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Variant clinical courses of 2 patients with neonatal intrahepatic cholestasis who have a novel mutation of *SLC25A13*

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Abstract

Deficiency of citrin due to mutations of the *SLC25A13* gene causes not only adult-onset type II citrullinemia, but also neonatal intrahepatic cholestasis. Neonatal intrahepatic cholestasis is a self-limiting condition and spontaneously disappears by 12 months of age without special treatment. The natural history of patients with *SLC25A13* mutations is not clear. Two patients with infantile hepatic dysfunction were found to have a novel mutation of the *SLC25A13* gene. DNA analyses of *SLC25A13* disclosed that the first patient was a compound heterozygote for the Ex16+74_IVS17-32del516 (del516-Ex16/IVS17) and IVS11+1G→A mutations and the second one a homozygote for the del516-Ex16/IVS17 mutation. It is predicted that the 516–base pair deletion mutation leads to a frameshift from codons 556 to 564, a premature termination at codon 565, and a truncated form of the citrin protein (normal, 675 amino acids). The first patient had disseminated intravascular coagulation associated with hepatic dysfunction in the neonatal period. The other patient had persistent cholestatic jaundice and underwent an operation to rule out bile duct atresia. Without specific treatment, both patients had a favorable clinical course. In conclusion, citrin deficiency resulting from the mutation of *SLC25A13* presented variant clinical courses, followed by hypercitrullinemia and intrahepatic cholestasis in infancy. The conditions in the patients were self-limiting and spontaneously disappeared.

1. Introduction

Citrin encoded by *SLC25A13* is expressed abundantly in the liver [1] and functions as a Ca^{2+}-regulated aspartate-glutamate transporter in mitochondria [2]. *SLC25A13* was determined as the disease-causing gene of adult-onset type II citrullinemia (CTLN2; OMIM 603471) [1]. Several different DNA sequence alterations in the gene have been reported [1,3-8]. Citrin deficiency causes not only CTLN2, but also neonatal intrahepatic cholestasis (NICCD; OMIM 605814) [4,9]. Adult-onset type II citrullinemia is characterized by the sudden appearance of behavioral aberrations, restlessness, disorientation, and coma, which can occur at any age (11-79 years) in life, but usually occurs in adulthood [1,3,4,7,9-11]. However, NICCD is characterized by intrahepatic cholestasis and shows milder symptoms without specific treatment [5,8,12-17], except in some cases [15]. Some of the individuals carrying *SLC25A13* gene mutations in both alleles, however, may develop CTLN2 with neuropsychiatric symptoms several decades later [9,11,14].

Here, we described 2 patients with NICCD who have a novel mutation of the *SLC25A13* gene, who had variant clinical courses in the neonatal period and infancy. The first patient is a compound heterozygote whose genotype included the common mutation in combination with the presently described novel one. The second patient is a

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Table 1
Laboratory data on admission

	Patient 1	Patient 2	Reference value
SLC25A13 mutation	II/XX	XX/XX	
Total bilirubin level (mg/dL)	4.3	11.9	3-21
Direct bilirubin level (mg/dL)	2.4	7.4	<12
Aspartate aminotransferase (IU/L)	147	228	12-30
Alanine aminotransferase (IU/L)	26	119	3-24
Alkaline phosphatase (IU/L)	906	5031	107-323
γ-Glutamyl transpeptidase (IU/L)	71	254	8-45
Total bile acid (μmol/L)	235	325	<10
Antithrombin III (%)	34	36	80-120
FDP dimer (μmol/L)	2.7	ND	<0.8
Fibrinogen (mg/dL)	148	86	150-350
APTT (s)	65	38	23-32
Prothrombin time (%)	19	51	78-100
PIVKA II (mAU/mL)	<10	46	<10
RBC (per μL)	257×10^4	395×10^4	400×10^4 to 510×10^4
Hb (g/dL)	8.7	12.2	10.7-19.0
Ht (%)	26	38	34-60
WBC (per μL)	39 400	12 700	5.0×10^3 to 20.0×10^3
Platelet (per μL)	7700	28.5×10^4	16.0×10^4 to 34.0×10^4
Blood gas analysis			
pH	7.21	ND	7.33-7.43
P_{CO_2} (mm Hg)	24.1	ND	32-45
P_{O_2} (mm Hg)	61.3	ND	80-100
HCO_3^- (mmoL/L)	9.5	ND	21-27
Base excess (mmoL/L)	−16.5	ND	−2.0 ± 2.0

SLC25A13 mutations II and XX were IVS11+G→A and del516-Ex16/IVS17, respectively.
FDP indicates fibrin degradation products; APTT, activated partial thromboplastin time; PIVKA II, protein induced by vitamin K absence; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; WBC, white blood cell count; ND, not done.

homozygote for the new mutation, which defines a clinical picture of the novel abnormality.

2. Case reports

2.1. Patient 1

A boy was born to a gravida II, 31-year-old mother at 38 weeks of gestation by cesarean delivery. The parents were unrelated. His birth weight was 2825 g, and the Apgar scores were 5 (1 minute) and 9 (5 minutes), respectively. The placenta was adhered to surrounding tissues but not ruptured. Because of a massive bleeding from the placenta at delivery, the patient had hypovolemic shock. Laboratory data on admission (23 hours of age) were compatible with disseminated intravascular coagulation (DIC) (Table 1). Serum ammonia concentration was normal (77 μg/dL), and metabolic acidemia and hyperbilirubinemia persisted. The patient was treated with an intravenous frozen plasma and blood transfusion and fully recovered from DIC at 70 days of life, but metabolic acidosis persisted. Hypergalactosemia (11.1 mg/dL by the Guthrie test) was detected at 39 days of age by neonatal mass screening. Conventional treatment of metabolic acidosis and feeding with lactose-free formulas were effective. Serum citrulline level elevated markedly to 104 nmol/mL (reference range, 9-29 nmol/mL) at 35 days of age with a decreased Fischer [(valine + leucine + isoleucine) (tyrosine + phenylalanine)] ratio of 1.30 (reference range, 2.43-4.40) (Table 2) [18].

2.2. Patient 2

A girl weighing 2364 g at birth was born to a 24-year-old primigravida mother at 39 weeks of gestation by spontaneous delivery. Apgar scores were 9 (1 minute) and 10 (5 minutes), respectively. Family history showed no record of hepatic or neurological diseases, and her parents were not related. Results of neonatal screening (at 5 days of age) for metabolic diseases (galactosemia, homocystinuria, phenylketonuria, and maple syrup urine disease) and endocrine diseases (hypothyroidism and adrenal hyperplasia) were negative. She was referred to us at 65 days of age because of hyperbilirubinemia. Her weight was 3866 g, and her height was 51.4 cm. Her appetite and weight gain were poor. She had been breast-fed and on artificial milk for the first 2 months of life, but only on artificial milk thereafter. Pertinent laboratory findings were shown in Table 1. There was no serological evidence of hepatitis-related viral infection. The patient received a nonspecific diagnosis of neonatal hepatitis. The patient underwent a liver biopsy at 75 days of age. The specimen showed no giant cell transformation of hepatocytes or fatty liver,

Table 2
Amino acid (nmol/mL) analysis

Age	Citrulline	Methionine	Phenylalanine	Tyrosine	Arginine	Serine	Citrulline/serine	Fischer ratio
Patient 1								
Plasma amino acid analysis (mo)								
1	104	48	33	134	123	292	0.36	1.30
8	33	60	99	160	90	197	0.17	2.80
13	38	92	62	72	82	142	0.27	5.17
23	34	49	83	179	71	148	0.23	2.63
36	34	26	68	107	56	46	0.74	2.74
Patient 2								
Paper blood amino acid analysis								
2 d	41	25	37	98	40	135	0.30	ND
6 mo	111	135	46	61	48	226	0.49	3.25
Plasma amino acid analysis (mo)								
16	16	35	72	70	52	58	0.28	2.76
20	25	18	68	101	80	45	0.56	2.94
Reference value	9-29	3-29	23-69	11-122	11-65	24-172	0.09-0.30	2.43-4.40

Reference values adopted from Soupart P. In: Hoden JT, editor. *Amino acid pools*. Amsterdam: Elsevier; 1962. p. 220.

although mild inflammatory infiltration and bile ducts were relatively scanty.

3. DNA diagnosis of known mutations and identification of a novel mutation

Informed consent for genetic analysis was obtained from their parents. Genetic analysis was authorized by the Medical Ethics Committee, Faculty of Medicine, Kagoshima University.

The procedure for DNA diagnosis of *SLC25A13* mutations was previously reported in detail [1,3,4]. Nine *SLC25A13* found frequently in Japanese patients with citrin deficiency [6,7,9] were screened by using a genetic analyzer with GeneScan software and a single primer extension procedure (SNaPshot) [4]. Of them, mutation (II): IVS11+1G→A was detected only in an allele of patient 1 by using the SNaPshot method (Fig. 1A). On the other hand, patient 2 showed no polymerase chain reaction (PCR) bands derived from 2 mutations ([III] in exon 16 and [VI] in

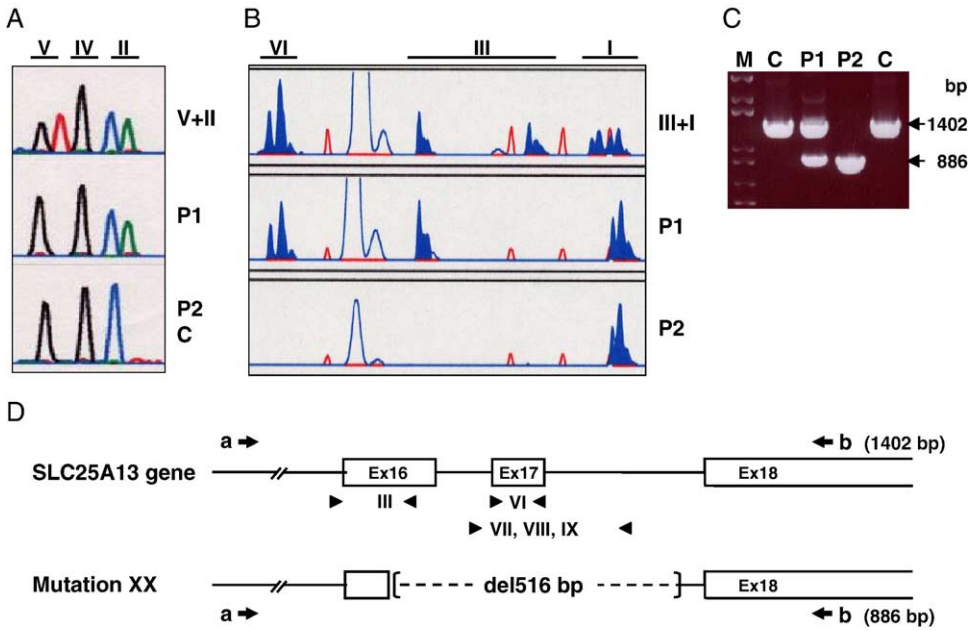

Fig. 1. DNA diagnosis of known mutations and identification of a novel mutation in *SLC25A13* gene. Detection of 3 mutations: II, IVS11+1G→A; IV, S225X; V, IVS13+1G→A, by SNaPshot method (A); I, 851del4; III, 1638ins23; VI, 1800ins1, by GeneScan method (B) [4]. C, Detection of a novel deletion mutation, XX: del516 bp, by agarose gel (1.2%) electrophoresis of the PCR products amplified with genomic DNA and primer set a/b, as shown in D. D, Schema of a novel mutation, XX: Ex16+74_IVS17-32del516, and other known mutations found in the region of exons 16 to 18. Combinations of arrowhead indicate primer sets to detect known mutations (see Reference [4]). Abbreviations: P1, patient 1; P2, patient 2, C, control; M, DNA size marker.

exon 17) in GeneScan method to detect 3 mutations (I, III, and VI) simultaneously (Fig. 1B) and also no bands (data not shown) in SNaPshot method to detect 3 mutations (VII, VIII, and IX) in exon 17 (see Fig. 1D). These results suggest that patient 2 has a deletion mutation in the region involving exons 16 and 17.

To identify the unknown mutations, regions containing exons 16 to 18 of the *SLC25A13* gene were amplified by PCR using genomic DNA and suitable primer sets (a, 5′-GGTGTAACTGGAGAGGTTGG-3′; b, 5′-TGCTTCATTCCCAGGAGGGA-3′; see Fig. 1D). The agarose gel electrophoresis detected the small size of PCR products in both our patients, homozygously in patient 2 and heterozygously in patient 1 (Fig. 1C). The sequencing analysis of the small band revealed that the deleted region was 516 base pairs from +74 in exon 16 to −32 in intron 17 (Fig. 1D), designated mutation (XX): Ex16+74_IVS17-32del516 (del516-Ex16/IVS17). Finally, we found that patient 1 was a compound heterozygote for the Ex16+74_IVS17-32del516 (del516-Ex16/IVS17) and IVS11+1G→A mutations, and patient 2 was a homozygote for the Ex16+74_IVS17-32del516 mutation.

4. Clinical course

Ursodesoxycholic acid and supplementation of fat-soluble vitamins were started 5 weeks after admission and continued to 15 (patient 1) and 18 months (patient 2), respectively. Patient 1 had received lactose-free milk after 6 weeks of admission to 1 year and had a favorable clinical course. Transaminase level normalized at 10 months of age. Serum total bile acid was extremely high (235 and 123 μmol/L on admission and at 1 month of age, respectively), persisted high (15-18 μmol/L) until 20 months of age, and normalized (7.5 μmol/L) at 36 months of age. Patient 1 had DIC and was supported by total parenteral nutrition. From 24 days of age, the patient was fed with breast milk. At 39 days of age, hypergalactosemia was suspected by mass screening and was confirmed (11.1 mg/dL). We then started feeding with lactose-free formulas. In patient 2, on the other hand, results of neonatal screening (at 5 days of age) for metabolic and endocrine diseases were negative. An aminogram was performed at 5 months of age to diagnose NICCD. Patient 2 was started on lactose-free milk at 6 months of age, which was discontinued 3 months later. Patient 2 still had a persistent increase in serum aspartate aminotransferase (140-200 IU/L), alanine aminotransferase (60-100 IU/L), type IV collagen (440-670 ng/mL; reference range, 0-150), and hyaluronic acid (87-94 ng/mL; reference range, 0-50) at 16 months of age. At 20 months of age, liver function normalized without special therapy. Two patients have been followed for 4 and 2 years, respectively, and both are alive and show no developmental delay or neurological abnormalities. There is no tendency for these patients to show an extraordinary craving for protein/lipid-rich food such as beans, peanuts, egg, fish, and beef and aversion for glucose or sweets as was previously reported [7,9,10]. It is not clear whether the patients have particular craving for some foods because their eating patterns and habits have not developed during the weaning period.

The changes of concentration of relevant amino acids are shown in the Table 2. In both patients, highly elevated serum citrulline concentrations were observed at 1 month (patient 1) and 2 days (patient 2) of life, respectively. However, the concentrations decreased at 1 year of age (Table 2).

5. Discussion

Two patients with infantile hepatic dysfunction were found to have a novel mutation of the *SLC25A13* gene. Clinical features of our patients were similar to those infants with *SLC25A13* mutation who presented with intrahepatic cholestasis in the neonatal period [5,8,12-17], except that patient 1 had bleeding tendency with DIC. The effect of citrin deficiency may begin during the intrauterine period, such as intrauterine growth retardation or blood coagulation defect. Patient 2 had failure to thrive. The levels of transaminase were persistently elevated, and alkaline phosphatase was extremely high. Newborn mass screening for citrin deficiency should be realized for early detection of NICCD as well as for prospect of CTLN2 [17]. Ohura et al [12] reported that a patient with NICCD and with galactosemia showed normal activities of galactose-metabolizing enzymes. Galactose metabolism does not seem to involve nicotinamide adenine dinucleotide (NAD) as cofactor. Uridine diphosphate galactose 4-epimerase, however, requires NAD^+ and is inhibited by NADH. This property of the enzyme may be the cause of galactosemia in patients with NICCD.

Most of the patients with NICCD showed milder symptoms by 12 months of age without special treatment other than feeding programs [8,12-16]; formulas containing middle-chain triglyceride or lactose-free formula and supplementation of fat-soluble vitamins have been used. Lack of bile acid induces malabsorption of lipid from intestines. Lactose-free formula or middle-chain triglyceride milk is easily absorbed and prevents malnutrition.

The aminogram of patient 1 showed elevations not only of serum citrulline, but also of methionine, threonine, tyrosine, and arginine. Because branched-chain amino acid concentrations were decreased, Fischer ratio was decreased [18]. Tamamori et al [17] reported that citrulline-serine ratio is also increased and serves as a marker of NICCD. In patient 1, citrulline-serine ratio was moderately high at 1 month of age, but became normal after 8 to 23 months. At 36 months of age, the ratio increased again, which might be explained by the decrease of the serine value. The level of transaminase in patient 2, which remained elevated till

16 months of age, was normalized at 20 months of age without specific treatment. Some metabolisms in the early infancy might have changed during 6 to 8 months when weaning was started.

The pathogenesis of transient and self-limited cholestasis in these infants with *SLC25A13* mutations remains unsolved. Citrin, a liver-type mitochondrial aspartate-glutamate carrier, is an essential component of the malate-aspartate NADH shuttle [2,7,9]. Deficiency of citrin may lead to an increase in cytosolic NADH/NAD^+, which induces metabolic abnormalities [9]. Citrin deficiency alone may not be sufficient to produce a CTLN2-like phenotype in knockout mice [19]. *SLC25A13* gene mutations and additional environmental and/or genetic factors may modify the symptoms of NICCD. Elucidation of the function of citrin should permit a fuller understanding of these phenomena.

We presume that some individuals with citrin deficiency may receive an erroneous diagnosis, such as infantile hepatitis. Liver dysfunction attributed to hypercitrullinemia may also induce bleeding tendency around the neonatal period. Deficiency of citrin should also be included in the differential diagnosis of NICCD or bleeding tendency. Neonatal intrahepatic cholestasis can be diagnosed by an analysis of the DNA mutation. For a patient with NICCD, an operation should not be performed to confirm biliary duct atresia because the condition is self-limiting and spontaneously disappears.

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