Two siblings with vitamin B6-nonresponsive cystathionine β-synthase deficiency and differing blood methionine levels during the neonatal period

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Abstract : We present two siblings with vitamin B6-nonresponsive homocystinuria due to a deficiency of cystathionine β-synthase who had different levels of methionine in the blood during the neonatal period, even though they had the same genetic defect. One of them was missed in the screening of newborns for homocystinuria. Special care should be taken in screening neonates for homocystinuria using the blood level of methionine. J. Med. Invest. 44 : 95-97, 1997

Key Words : *homocystinuria, newborn screening, cystathionine β-synthase, methionine, homocyst(e)ine*

INTRODUCTION

It had been thought that patients with homocystinuria, due to cystathionine β-synthase (CBS) deficiency, showed elevated concentrations of both homocyst(e)ine and methionine in the blood and urine even in the neonatal period. For this reason, the blood level of methionine is routinely measured in neonates in Japan in the mass screening for CBS deficiency. We report two siblings with vitamin B6-nonresponsive CBS deficiency with differing blood methionine levels, one of which was missed in the screening of newborns for homocystinuria.

PATIENTS

Case 1 : A 9-year-old boy had CBS deficiency, as previously reported(1). His parents were not consanguineous. He was fed with normal milk formula and his protein intake was about 2.5 g/kg body weight/day at 5 days old. Although hypermethioninemia was detected on routine screening at 5 days old, diagnosis of homocystinuria was not made until he was 3 months old because of undetectable urinary levels of homocystine, and of undetectable plasma levels of non-protein-bound homocyst(e)ine. Homocystine was detected in urine at 3 months old and a marked reduction in CBS activity was observed in cultured skin fibroblasts (Table 1 and 2). A marked elevation of total plasma homocyst(e)ine was present at the age of 19 and 59 days, as determined in specimens that had been frozen at -20℃ for 3 years (71.7 and 66.9 μmol/l, respectively ; normal 6.4-10.2 μmol/l) (Table 1). Since this patient did not respond to high dosages of vitamin B6 (500 mg daily for 10 days), a low methionine diet and betaine were initiated. His subsequent physical and psychomotor development was normal.

Case 2 : A 7-year-old girl, the younger sister of case 1. She had been delivered after 41 weeks of gestation with no complication. She weighted 3,352 g at birth. She was fed with normal milk formula and her protein intake was about 2.5 g/kg body weight/day at 5 days old. However, her blood concentration of methionine was below the normal cut-off value in mass screening at 5 days old ; i.e., less than 67 μmol/l (Table 1). While her physical development was normal, she exhibited retardation, especially of speech development. At the age of 2.5 years she visited Tokushima University Hospital. At that time her development quotient was 57. A test for cyanide-nitroprusside in urine was positive. Analysis of amino acids in plasma and urine gave the following values : plasma methionine and plasma total homocyst(e)ine, 556 μmol/l (normal ; 20-50 μmol/l) and 95.7 μmol/l (normal ; 6.4-10.2 μmol/l) ; urinary levels of methionine and homocystine, 0.59 μmol/mg creatinine (normal ; trace-0.10 μmol/mg creatinine) and 1.03 mmol/mg creatinine (normally undetectable). No activity of CBS was detected in cultured skin fibroblasts (Table 2). Since the patient failed to respond to high doses of vitamin B6 (500 mg daily for 8 days), she was administered a low methionine diet and betaine. Her IQ rose from 57 at age of 2.5 years to 99 at age of 6.5 years.

DISCUSSION

Newborn infants with homocystinuria are classified into three groups according to the blood level of methionine and the urinary level of homocystine : Group 1 - patients with hypermethioninemia and homocystinuria in the

Received for publication July 3, 1997 ; accepted July 22, 1997.

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[2] Abbreviation used in this paper : CBS ; Cystathionine β-synthase

Table 1. Biochemical features of two siblings with homocystinuria during neonatal period and childhood

	Neonatal period			Childhood[f]	
	Case 1		Case 2	Case 1	Case 2
Age	5 D	19 D	5 D	1-3 M	2.5 Y
Blood					
methionia[a] (μmol/l)	536	1,421	<67[e]	1,495-1,796	556
total homocyst(e)ine[b] (μmol/l)	n.e.[c]	71.7[d]	n.e.	66.9 d	95.7
Urine					
cyanide-nitroprusside test	n.e.	negative	n.e.	negative-positive	stongly positive

a : normal range ; 20-50 μmol/l
b : normal range ; 6.4-10.2 μmol/l
c : not examined
d : specimens frozen at-20℃ for 3 years
e : below cut off value for normal in neonatal screening
f : before treatment

Table 2. Cystathionine β-synthase activities in cultured skin fibroblasts

	Cystathionine β-synthase activity (nmol of formed cystathionine/ hr/ mg protein)
Case 1	0.065
Case 2	n.d.[a]
Controls (n=7)[b]	4.55±2.13 (2.90-8.30)[c]

a : not detectable
b : number of subjects
c : range

neonatal period (2) ; Group 2 - patients with hypermethioninemia but no homocystinuria in the neonatal period, but who clearly excrete homocystine in urine later (1, 3), and Group 3 - patients with a normal serum concentration of methionine in the neonatal period, but who subsequently exhibit hypermethioninemia and homocystinuria (3). The metabolism of methionine and homocyst(e)ine is catalyzed in the fetus and adults by several enzymes with quantitatively differing activities. The specific activities of methionine adenosyltransferase and betaine-homocysteine methyltransferase are lower in the fetal liver than the adult liver, whereas the activity of 5-methyltetrahydrofolate-homocysteine methyltransferase is higher in fetal tissue than adult tissue (4, 5). This fetal enzyme pattern should direct a large portion of the available homocysteine to the 5-methyltetrahydrofolate-dependent methylation cycle rather than toward the synthesis of cystathionine, then change to an adult pattern during development. The failure to detect homocystinuria or hypermethioninemia in some newborn infants with a deficiency of CBS may therefore be explained by a quantitative difference in the activities of enzymes involved in the metabolism of methionine and homocysteine for first few weeks or months after birth (6).

Case 1 belonged to Group 2, while case 2 belonged to Group 3. Hypermethioninemia was found during the neonatal period in case 1, whereas the urinary excretion of homocystine was so slight that it was undetected until the age of 3 months. It is known that homocystine is easily bound to plasma protein (7, 8). No plasma non-protein-bound homocystine was detected in case 1, even though the plasma level of total homocyst(e)ine had increased markedly (close to a 10 fold increase over the normal level) at 19 and at 59 days old. Several patients with a deficiency in CBS had no detectable or only mild increases in plasma non-protein-bound homocyst(e)ine when the plasma level of protein-bound homocyst(e)ine was 10-fold higher than the normal level (7, 9).

No examination of homocyst(e)ine was performed in case 2 during the neonatal period, because her methionine level was not elevated. The assay of enzyme activity in the cultured skin fibroblasts confirmed a deficiency in CBS. Most vitamin B6-responsive patients with CBS deficiency are missed on newborn screening, because the blood level of methionine is not sufficiently elevated in the plasma during the first few days of life (6). However, our patients did not have a vitamin B6-responsive form of CBS deficiency.

As shown by our patients, their blood methionine levels in the neonatal period seemed to fluctuate, even though they were siblings with the same genetic defect. These findings indicated that in homocystinuric neonates the blood levels of methionine were determined not only by the genetic defect in CBS, but also by the activity of other enzymes involved in methionine metabolism and dietary intake of methionine. Poor protein intake can be excluded as a cause in our patient. Therefore, we suggest that the activities of enzymes other than CBS in methionine metabolism probably differed in the neonatal period in these patients. Thus, genotype may not always be consistent with phenotype in patients with inherited diseases (10).

Considering its position in the metabolic pathway, homocyst(e)ine is nearest to CBS, whereas methionine is distal to it. Accordingly, we suggest that a defect in CBS leads to a more obvious rise in homocyst(e)ine vs. that of methionine. It is probable that blood homocyst(e)ine closest to the block at this enzyme had increased in the neonatal period in case 2, despite a normal blood level of methionine. Therefore, in the newborn screening of CBS deficiency, a new method for determining the blood level of homocyst(e)ine should be developed and evaluated properly to avoid missing patients with CBS deficiency.

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