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Two Siblings of Hyperphenylalaninemia : Suggestion to a Genetic Variant of Phenylketonuria

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A sibling case of hyperphenylalaninemia without phenylpyruvic aciduria was described. An oral loading of phenylalanine revealed a delay of clearance of serum phenylalanine, a slight elevation of serum tyrosine, and an increased urinary excretion of phenylpyruvic acid and *o*-hydroxyphenylacetic acid. Phenylalanine hydroxylase in the liver was found to be of about one-tenth the normal activity.

These findings suggest that hyperphenlyalaninemia may be a genetic variant of phenylketonuria.

The screening program for phenylketonuria (PKU) which has been widely going on in the neonatal period revealed the existence of hyperphenylalaninemia. The latter is differentiated from classical PKU in that the elevation of blood phenylalanine is milder and the ferric-chloride test for urinary phenylpyruvic acid is negative. The biochemical mechanism through which this peculiar hyperphenylalaninemia is caused remains obscure, although several possibilities have been postulated.[1]

This paper describes the results of biochemical studies on two siblings of hyperphenylalaninemia.

Case Report

Case N.S., a boy was born with a weight of 2,620 g after a full-term pregnancy. Blue asphyxia was seen at birth. Neonatal period was uneventful. Since three months of age he began to show convulsions like infantile spasm. At the age of three years, the patient was admitted to our hospital because of frequent episodes of convulsions. He showed signs of spastic tetraplegia. IQ was found to be below 30. EEG showed findings of hypsarrhythmia.

Analyses of free amino acids in serum showed an elevation of phenylalanine ranging from 6 to 12 mg/100 ml and other amino acids including tyrosine were within normal limits (cf. Table 1). Urinary ferric-chloride test was never positive. The parents were healthy but consanguineous (first cousins). There were two siblings, one being healthy and the other found to have hyperphenylalaninemia (*Case K. S.*).

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TABLE 1. *Serum amino acid levels in the patients with hyperphenylalaninemia (mg/100 ml)*

Amino acids	Case N.S.	Case K.S.	Father	Mother	Brother	Normal range
Aspartic acid	0.47	0.61			0.37	Trace-0.48
Threonine	0.79	1.30	1.01	0.67	0.82	0.37-1.05
Serine (+Glutamine)	3.22	5.56	3.82	4.58	5.27	2.06-6.67
Glutamic acid	0.90	2.76	2.88	1.47	1.86	0.42-2.49
Proline	2.04	2.11	2.83	1.67	2.03	0.66-2.19
Glycine	1.42	1.16	1.90	1.46	1.67	1.23-3.44
Alanine	2.22	2.47	4.04	3.15	2.71	1.30-3.91
Valine	2.12	3.34	2.88	2.50	2.20	0.94-2.87
Methionine	0.20	0.45	0.53	0.36	0.38	Trace-0.50
Isoleucine	0.63	0.94	0.98	0.81	0.73	0.28-0.94
Leucine	1.13	2.01	1.72	1.43	1.23	0.79-1.53
Tyrosine	0.69	1.56	1.03	0.69	0.85	0.36-1.25
Phenylalanine	8.81	12.40	0.80	0.68	1.12	0.50-1.14
Lysine	1.37	0.98				1.10-3.74
Histidine	0.62	0.66				0.33-1.05

Case K.S., a younger brother of Case N.S., was born after a full-term pregnancy and spontaneous delivery with a weight of 3,020 g. His neonatal history was uneventful. At the age of 30 days, the patient had the first attack of convulsions like infantile spasm. At the age of 40 days, the patient was admitted to our hospital, and physical examination revealed a moderately nourished boy with eczema on the head and face. There was no neurologically abnormal signs. DQ was fond to be 79. EEG showed findings of hypsarrhythmia.

Determination of serum amino acids showed an elevation of phenylalanine ranging from 12 to 15 mg/100 ml and normal range of the other amino acids. Urinary ferric chloride test was mostly negative, sometimes weakly positive.

Since 45 days of life, the treatment with a low phenylalanine diet was started. During the treatment, levels of serum phenylalanine were maintained between 2 to 6 mg/100 ml. At the age of 20 months, the patient showed satisfactory development, DQ being of 83. The parents and a healthy sibling showed normal levels of serum phenylalanine (cf. Table 1).

SPECIFIC STUDIES

An oral phenylalanine loading test (0.1 g/kg) in Case N.S. showed a higher peak of serum phenylalanine and its delay in returning to the pre-loading level, following the test dose (cf. Fig. 1).

Urine specimens following the phenylalanine loading of Case N.S. and Case K.S. gave positive reaction for both the $FeCl_3$ test and the dinitrophenylhydrazine test, indicating an increased excretion of phenylpyruvic acid. *o*-Hydroxyphenylacetic acid (*o*-HPAA) was investigated by Berry's paperchromatographic procedure[2]. A spot of *o*-HPAA was undetectable in urine from Case K.S. under the ordinary diet but it appeared in urine following the phenylalanine loading.

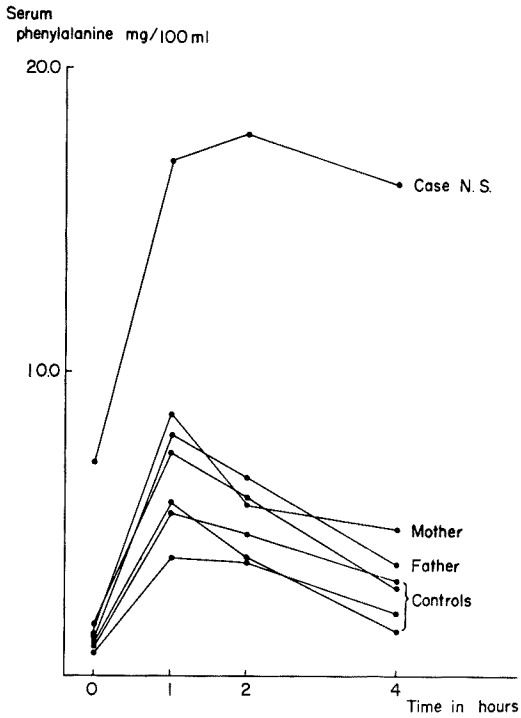

Fig. 1. Oral phenylalanine loading test (0.10 g/kg).

TABLE 2. *Serum tyrosine levels following the phenylalanine loading (0.2 g/kg)*

	Serum tyrosine (mg/100 ml)	
	Before the loading	4 hours following the loading
Case K.S.	0.67	0.91
Classical PKU	0.23	0.20
Father of Case K.S.	1.03	1.72
Mother of Case K.S.	0.69	2.21
Control A	1.10	5.60
" B	0.60	1.49

Serum tyrosine levels after the phenylalanine loading were determined in Case K.S. by an automatic amino acid analyzer (cf. Table 2). A slight rise in serum tyrosine after the loading was found in Case K.S., in contrast to no rise in a case of classical PKU. These results suggest a residual activity of phenylalanine hydroxylase in our case of hyperphenylalaninemia.

In order to confirm this point, the activity of phenylalanine hydroxylase in the liver was determined by Justice *et al.*'s method[3] as follows. Liver specimens obtained by the needle-biopsy from the patients were immediately homogenized

with three volumes of 0.15 M potassium chloride. The homogenate was centrifuged at 16,000 g for 15 minutes, and the supernatant was immediately used for assay of phenylalanine hydroxylase activity. The incubation mixture contained 0.8 μ mole L-phenylalanine (including 0.2 μc of uniformly labeled ^{14}C-phenylalanine), 1.0 μmole NADH, 2 μmole nicotinamide, 0.04 ml of liver extract and 0.2 M phosphate buffer, pH 7.0, with a final volume of 0.6 ml. Incubation was carried out at 25°C for 45 minutes and stopped by the addition of 0.6 ml of 10% trichloroacetic acid. The reaction mixture was centrifuged at 2,000 g and an aliquot of the supernatant was applied to Toyoroshi No. 51. Ascending chromatography was carried out with a solvent system consisting of 2-propanol/water/ammonium hydroxide (8: 1: 1 v/v) for 18 hours. The paper was scanned for radioactivity using a strip counter. Chromatograms were compared with authentic L-phenylalanine and tyrosine standards for mobility and position.

Results are shown in Fig. 2; the patient of classical PKU showed no peak of radioactivity in tyrosine fraction.

Case K.S. showed a slight but significant peak in tyrosine fraction, correspondent to about one-tenth to one-fifteenth that in controls.

These results indicate that our patient with hyperphenylalaninemia has a partial defect in the activity of phenylalanine hydroxylase of the liver.

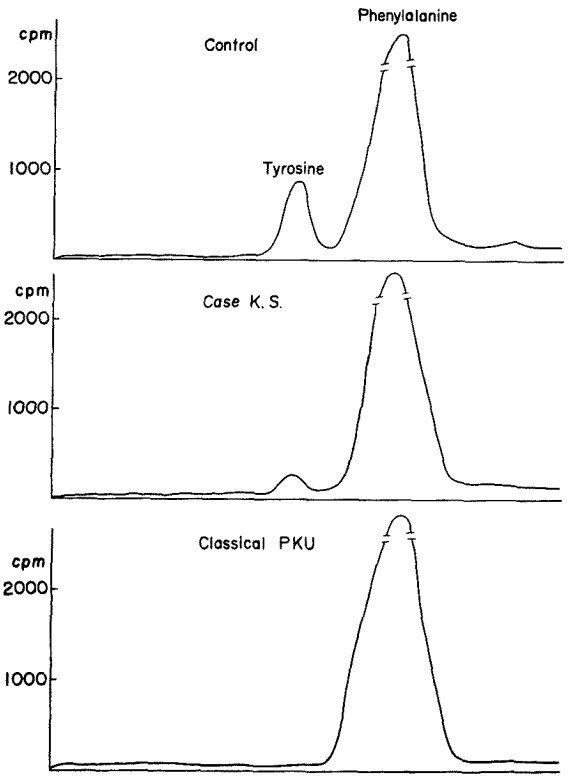

Fig. 2. Activity of phenylalanine hydroxylase in the liver

Comment

Hyperphenylalaninemia without phenylpyruvic aciduria could be caused by any of the following mechanisms; a defect in phenylalanine transaminase, heterozygote of classical PKU, a delayed maturation of tyrosine oxidation system or a partial defect of phenylalanine hydroxylase.[1]

In our patients with hyperphenylalaninemia, a defect in phenylalanine transaminase can be excluded because these patients were found to excrete phenylpyruvic acid after the phenylalanine loading. The absence of urinary phenylpyruvic acid is, therefore, ascribed to relatively low phenylalanine levels in blood as compared with those in classical PKU.

This explanation would be consistent with the observation that phenylpyruvic aciduria disappeared in the course of dietary treatment of PKU with the decrease of serum phenylalanine levels and that critical level of serum phenylalanine for positive $FeCl_3$ test was thought to be about 15 mg/100 ml.[4]

The possibility of heterozygosity for PKU is also unlikely, because most heterozygotes for PKU show normal levels of serum phenylalanine in the fasting state.

Normal levels of serum tyrosine in our patients with hyperphenylalaninemia deny the possibility of delayed maturation of tyrosine oxidation system.

A partial defect of phenylalanine hydroxylase was demonstrated in two cases of hyperphenylalaninemia by Justice *et al.*[3]

The present studies of ours on siblings of hyperphenylalaninemia not only suppport their findings, but also strongly suggest that hyperphenylalaninemia, at least persistent form, may be a genetic variant of PKU.

Most of the patients with hyperphenylalaninemia reported so far have no mental retardation. Our siblings with hyperphenylalaninemia showed, however, mental retardation. There is a possibility that the patients with hyperphenylalaninemia might be susceptible to brain damage.

References

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