siblings had sublingual thyroid glands and hypothyroidism. A third sibling had a left lobe agenesis of the thyroid, but normal function of the gland. This is the second such family to be described.

The ectopic position of the thyroid gland is not an uncommon anomaly, with an incidence of about 1 in 4000 of all thyroid diseases.¹ Generally only one sibling is affected but familial occurrences have occasionally been reported.²⁻⁵ This anomaly is the

most frequent cause of hypothyroidism in babies, with an incidence of between 36 and 68%.⁶⁻⁷ A family is described with 3 affected siblings; 2 siblings had sublingual thyroids and the third had hemiagenesis of the gland.

Case reports

Case 1. A 4-year-old girl was examined because of stunted growth and slow physical and psychomotor development. She was the product of a term, uncomplicated pregnancy and delivery. There was no