

<Case Report>

## A case of untreated hyperphenylalaninemia in Japanese adult

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**Summary** We present herein a case of untreated hyperphenylalaninemia (HPA) in a Japanese adult. The patient's plasma concentration of phenylalanine (Phe) was 1245 nmol/mL, and that of tyrosine (Tyr) was 26 nmol/mL. In spite of the lower Tyr nutriture, the patient's protein nutritional status assessed by retinol-binding protein (RBP), transthyretin (TTR), transferrin (TRF), and albumin was within normal levels. To prevent further disease progression, dietary management, i.e., dietary restriction of Phe, was required, while taking care that the patient did not suffer from protein malnutrition.

**Key words:** Phenylketonuria, Phenylalanine hydroxylase, Tyrosine, Tetrahydrobiopterin, Congenital metabolic disease

### 1. Introduction

This study outlines a Japanese case of patient diagnosed with hyperphenylalaninemia (HPA) at age 49, who was not treated with dietary management.

HPA<sup>1,2</sup> is an inherent autosomal recessive disease caused by phenylalanine hydroxylase (PAH, EC 1.14.16.1) enzyme deficiency on 12q22-q24.2, or by defects in the regeneration of the cofactor tetrahydrobiopterin (BH4) (Fig. 1). Nowadays, more than 500 mutations in the PAH gene have been identified<sup>1-5</sup>. Most PAH gene mutations (60.1%) were missense mutations at the PAH database<sup>6</sup>. This mutation did not prevent transcription and translation. The mutations in PHA change single amino acid, and the most common mutation replaces the amino acid arginine (Arg) with the amino acid tryptophan (Trp) at position 408. Since

PAH converts phenylalanine (Phe, an essential amino acid) to tyrosine (Tyr, a nonessential amino acid), the lack of enzyme PAH activity results in a lowered plasma concentration of Tyr and an elevated plasma concentration of Phe. Accumulated Phe in plasma enters the central nervous system by transporter across the blood-brain barrier. Phe not only behaves as the neurotoxic molecule forming amyloid fibrils<sup>7</sup>, but also competes with other neutral amino acids and tryptophan via the same transporter. Toxic levels of Phe damage the brain<sup>6,7</sup>, and insufficient levels of Tyr interfere with synthesis of neurotransmitters (dopamine, norepinephrine, and epinephrine) in the brain. Because untreated patients with HPA will progress to manifest neurological problems and mental retardation, the disease should be detected by a newborn screening test performed 2-7 days after

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birth<sup>1, 2, 8, 9</sup>. Once diagnosed as having this inherent disease, the patient must be placed on dietary management, i.e., intake of Phe-restricted foods as soon as possible, before Phe affects brain development and function.

## 2. Case history

The patient was diagnosed with HPA at age 49 when he was subjected to nutritional assessment. At age 1, he started showing signs of behavioral and psychiatric problems. He had remained hospitalized for more than 30 years. During that time, he was diagnosed with mental retardation and reactive psychosis. Written informed consent was obtained from all subjects or families of patients, and our study was approved by the guidelines established by the Protection of Human Subjects Committee of Aizunishi Hospital.

Physical examination indicated that his height was 155.0 cm and he had a body weight of 52.9 kg

(22.5 body mass index). He now received a diet formula based on the Dietary Reference Intakes (DRIs) of Japan<sup>10</sup>, comprised of Phe and Tyr in the amount of 25 mg/kg/day. Nutritional assessment revealed normal protein nutriture (Table 1); that he had 4.2 g/dL albumin (RR: >3.5 g/dL), 32.6 mg/dL transthyretin (TTR, RR: >20 mg/dL), 5.2 mg/dL retinol-binding protein (RBP, RR: >2.0 mg/dL), and 220 mg/dL transferrin (TRF, RR: >200 mg/dL), however, he had a marked elevation in plasma Phe concentration (1245 nmol/mL, RR: 43-76 nmol/mL) and a lowered Tyr concentration (26 nmol/mL, RR: 40-90 nmol/mL). Plasma amino acid concentrations were measured by HPLC using post-column ninhydrin detection, and plasma specimens were collected after over night fasting. Figure 2 shows the correlation between plasma concentrations of Phe and Tyr in this patient and in 49 other patients having the same formula diet as he. In the 49 patients, plasma concentrations of Tyr significantly correlated with those of Phe ( $r=0.652$ ,  $p<0.001$ ), indicating that plasma levels

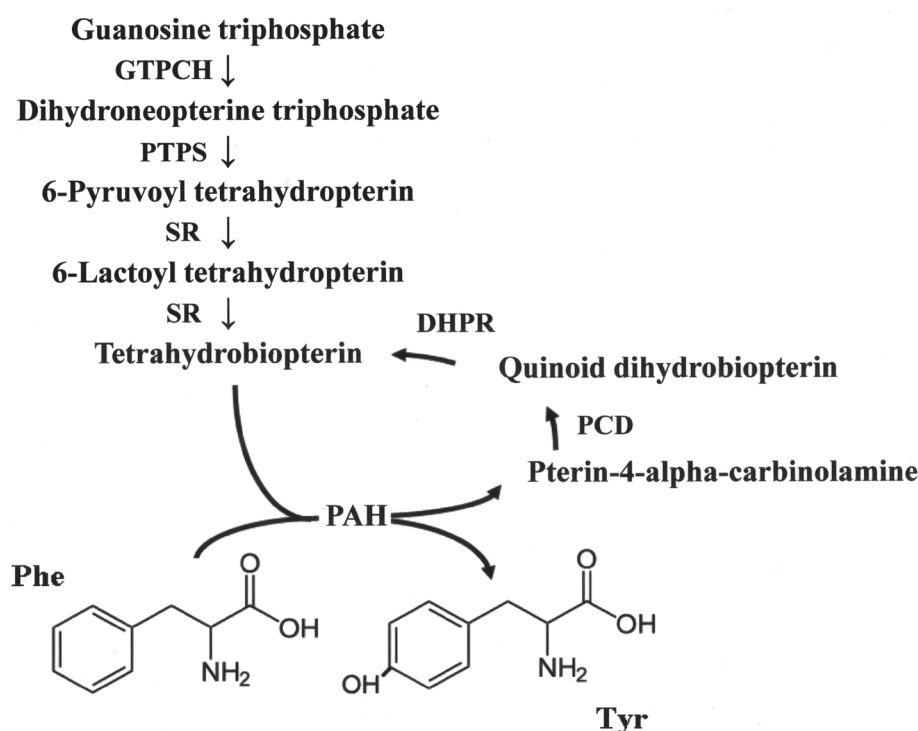


Fig. 1 Biosynthesis and regulation of tetrahydrobiopterin (BH4).

GTPCH: GTP cyclohydrolase (EC 3.5.4.16); PTPS: 6-pyruvoyl tetrahydropterin synthase (EC:4.2.3.12); SR: sepiapterin reductase (EC 1.1.1.153); PCD: pterin-4-alpha-carbinolamine dehydratase (EC:4.2.1.96), DHPR; dihydropteridine reductase (EC 1.5.1.34); PAH: phenylalanine hydroxylase (EC 1.14.16.1)

of Phe were nearly equal to Tyr (0.93 on average). It was natural that the ratio of Phe to Tyr was markedly higher in the patient (48.26). In addition, this patient's plasma levels of other aromatic amino acid (Trp) and branched-chain amino acids (Val, Ile, and Leu) were significantly lower than those from the 49 other patients (Table 1).

### 3. Discussion

A newborn mass-screening program for the early detection of phenylketonuria (PKU), maple syrup urine disease (MSUD), homocysteinemia,

histidinemia, and galactosemia, using filter paper blood specimens, was started throughout Japan in 1977<sup>11</sup>, and the prevalence of HPA is now 1 in 82,000 live births. Because the current patient was born at that time, it is obscure whether he skipped the screening or was misdiagnosed.

Nowadays, HPA is classified into three groups: classic PKU, non-PKU HPA, and variant PKU<sup>1, 2</sup>. Classic PKU is caused by a complete or nearly-complete deficiency of PAH activity, accompanied with severe HPA of more than 1000 nmol/mL in untreated cases, which develops profound and irreversible neurological damage. Non-PKU HPA

Table 1 Assessment of protein nutritional status

	A patient with HPA	49 patients without HPA
Serum protein		
Albumin (3.9-4.9 g/dL) <sup>a</sup>	4.2	4.4 (3.4-5.2) <sup>b</sup>
TTR (23-42 mg/dL)	32.6	26.8 (16.7-40.8)
RBP (3.6-7.2 mg/dL)	5.2	3.6 (1.8-5.8)
TRF (190-320 mg/dL)	220	246 (165-409)
Plasma AAA		
Phe (43-76 nmol/mL) <sup>a</sup>	1245	52 (30-85) <sup>b</sup>
Tyr (40-90 nmol/mL)	26	59 (36-113)
Trp (37-75 nmol/mL)	33	50 (6-92)
Plasma BCAA		
Val (150-310 nmol/mL) <sup>a</sup>	146	213 (123-353) <sup>b</sup>
Leu (78-180 nmol/mL)	56	109 (47-176)
Ile (40-110 nmol/mL)	32	59 (24-92)

<sup>a</sup>Reference range (RR), <sup>b</sup>median (and range).

TTR: transthyretin; RBP: retinol-binding protein; TRF: transferrin.

AAA: aromatic amino acid; BCAA: branched-chain amino acid.

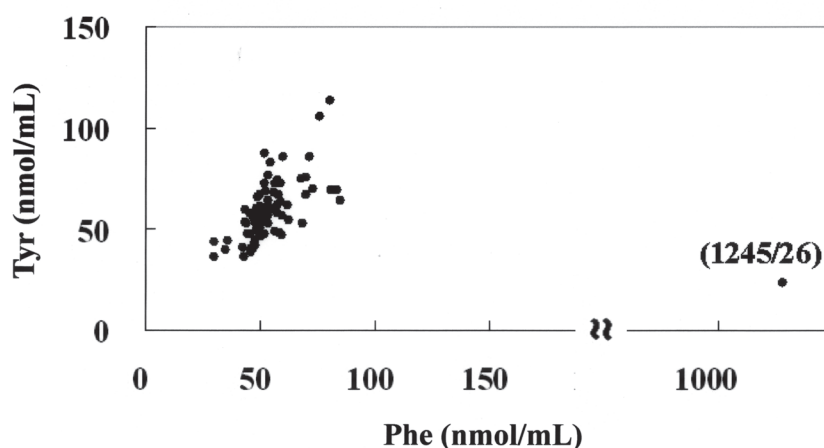


Fig. 2 Comparison of plasma concentrations of Tyr to Phe in a patient (in parentheses) with HPA, and 49 patients without HPA.

exhibits mild HPA (120-1000 nmol/mL) and in those who have it about 35% of PHA activity remains<sup>4</sup>. HPA in variant PKU is caused by BH4 deficiency. Prevalence of the two former types is considered to be among 98% of cases and that of variant PKU is 2%<sup>2</sup>. Although the BH4 loading test<sup>2,4</sup> was not performed, we considered that our patient had classic PKU given the evidence such as his higher plasma Phe concentration (>1000 nmol/mL) and the fact that the worldwide prevalence of BH4 is low.

A significant observation of this patient is that he had normal melanin (a polymer of Tyr) metabolism as observed from his hair pigmentation. His plasma Tyr which came directly from food and not from Phe, was 26 nmol/mL (65% of the lower limit) that capable of fulfilling the melanin synthesis. In addition, serum concentrations of albumin (Genbank accession number: AAA98797), TTR (NP\_000362), RBP (NP\_006735), and TRF (AAA 61140), which respectively comprised 18, 20, 8, and 26 mol of Tyr<sup>12</sup>, were all observed as within the normal limit. Tyr is also indispensable for the configuration of tetrameric TTR<sup>13</sup>. Other defected amino acids (i.e., Val, Ile, Leu, and Trp) in the patient were necessary for the synthesis of somatic and visceral protein. At this time, we conclude that melanin metabolism and protein nutrition were barely maintained by such levels of amino acids. However, a close relationship between plasma amino acid levels and serum albumin, together with rapid turnover proteins (i.e., TTR, RBP, and TRF) was left to be further investigated in the 49 patients. It will be reported in the future.

Individuals with classic HPA are reported to tolerate less than 300 mg/day of dietary Phe, but our patient had been taking intolerable amounts of Phe (ca. 600 mg/day)<sup>1,2</sup>. Therefore, to prevent further disease progression, dietary management, i.e., dietary restriction of Phe, must be started for the patient while ensuring that he does not succumb to protein malnutrition, because almost all meat foodstuff and dairy products are composed of equimolar Tyr to Phe.

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