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ORIGINAL ARTICLE

Biochemical and molecular characteristics of citrin deficiency in Korean children

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Mutations in *SLC25A13* cause citrin deficiency, which has three phenotypes: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) and adult-onset type 2 citrullinemia (CTLN2). The purpose of this study was to determine the mutation spectrum and the clinical and biochemical characteristics of citrin deficiency in Korean patients. Thirty-four patients were diagnosed with citrin deficiency based on mutations in *SLC25A13*, as verified by direct sequencing and long PCR screening of a large transposon insertion. A total of 66 alleles from 33 unrelated families of 34 patients with citrin deficiency (27 NICCD, 2 FTTDCD and 5 CTLN2) were retrospectively identified. The common pathogenic alleles were IVS16ins3kb (33%), c.851_854del (30%) and c.1177+1G>A (12%), and three novel variants were identified. Levels of citrulline, threonine, methionine, tyrosine and arginine and the threonine-to-serine ratio were higher in children with neonatal intrahepatic cholestasis caused by NICCD compared with that in patients with idiopathic neonatal hepatitis (INH). We concluded that Korean patients with citrin deficiency showed the highest frequency of the IVS16ins3kb mutation and that plasma amino-acid profiles can be used to differentiate between NICCD and INH.
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INTRODUCTION

Citrin deficiency causes adult-onset type 2 citrullinemia (CTLN2), failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD) and neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD; OMIM 605814).[1–3] NICCD is characterized by multiple aminoacidemia, galactosemia, hypoglycemia, hypoproteinemia and fatty liver in affected patients. In general, the mild cholestatic feature seen in infants with NICCD spontaneously resolves before 1 year of age without medical treatment. Adults with CTLN2 present with severe hyperammonemia, hepatic encephalopathy and liver failure, and often require liver transplants.

Initially, mutations in *SLC25A13*, on chromosome 7q21.3, were shown to cause CTLN2 by Kobayashi *et al.*[1] Soon thereafter, Ohura *et al.*[2] and Tazawa *et al.*[3] found that mutations in *SLC25A13* not only caused CTLN2 but also caused NICCD in cholestatic Japanese infants.[2,3] The pathogenic variants of *SLC25A13* were carried by 1.4% of the Japanese population.[4] A total of 95 pathogenic allelic variants have been identified.[5–7] Among the two largest cohorts, two pathogenic mutations (c.851_854del and c.1177+1G>A) were mainly seen (30–70%) in the Japanese population,[7] whereas the c.1177+1G>A mutation was rare in Chinese patients of Song's group,[8] thereby suggesting the genetic heterogeneity of *SLC25A13*.

Here, we report the clinical, biochemical and genetic features of Korean patients with citrin deficiency.

MATERIALS AND METHODS

Subject

During the period between 2006 and 2014, DNA samples from patients with citrin deficiency were collected at the Asan Medical Center with informed consent. Citrin deficiency was diagnosed based on clinical and biochemical characterization, and mutation identification. Patients were classified to NICCD, FTTDCD or CTLN2 according to clinical phenotypes. This study was approved by the Institutional Review Board of the Asan Medical Center.

Mutation identification

Citrin deficiency was confirmed by the identification of mutations in *SLC25A13*. Eighteen exons of *SLC25A13* and their intronic flanking sequences were amplified by PCR. Large transposon insertions, such as IVS16ins3kb, were identified using a method described elsewhere.[7] In this study, we sequenced the inserted sequence frame (2667 nucleotides), which is an antisense strand from C6orf68 (NM_138459). Identification of known pathogenic variants was based either on a recently updated review article written by Kobayashi *et al.*[5] or on publically available mutation databases.

Comparison with idiopathic neonatal hepatitis

Infants with idiopathic neonatal hepatitis (INH) were included as the control group. The definition of INH, which is persistent cholestatic hepatitis without apparent cause, such as infections, metabolic disorders or pancreaticobiliary anomaly, despite extensive evaluation, was based on the guidelines of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition.[9] Genetic analysis of *SLC25A13* was also performed to exclude NICCD.

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RESULTS

Patient characteristics

A total of 34 patients from 33 unrelated Korean families were diagnosed with citrin deficiency: 27 infants with NICCD, 2 older children with FTTDCD and 5 adults with CTLN2. The median age of onset for the NICCD children and the CTLN2 patients was 24 days (range: 4–45 days) and 20 years (range: 15–40 years), respectively. Twenty-one (62%) were males. Five CTLN2 patients initially presented with hyperammonemia ($n=5$) and hepatic encephalopathy. Two siblings with FTTDCD presented with nonalcoholic fatty liver disease at the age of 10.6 and 11.7 years.

SLC25A13 sequencing

A total of 11 different *SLC25A13* mutations were identified, and Table 1 shows a detailed variant profile of *SLC25A13* in the 66 alleles of citrin deficiency. Of note, the most common variant among the 66 alleles was IVS16ins3kb (33%), followed by c.851_854del (30%), c.1177+1G>A (12%) and p.Ser225* (9%). Three novel variants of *SLC25A13* were also identified: c.1645C>T (p.Gln549*) ($n=1$), c.1763G>C (p.Arg588Pro) ($n=1$) and c.221C>T (p.Ser74Phe) ($n=1$). None of these novel mutations are observed among normal populations, as determined when we searched the public databases, 1000 genomes (http://browser.1000genomes.org/index.html) and Exome Aggregation Consortium (ExAC) (Cambridge, MA, USA) (http://exac.broadinstitute.org). The c.1645C>T mutation causes a premature stop codon (p.Gln549*) in exon 16 and is expected to result in a truncated mutant protein. The other two missense mutations (p.Ser74Phe and p.Arg588Pro) are located at highly conserved loci (SIFT (sorting intolerant from tolerant)=0.00 and 0.00, respectively). PolyPhen-2 predicted both c.1763G>C and c.221C>T to be deleterious pathogenic mutations.

Comparison with INH

Sixteen children with NICCD and 24 children with INH were enrolled in our study to allow comparison of their clinical and biochemical characteristics (Table 2). At the time of diagnosis, a large percentage of the NICCD patients presented with a failure to thrive, although this improved substantially when they reached the age of 1 year. However, at the age of 1 year, a large percentage of INH patients still presented with a failure to thrive, as well as chronically persistent cholestasis.

Serum amino-acid analysis showed elevated levels of methionine (261 ± 193 μmol l^{-1}; normal range, at age <4 months, 10–60 μmol l^{-1}) and threonine (506 ± 278 μmol l^{-1}; normal range 24–174 μmol l^{-1}) in NICCD patients (Table 3). The threonine-to-serine ratio differed significantly between the two groups ($P<0.001$), while the Fischer ratio (valine+leucine+isoleucine/tyrosine+phenylalanine) did not.

DISCUSSION

The mutation spectrum of Korean NICCD patients indicated a unique genetic heterogeneity; for example, the allele frequency of mutation [XIX]:IVS16ins3 kb was 33.3%, which was slightly lower than that

Table 2 Biochemical and clinical characteristics of NICCD vs INH

Variables	*NICCD*, N=*16*	*INH*, N=*24*	P-*value*
Initial presentation			
Birth weight	2.57 ± 0.60	2.93 ± 0.61	0.055
Small for gestational age	19% ($n=3$)	13% ($n=3$)	0.023
Cholestasis	88% ($n=15$)	100% ($n=24$)	0.43
Neonatal screening	50% ($n=8$)	8% ($n=2$)	0.021
Failure to thrive	56% ($n=9$)	21% ($n=5$)	0.036
Fatty change/hepatitis (USG/liver biopsy)	75% ($n=12$)	100% ($n=24$)	0.062
Laboratory			
Prothrombin time, INR	1.35 ± 0.32	1.22 ± 0.21	0.12
AST (IU l^{-1})	94 ± 61	411 ± 603	0.056
ALT (IU l^{-1})	56 ± 54	278 ± 590	0.008
Bilirubin, total (mg dl^{-1})	5.7 ± 3.7	10.8 ± 6.5	0.001
Bilirubin, direct (mg dl^{-1})	2.9 ± 2.1	6.3 ± 3.6	<0.001
Albumin (g dl^{-1})	3.2 ± 0.6	3.0 ± 0.7	0.63
Alkaline phosphatase (IU l^{-1})	770 ± 236	649 ± 415	0.23
α-Fetoprotein (ng ml^{-1})	$114\,228\pm169\,792$	$91\,049\pm14\,792$	0.37
Ammonia (μmol l^{-1})	110 ± 112	75.0 ± 32.8	0.33
Galactosemia (mg dl^{-1})	5.24 ± 12.3	1.11 ± 0.28	0.009
Follow-up			
Cholestasis	0% ($n=0$)	66% ($n=16$)	0.001
Failure to thrive	16% ($n=1$)	54% ($n=13$)	0.022

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; INH, idiopathic neonatal hepatitis; NICCD, intrahepatic cholestasis caused by citrin deficiency; USG, ultrasonography.

Table 1 Variants of *SLC25A13* among children with citrin deficiency

				No. of alleles			
Mutation name	*Variant*	*Loci*	*Type*	N=*66*		*Japanese Cohort*[7]	*Chinese Cohort*[8]
[XIX]	IVS16ins3kb	Intron 16	Insertion	22	33.3%	4.4%	7.5%
[I]	c.851_854del (p.Met284Prosfs*)	Exon 9	Deletion	20	30.3%	32.8%	58.4%
[II]	c.1177+1G>A (p.340_392del)	Intron 11	splicing	8	12.1%	36.3%	1.3%
[IV]	c.674C>A (p.Ser225*)	Exon 7	Nonsense	6	9.1%	3.3%	0%
[XVI]	c.1592G>A (p.Gly531Asp)	Exon 16	Missense	2	3%	1.1%	0%
[III]	c.1638_1660dup (p.Ala554Glyfs*)	Exon 16	Duplication	2	3%	4.6%	8.9%
[VII]	c.1813C>T (p.Arg605*)	Exon 17	Nonsense	2	3%	0.5%	0%
NA	c.1399C>T (p.Arg467*)	Exon 11	Nonsense	1	1.5%	0%	2.2%
NA: novel	c.221C>T (p.Ser74Phe)	Exon 4	Missense	1	1.5	0%	0%
NA: novel	c.1645C>T (p.Gln549*)	Exon 16	Nonsense	1	1.5	0%	0%
NA: novel	c.1763G>C (p.Arg588Pro)	Exon 17	Missense	1	1.5	0%	0%

Abbreviation: NA, not available.

Table 3 Serum amino-acid analysis of NICCD vs INH

Variables	*NICCD*, N = *16*	*INH*, N = *24*	P-*value*
Citrulline (μmol l^{-1})	309 ± 255	23.9 ± 35.1	< 0.001
Threonine (μmol l^{-1})	506 ± 278	216 ± 77.2	< 0.001
Methionine (μmol l^{-1})	261 ± 193	51 ± 19.3	0.001
Tyrosine (μmol l^{-1})	219 ± 172	108 ± 41.8	0.022
Arginine (μmol l^{-1})	200 ± 125	81 ± 35	0.02
Phenylalanine (μmol l^{-1})	78.1 ± 38.6	64 ± 14.8	0.19
Serine (μmol l^{-1})	294 ± 347	170 ± 39.7	0.17
Threonine-to-serine ratio	2.78 ± 1.20	1.3 ± 0.41	<0.001
Fischer ratio	1.63 ± 1.09	2.2 ± 0.9	0.1

Abbreviations: INH, idiopathic neonatal hepatitis; NICCD, intrahepatic cholestasis caused by citrin deficiency.

reported by Tabata *et al.*[7] IVS16ins3kb is a large insertion mutation resulting in premature stop codon before exon 17. Tabata *et al.*[7] reported the allele frequencies of mutation [XIX] according to ethnicity in East Asian patients with citrin deficiency: 3.6% in Japanese; 6.4% in Chinese; and 45.5% in Korean patients. The next most common mutations were c.851_854del:[I] and c.1177+1G>A:[II] in this study. These are the most common pathogenic mutations in Japanese NICCD patients.[5–7] Mutation [X]:IVS6+5G>A is uniquely identified in Chinese patients (8%).[8] In this study, mutation [X] was never identified among Korean patients with citrin deficiency.

Despite the genetic heterogeneity of Korean patients with citrin deficiency, the clinical features of the Korean patients in our study were not unique when compared with the disease spectrum in known NICCD patients.[4–12] In addition, the amino-acid profiles also were similar to those of East Asians.[13] Typical profiles of multiple aminoacidemia, such as high citrulline, threonine, methionine and tyrosine, in such patients seem to be relatively characteristic (Table 3). These profiles of NICCD were significantly different from patients with INH and biliary atresia.[13,14] However, both cholestatic hepatitis and neonatal liver failure often show nonspecific multiple aminoacidemia.[15] Furthermore, strikingly high levels of citrullinemia are not found in every NICCD patient. Contrary to previous reports,[5,13] the Fischer ratio did not differ significantly between the two groups; five NICCD patients had normal Fischer ratios in this study. Thus, it may be difficult to differentiate between INH patients and NICCD patients based only on amino-acid profiles. In addition, the diagnostic accuracies of the threonine-to-serine ratio and the Fischer ratio have not been well studied.[13,16]

In this study, lower AST, ALT and bilirubin were noted in NICCD patients. These results must be interpreted cautiously because of the possibility of selection bias. Among 174 infants with suspected INH, only 24 had both a genetic test for *SLC25A13* and a liver biopsy. In the clinical context, clinicians tend to conduct more extensive evaluations, such as biopsy and gene tests, for children who have chronically severe and persistent cholestasis. The scope of our study was also limited by the following challenges. The study was carried out retrospectively and the patient number was relatively small. Based on the previously known carrier frequency of *SLC25A13* (1/115 in Korean),[17] citrin deficiency seems to be underdiagnosed in Korea because physicians are unaware of the disease.

In conclusion, this study describes the clinical characteristics of citrin deficiency in 34 Korean patients and the mutation spectrum of *SLC25A13*. The genetic heterogeneity of Korean patients was characterized by the highest frequency of mutation [XIX]: IVS16ins3kb, and three novel mutations of *SLC25A13* were identified. Comparing biochemical and clinical characteristics in NICCD and INH, serum amino-acid profiles seem to be more reliable for differentiating between NICCD and INH. Early genetic testing would be of value.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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