J Korean Med Sci 2007; 22: 952-6
ISSN 1011-8934
DOI: 10.3346/jkms.2007.22.6.952

Neonatal Intrahepatic Cholestasis Caused by Citrin Deficiency in Korean Infants

Citrin is a liver-type mitochondrial aspartate-glutamate carrier encoded by the *SLC25A13* gene, and its deficiency causes adult-onset type II citrullinemia and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). Here, the authors investigated clinical findings in Korean infants with NICCD and performed mutation analysis on the *SLC25A13* gene. Of 47 patients with neonatal cholestasis, three infants had multiple aminoacidemia (involving citrulline, methionine, and arginine) and galactosemia, and thus were diagnosed as having NICCD. Two of these three showed failure to thrive. The laboratory findings showed hypoproteinemia and hyperammonemia, and liver biopsies revealed micro-macrovesicular fatty liver and cholestasis. The three patients each harbored compound heterozygous 1,638-1,660 dup/ S225X mutation, compound heterozygous 851del4/S225X mutation, and heterozygous 1,638-1,660 dup mutation, respectively. With nutritional manipulation, liver functions were normalized and catch-up growth was achieved. NICCD should be considered in the differential diagnosis of cholestatic jaundice in Korean infants.

Key Words : *Cholestasis; Citrin; Citrullinemia; SLC25A13; Mutation*

Jae Sung Ko, Jung Han Song*, Sung Sup Park*, Jeong Kee Seo

Departments of Pediatrics and Laboratory Medicine*, Seoul National University College of Medicine, Seoul, Korea

Received : 15 January 2007
Accepted : 15 March 2007

Address for correspondence
Jeong Kee Seo, M.D.
Department of Pediatrics, Seoul National University Hospital, 28 Yeongeon-dong, Jongro-gu, Seoul 110-744, Korea
Tel : +82.2-2072-3627, Fax : +82.2-743-3455
E-mail : jkseo@snu.ac.kr

*This study was supported by grant no. 0420060800 from SNUH Research Fund.

INTRODUCTION

The *SLC25A13* gene on chromosome 7q21.3 is known to be responsible for adult-onset type II citrullinemia (CTLN2). The *SLC25A13* gene consists of 18 exons and encodes a liver-type mitochondrial aspartate-glutamate carrier named citrin (1, 2), which plays an important role in malate-aspartate NADH shuttling, urea synthesis, and gluconeogenesis. CTLN2 is characterized by late-onset hyperammonemia and neuropsychiatric symptoms, such as disorientation, delirium, delusion, and disturbed consciousness (3). Ohura et al. (4) and Tazawa et al. (5) demonstrated that mutations in the *SLC25A13* gene cause neonatal intrahepatic cholestasis and transient citrullinemia, which has been designated as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD; OMIM 605814) (6). NICCD is characterized by multiple amino acidemia (involving citrulline, threonine, methionine, arginine, and tyrosine), galactosemia, hypoproteinemia, hypoglycemia, cholestasis, and fatty liver. Most NICCD patients show ameliorated symptoms within 1 yr of age and require no further special treatment other than feeding programs. However, several decades later some patients develop CTLN2 (7, 8).

Initially, NICCD was identified exclusively in the Japanese population, but recently the disease has been reported in Palestinians (9), Vietnamese (10), and Taiwanese infants (11). Furthermore, a population analysis showed that the carrier rate of mutations in *SLC25A13* among Koreans was 1 in 112 (11). There has been no report of NICCD in Korea. In the present study, the authors investigated clinical findings of Korean infants with NICCD and performed mutation analysis on the *SLC25A13* gene.

MATERIALS AND METHODS

Patients

Between 2005 and 2006, 47 patients were admitted to the Department of Pediatrics in Seoul National University Children's Hospital for neonatal cholestasis. Examinations included abdominal ultrasonography, duodenal intubation, a hepatobiliary scan, and liver biopsy. Total parenteral nutrition, drug-related cholestasis, and cholestasis secondary to sepsis were excluded.

NICCD was diagnosed based on the presence of hyperamino acidemia, galactosemia, fatty liver, and on the results of the genetic study detailed below. Patients with NICCD were followed until at least 18 months of age.

Genetic study

Genomic DNA was extracted from peripheral blood using Wizard genomic DNA purification kits, according to the manufacturer's instructions (Promega, Madison, WI, U.S.A.). Informed consent was obtained from all parents. Mutations of the *SLC25A13* gene were detected by using previously described primers (11, 12). Seven different primer sets were used to detect the 12 known mutations of *SLC25A13* by polymerase chain reaction (PCR), namely, IVS8F2 and Ex9B, mutation [I] (851del4); Ex11F2 and IVS11Bm2, mutation [II] (IVS11+1G>A); IVS15F2 and Ex16B, mutation [III] (1,638-1,660 dup); IVS6F and IVS7B, mutation [IV] (S225X); Ex13F and IVS13Bm, mutation [V] (IVS13+1G>A); IVS16F and IVS17B, mutation [VI] (1800ins1), mutation [VII] (R605X), mutation [VIII] (E601X), and mutation [IX] (E601K); IVS5NF and IVS6NB, mutation [X] (IVS6+5G>A), mutation [XI] (R184X), and mutation [XIV] (IVS6+1G>C). PCR was performed using 1 cycle at 94℃ for 1 min, 30 amplification cycles (94℃ for 30 sec, 50℃ for 30 sec, and 72℃ for 30 sec), followed by 72℃ for 5 min. Forward and reverse strands of PCR products were direct-sequenced using the above-mentioned PCR primers on the ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). Sequences obtained were compared with the reference sequence NM-014251 registered in the National Center for Biotechnology Information database (http://www. ncbi.nlm.nih.gov).

Table 1. Differential diagnosis of neonatal cholestasis

Diagnosis	No (%)
Extrahepatic biliary atresia	11 (23)
TORCH infection	4 (9)
NICCD	3 (6)
Progressive familial intrahepatic cholestasis	2 (4)
ARC syndrome	2 (4)
Dubin-Johnson syndrome	2 (4)
Alagille syndrome	1 (2)
Non-syndromic bile duct paucity	1 (2)
Idiopathic	21 (45)

NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; ARC syndrome, arthrogryposis, renal dysfunction, cholestasis syndrome.

RESULTS

Among the 47 patients with neonatal cholestasis presented during the two-year period, extrahepatic biliary atresia was diagnosed in 11, congenital infection with TORCH in 4, progressive familial intrahepatic cholestasis (PFIC) in 2, ARC (arthrogryposis, renal dysfunction, cholestasis) syndrome in 2, neonatal Dubin-Johnson syndrome in 2, Alagille syndrome in 1, and non-syndromic bile duct paucity in 1. No etiology was identified in 21 patients (Table 1).

NICCD was diagnosed in three patients. Table 2 summarizes the genotype and clinical and laboratory findings of these three patient. Birth weights ranged from 2.7 to 3.1 kg. The chief complaint was cholestatic jaundice in all patients, and all were referred for suspected neonatal hepatitis or biliary atresia. Two infants were referred at 3 months of age and one infant at 7 months. Two infants showed failure to thrive at initial presentation. Newborn tandem mass screening performed on case 2 at 1 week of age was normal. Total serum protein levels were reduced in two patients. Blood ammonia levels were slightly increased in all three, and gamma glutamyltranspeptidase and alkaline phosphatase were markedly elevated in all three, whereas alanine aminotransferase levels were slightly increased in two. Hypoglycemia was present in case 1. Alpha-fetoprotein levels were high and galactosemia was detected in all patients. Plasma amino acid analyses showed that citrulline, methionine, and arginine were significantly elevated in all three. Plasma tyrosine, threonine, and lysine were elevated in two (Table 3). No patient had a bleeding tendency or a cataract.

Table 3. Plasma amino acid and galactose levels in the 3 NICCD patients

Case	Citrulline	Methionine	Threonine	Arginine	Tyrosine	Lysine	Galactose
1	301	73	553	397	215	332	162
2	186	106	216	215	248	246	14
3	93	64	168	120	100	166	26
Reference	8-47	3-43	20-204	12-112	19-119	66-270	<8

NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; amino acid, μM/L; galactose, mg/dL.

Table 2. Clinical and laboratory findings, and the genotypes of the 3 NICCD patients

Case	Age (mo)	Weight (kg)	TB/DB (mg/dL)	AST/ALT (IU/L)	γ-GT (IU/L)	Protein (g/dL)	NH_3 (μg/dL)	α-fetoprotein (ng/mL)	Genotype
1	3	5.7	5.4/3.2	145/48	135	5.2	109	84,000	III/(-)
2	3	4.6	5.2/3.2	238/158	263	5.1	89	29,500	I/IV
3	7	6.4	4.5/2.7	115/70	148	6.1	76	44,700	III/IV
Normal value			<1.2/<0.5	<40/<40	< 63	>6.0	<75	<55	

NICCD, neonatal intrahepatic cholestasis caused by citrin deficiency; TB, total bilirubin; DB, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
SLC25A13 mutations I, III, and IV are 851del4, 1,638-1,660 dup, and S255X, respectively.

Liver biopsy was performed in two patients. Cholestasis and micro-macrovesicular fatty change were observed in these two, and moderate periportal fibrosis was present in case 3.

Five of the six alleles (83%) examined showed mutations. In detail, compound heterozygous 1,638-1,660 dup/S255X mutation (mutation [III]/[IV]), compound heterozygous 851del4/ S225X mutation (mutation [I]/[IV]), and heterozygous 1,638-1,660 dup mutation (mutation [III]) were found in each patient. The allele frequencies of mutations [I], [III], and [IV] were 17%, 33%, and 33%, respectively.

Lactose-free soy formula and a medium chain triglyceride (MCT) formula were fed to all patients. Fat-soluble vitamins were added. Within 2 months of diagnosis, plasma amino acid profiles were normalized, whereas liver functions were nearly normalized at the age of 9 to 13 months. At the age of 18 months, catch-up growth had been achieved by all three patients.

DISCUSSION

Citrullinemia is categorized as CTLN1 (classical neonatal onset) or as CTLN2 (adult onset). CTLN1 is an autosomal recessive disease that is caused by a deficiency of arginiosuccinate synthetase, and is characterized by a neonatal-onset and severe hyperammonemia, irritability, lethargy, poor feeding, and tachypnea (13). On the other hand, CTLN2 is an autosomal recessive disease caused by citrin deficiency, and is characterized by late onset (11 to 79 yr), frequent attacks of hyperammonemia, and mental derangement, and leads to death within a few years from the onset (3). The *SLC25A13* gene, which encodes citrin, has been found to be defective in CTLN2. In the liver, citrin participates in the urea cycle by supplying aspartate from mitochondria to the cytosol for the incorporation into argininosuccinate. Lack of aspartate for argininosuccinate synthesis under citrin deficiency probably causes citrulline accumulation (14). Previous DNA analyses have shown that some infants with idiopathic neonatal hepatitis harbor the same mutations in *SLC25A13* as CTLN2 patients (4, 5), and these infants had cholestasis, hyperaminoacidemia, galactosemia, hypoproteinemia, and hepatic steatosis. On newborn tandem mass screening, about a half of NICCD patients were detected because of high citrulline, galactose, phenylalanine, and/or methionine concentrations (15, 16).

In the present study, NICCD accounted for approximately 6% (3/47) of our cohort of neonatal cholestatic liver disease. Other hereditary intrahepatic cholestasis including PFIC, Alagille syndrome, ARC syndrome, and Dubin-Johnson syndrome were found in 15%. The study shows that genetic intrahepatic cholestasis is a common cause of neonatal cholestasis in Korean infants. The initial evaluations of infants with cholestasis include ultrasonography, duodenal intubation, and percutaneous liver biopsy, in order to exclude biliary atresia. Liver biopsy is also useful for detecting Alagille syndrome, Dubin-Johnson syndrome, and PFIC. Serum γ-GT is also helpful because low levels of γ-GT in the presence of cholestasis suggest PFIC or ARC syndrome. Our data suggest that plasma amino acid analysis is useful for the screening for metabolic diseases, such as NICCD.

In the present study, NICCD patients presented with cholestatic jaundice and failure to thrive. Laboratory findings showed mild hyperammonemia, hypoproteinemia, and galactosemia. Citrin deficiency blocks the malate-aspartate shuttle, which may increase the cytosolic NADH-to-oxidized nicotinamide adenine dinucleotide ratio ($NADH/NAD^+$). Since uridine diphosphate-galactose epimerase, which requires NAD^+ as a cofactor, is strongly inhibited by NADH, a high level of NADH in liver cytosol may underlie the etiology of galactosemia in NICCD (14).

Hypoglycemia was detected in one patient. Citrin functions to provide substrates for gluconeogenesis as a component of the pathway responsible for converting amino acids to glucose (3). In the present study, plasma amino acid analysis showed that the concentrations of citrulline, methionine, threonine, arginine, and/or tyrosine were elevated. One of our three patients did not show an abnormal amino acid profile on newborn mass screening at 7 days of age. Tandem mass screening data in Japan suggest that the incidence of NICCD is approximately 1 in 34,000 (15), but the frequency of homozygotes with mutated *SLC25A13* has been estimated to be 1 in 19,000 (17). Tamamori et al. (16) demonstrated that NICCD patients with negative newborn mass screening findings have lower total amino acid levels, and that citrulline/total amino acids ratios can offset the effect of low total amino acids.

This is the first report of genetically confirmed NICCD in a Korean cohort. The mutation rate observed in three NICCD patients was 83%, which is similar to that reported in Taiwanese patients (18). In the present study, the allele frequencies of mutation [I], [III], and [IV] were 17%, 33%, and 33%, respectively. Screening of 12 mutations in Korean control individuals showed that mutation [I] and [II] were found to be most common, and mutation [III] accounted for 5% of total mutations, but mutation [IV] was absent (11). A Japanese study found that the frequency of mutation [IV] differs in NICCD patients and controls (6). However, since the number of patients with NICCD was comparatively small in the present study, further mutation studies are needed. To date only one adult case of CTLN2 has been reported in Korea (19); the patient presented with insomnia and lethargy at 55 yr of age. However, the theoretical frequency of homozygotes calculated from carrier rates in Koreans is 1 in 50,000 (11), which suggests that NICCD and CTLN2 are substantially under diagnosed in Korea.

The management of NICCD is directed toward treating the consequences of cholestasis and galactosemia. MCT formula and supplementation with fat-soluble vitamins have been used to prevent complications of prolonged cholestasis. It should be noted that lactose may be toxic to NICCD patients and should be avoided while cholestasis persists (20).

The prognosis of our patients was good because liver functions were normalized before 13 months of age and catch-up growth was achieved at 18 months. The amelioration of NICCD symptoms over a year suggests hepatocyte maturation and some adaptation or compensation by other mitochondrial carriers (14). One of our three patients showed moderate periportal fibrosis on liver histology. Mild to moderate portal fibrosis has been previously observed in NICCD (5, 7). Liver function was normalized in this patient, but his clinical course needs to be carefully monitored. One NICCD patient developed liver failure and underwent liver transplantation at 11 months of age (21). It should be noted that some NICCD patients develop CTLN2 at their twenties or even several decades later (7, 8). Although the prognosis of CTLN2 is poor, liver transplantation is remarkably effective (3). As yet, it cannot be determined which NICCD patients will later develop CTLN2, and thus, all NICCD patients should be followed for signs of development of CTLN2.

The present study indicates that NICCD should be considered in the differential diagnosis of cholestatic jaundice in Korean infants, and that plasma amino acid analysis should be included in the evaluation of infantile intrahepatic cholestasis. Moreover, the findings of *SLC25A13* genetic studies were found to be useful for confirming the diagnosis of NICCD.

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