Original Research

Blood glucose and insulin and correlation of *SLC25A13* mutations with biochemical changes in NICCD patients

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Impact statement

This study aims to compare FBG, FINS, C-P, other biochemical and clinical manifestations between NICCD and non-NICCD infants, and discuss differential diagnosis of NICCD and INC beyond the genetic analysis. And investigate the correlation between *SLC25A13* genetic mutations and biochemical changes. This work presented that incidence of hypoglycemia may be higher in small gestational age infants with NICCD. Low LDL-C may be one of the characteristics of dyslipidemia in NICCD infants. There was a correlation between *SLC25A13* gene mutations distribution and the GGT level.

Abstract

Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) is a hereditary metabolic disease arising from biallelic mutations of *SLC25A13*. This study aimed to explore the characteristics of fasting blood glucose (FBG), fasting insulin (FINS) and C-peptide (C-P) levels in NICCD infants, analyze their *SLC25A13* genetic mutations and further discuss the correlation between *SLC25A13* genetic mutations and biochemical changes. Seventy-two cases of infants with cholestasis disease were gathered. Among them, 36 cases with NICCD diagnosis were case group. Meanwhile, 36 cases with unknown etiology but excluded NICCD were control group. FBG, FINS, C-P, ALT, AST, GGT, ALP, TG, HDL-C, LDL-C and Non-HDL-C were collected from all subjects, and DNA was extracted from venous blood for *SLC25A13* mutations detection. The incidence of hypoglycemia was 3% in NICCD group. There were no significant statistical difference of FBG, FINS and C-P between NICCD and INC groups ($P > 0.05$). ALT, LDL-C and Non-HDL-C levels in NICCD group were lower than the INC group, while *SLC25A13* mutations were associated with the level of GGT ($P < 0.05$). Ten different *SLC25A13* genetic mutations were detected, among which, *851del4*, *IVS16ins3kb*, *IVS6+5 G > A* and *1638ins23* mutations made up 82% of all mutations. The incidence of hypoglycemia may be higher in small gestational age infants with NICCD. Low LDL-C may be one of the characteristics of dyslipidemia in NICCD infants. There was a correlation between *SLC25A13* gene mutations distribution and the GGT level, but the meaning of this finding remains to be further in-depth study.

Keywords: Neonatal intrahepatic cholestasis caused by citrin deficiency, fasting blood glucose, fasting insulin, *SLC25A13*, mutation

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Introduction

Infant cholestatic jaundice affects approximately 1 in every 2500 infants worldwide.[1] Infant cholestatic jaundice with unknown etiology is called idiopathic neonatal cholestasis (INC). The most common causes for infant cholestatic jaundice are biliary atresia, infection and hereditary metabolic abnormalities. Among hereditary metabolic diseases, citrin deficiency (CD), galactosemia and glycogen storage disease are more commonly seen.[2] CD is an autosomal recessive disease which caused by mutation of citrin protein encoding gene-*SLC25A13*.[3] Currently, there are more than 100 different *SLC25A13* pathogenic mutations that have been found in patients with CD worldwide.[4–8] Clinically, there are three age-related phenotypes – Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD,OMIM#605814),[9,10] adult onset citrullinemia type 2 (CTLN2,OMIM #603471)[3,11] and failure to thrive and dyslipidemia caused by CD (FTTDCD),[12–14] respectively. NICCD is primarily a neonatal or infantile onset, with main clinical manifestations of hepatomegaly, liver dysfunction and hypoglycemia.[15,16] As a major pediatric clinical phenotype of the CD, NICCD is very common in mainland China, it is now recognized as a worldwide pan-ethnic disorder.[11,17,18] Molecular epidemiological investigation shows that the

carrier rate of *SLC25A13* mutations is documented to be 1 in every 63 infants, where in the south of the Yangtze river, this number is up to 1 in every 48 infants.[15,16] NICCD is characterized by multiple amino acidemia, hypoglycemia, galactosemia, hypoproteinemia, cholestasis, and fatty liver.[5,10,19] Retrospective analysis of biochemical indices in 75 NICCD cases carried out by Ohura *et al*.[20] in Japan had found 18 cases (24%) with hypoglycemia, and concluded that hypoglycemia is common among neonatal-onset NICCD. By using gene knockout technology, Saheki *et al*.[21] who established mice with *SLC25A13* deficiency had shown hypoglycemia after fasting, in comparison to wild type mice. In mainland China, Zhang *et al*.[22] also discussed this issue, in her study of 20 NICCD cases, hypoglycemia was found in NICCD patients compared to a control group ($P < 0.05$) with the mean value of (2.78 ± 0.63) mmol/L. All studies above assumed that the pathogenesis of hypoglycemia was *SLC25A13*-mutation related, caused by a disturbance of gluconeogenesis, because the aspartate-glutamate carrier(citrin) provides substrates for gluconeogenesis as a part of the pathway for the conversion of amino acids to glucose.[11,23] It is widely considered that the mechanism of hypoglycemia in NICCD patients is liver dysfunction due to citrin functional defects.

Since NICCD was first described by Ohura *et al*.,[9] increasing numbers of cases have been reported, and studies of *SLC25A13* gene and genotypes distribution, especially the studies of *SLC25A13* genetic mutations and geographical distribution are relatively abundant.[6,12,16,24] However, in most international and domestic studies, fasting insulin (FINS) and C-peptide (C-P) were not tested synchronically with the fasting blood glucose (FBG). Therefore, there is still lack of correlation study between *SLC25A13* genetic mutations and biochemical indices such as FBG, FINS and C-P. As to whether NICCD patients have glycogen reserve shortage due to liver function impairment, or have insulin metabolic disorder, or hyperinsulinemia, these questions remain to be answered.

In summary, this study aims to compare FBG, FINS, C-P, other biochemical and clinical manifestations in the meanwhile between NICCD and non-NICCD infants, and discuss differential diagnosis of NICCD and INC beyond the genetic analysis. Subsequently, the correlation between *SLC25A13* genetic mutations and biochemical changes will be further discussed and explored.

Materials and methods

Subjects

Subjects were infants aged less than a year old (<1 year old) with unexplained intrahepatic cholestasis that were suspected with NICCD. Thirty-six infants with confirmed diagnosis of NICCD by *SLC25A13* gene analysis were in the case group (NICCD). Thirty-six infants who were genetically excluded NICCD with unknown etiology that is defined as INC were in the control group (INC). All 72 subjects were infants who came to the First Affiliated Hospital of Jinan University from 1 March 2013 to 31 October 2013.

NICCD was diagnosed based on the presence of hyperamino acidemia, galactosemia, fatty liver, and on the results of the genetic study detailed below.[19] Once diagnosed with NICCD, the patients were treated with lactose (galactose)-restricted and medium-chain triacylglycerol (MCT)-supplemented formula, meropenem, gamma globulin, human serum albumin (HSA), supplementation of fat soluble vitamins and low-carbohydrate, high-protein diet.

INC is defined as that begins within one year of age, including onset of the neonatal period. Serum total bilirubin (TBil) > 85 mmol/L and direct bilirubin (DBil) that accounted for 20% of the TBil, or TBil <85 mmol/L and DBil >17 mmol/L of unknown causes. The exclusion criteria were: (1) diseases affecting the extrahepatic biliary system, such as biliary atresia, choledochal cyst, tumor, inspissated bile, or hemangioma, among others, by imaging of the hepatobiliary system;[25] (2) patients with persistent cholestasis and low c-glutamyl transpeptidase (GGT; no more than 50 U/L), which may be indicative of progressive familiar intrahepatic cholestasis or bile salt synthesis defects;[26] (3) Patients with low free T4 and elevated thyroid stimulating hormone; (4) patients with obvious extrahepatic abnormalities, such as abnormal facies, heart disease, butterfly vertebrae, etc.; (5) patients with positive serology that may indicate infection of hepatitis B, hepatitis C, hepatitis A and E, toxoplasmosis, rubella, herpes simplex, human immunodeficiency virus-1 or syphilis. Patients with cytomegalovirus (CMV) infection were not excluded because it is highly prevalent in Chinese infants, and patients infected with CMV have the same outcome as those without the infection.[27]

Ethics statement

This study has been approved by the Medical Ethical Committee, the First Affiliated Hospital, Jinan University, China, and adheres to the World Medical Association Declaration of Helsinki (WMADH 2008).

In this study, *SLC25A13* genetic mutations analyses were conducted with the written informed consents from every patients' guardian, following the approval of the First Affiliated Hospital of Jinan University.

Methods

DNA extraction and *SLC25A13* gene diagnosis

Peripheral blood DNA from all subjects was prepared by using DNA extraction kit (Hangzhou, SIMGEN) ; DNA Marker, ordinary Tag and LA-Taq enzyme were purchased from Takara Company; primers were synthesized by Thermo Fisher Scientific (Shanghai) Co., Ltd. PCR reactions (PTC-200, Life Science Inc.) and gel electrophoresis conditions (PAC300, Bio-Rad) were performed based on Yasuda et al.[28] and Lin et al.[29] Screening for four high frequency *SLC25A13* genetic mutations was performed on all subjects. In those whom initial screening revealed only one mutated allele, all 18 exons and their flanking sequences in *SLC25A13* gene were further sequenced. Sequencing was based on the principle of Sanger dideoxy chain termination method, with the PCR primers as the sequencing primer (BigDye Terminator kit, BI3730 sequencer). Sequencing

was completed by using the Thermo Fisher Scientific (Shanghai) Co., Ltd Guangzhou Division.

Biochemical parameters examinations

A 3–4-h fast was required for all subjects before venous blood samples were collected. All following data were gathered for further biochemical analyses: FBG, FINS, C-P, alphafetoprotein (AFP), alanine aminotransferase (ALT), aspartate amino transferase (AST), glutamete transpeptidase (GGT), alkaline phosphatase (ALP), triglyceride (TG), HDL cholesterol (HDL-C) and LDL cholesterol (LDL-C).

In order to assessed the insulin sensitivity in NICCD patients, the homeostasis model of assessment for insulin resistance index(HOMA-IR) was evaluated by using FBG (mmol/L) and FINS (mU/L), HOMA-IR = FBG×FINS/22.5.[30] Fasting-cell function (FBCI) was calculated to evaluate the islet β-cell function in fasting state, FBCI= FINS / FPG.[30] The HOMA β-cell function index (HBCI) was calculated to evaluate the secretion of islet β cells after glucose overload, HBCI = 20 × FINS / (FBG-3.5).[30] Non-HDL cholesterol (Non-HDL-C) concentration was measured by subtracting HDL-C concentration (mmol/L) from total serum cholesterol (TC) concentration (mmol/L).[31] By using the method of chemiluminescence immunoassay, detection of FINS and C-P was performed by the Department of Nuclear Medicine, from the First Affiliated Hospital of Jinan University. The rest of the biochemical examinations were completed by the biochemistry laboratory of the First Affiliated Hospital of Jinan University.

Statistical analysis

Data were processed and analyzed using SPSS (version 13.0, SPSS Inc, Chicago, Illinois, USA). For normal distributed data such as age, FBG, TG, HLD-C, LDL-C and non-HDL-C, they were expressed as mean ± standard deviation (mean ± SD) respectively, while for skewed distributed data such as FINS, C-P, ALT, AST, GGT, ALP, they were expressed as median (minimum, maximum), respectively. T test and Wilcoxon rank sum test were used for analyzing measurement data; $\chi 2$ test was used to analyze the relationship between biochemical indices and the *SLC25A13* gene. A *P*-value of < 0.05 was considered statistically significant.

Results

General condition of subjects

There were 36 infants in case group (NICCD), with male/female ratio of 17:19 and age ranging from 1 to 12 months old. There were 36 infants in control group (INC); with male/female ratio of 18:18 and age ranging from 0.5 to 12 months old (Table 1).

Follow-up of NICCD patients

Thirty-six NICCD patients were followed up for three months. Five cases lost to follow-up. Among the other 31 NICCD patients, 1 patient had progressed to hepatic cirrhosis,[33] and the remaining 30 cases' liver function were returned to normal or improved significantly after diet treatment.

Features of FBG, FINS and C-P

In NICCD group, FBG fluctuated between 1.14 and 8.04 mmol/L with mean value of 5.0 ± 1.2 mmol/L. The FBG of the only NICCD infant who had hypoglycemia was 1.14 mmol/L, while the FINS and C-P were 0.5 mU/L and 0.69 ng/ml, respectively. In INC group, FBG fluctuated between 3.06 and 7.83 mmol/L with mean value of 5.1 ± 1.0 mmol/L, no hypoglycemia was observed. T test showed that there was no significant difference in blood glucose between NICCD and INC groups (t = 0.18, $P = 0.908$).

FINS level in NICCD group was 4.72 (0.50, 72.31) mU/L, and C-P was 1.17 (0.10, 6.64) ng/ml. There was one missing value. In INC group, FINS level was 5.87 (0.98, 39.18) mU/L, and C-P was 1.81 (0.31, 6.35) ng/ml. There were three missing values in INC group. Wilcoxon rank sum test showed that the differences of both FINS ($P = 0.302$) and C-P ($P = 0.$ 0.278) between NICCD and INC groups were not statistically significant (Table 1).

Features of other biochemical parameters in NICCD group

In NICCD group, liver function indices such as ALT, AST, GGT, ALP and AFP were tested by Wilcoxon rank sum test, respectively (Table 2). The results in Table 2 showed that there was significant difference of ALT level between NICCD and INC groups ($P < 0.05$), both ALT levels were higher than the normal reference range.[35] Lipid-related

Table 1 The level of blood glucose, insulin and C-P in NICCD and INC patients

Indices	NICCD group	INC group	Reference ange[32]	Z	*P*
Gender (M/F)	36 (17/19)	36(18/18)	—	—	—
Age (month)	6.5 ± 3.4[a]	4.6 ± 2.9[a]	—	—	—
FBG (mmol/L)	5.0 ± 1.2[a]	5.1 ± 1.0[a]	2.8–5.6	0.12[b]	0.908
FINS (mU/L)	4.72 (0.50, 72.31)[c]	5.87 (0.98, 39.18)[c]	5–25	1.03	0.302
C-P (ng/ml)	1.17 (0.10, 6.64)[c]	1.81 (0.31, 6.35)[c]	0.5–2	1.09	0.278

[a]Expressed as mean ± SD.
[b]Expressed as median (minimum, maximum).
[c]Expressed as T values.

Table 2 The laboratory parameters in NICCD and INC patients

Indices	NICCD group	INC group	Reference range[32,34]	*P*
ALT (U/L)	46.5 (11.0, 183.0)[a]	80.0 (10.0, 734.0)[a]	5–40	0.025*
AST (U/L)	68.0 (25.0, 261.0)[a]	98.0 (20.0, 495.0)[a]	5–40	0.087
GGT (U/L)	127.0 (16.0, 447.0)[a]	86.0 (9.0, 863.0)[a]	8–50	0.367
ALP (U/L)	463.5 (213.0, 1087.0)[a]	395.0 (220.0, 995.0)[a]	185–555	0.423
AFP (ng/mL)	2576.2 (5.0, 589102.0)[a]	692.7 (28.0, 237149.0)[a]	88.0 ± 87.0[b]	0.682
TG (mmol/L)	1.20 ± 0.56[c]	1.18 ± 0.59[c]	0.40–1.70	0.875
HDL-C (mmol/L)	1.10 ± 0.5[c]	1.00 ± 0.30[c]	0.91–1.42	0.600
LDL-C (mmol/L)	1.62 ± 0.63[c]	2.51 ± 1.08[c]	0–3.12	0.000*
Non-HDL-C(mmol/L)	2.24 ± 0.72[c]	3.01 ± 1.60[c]	1.97–3.77	0.034*

*$P < 0.05$ between two groups.
[a]Expressed as median (minimum, maximum).
[b]Just for children at 3 months, while 46.5 ± 19.0 for 5 months, 12.5 ± 9.8 for 6 months and 8.5 ± 5.5 for 8–12 months.
[c]Expressed as mean ± SD.

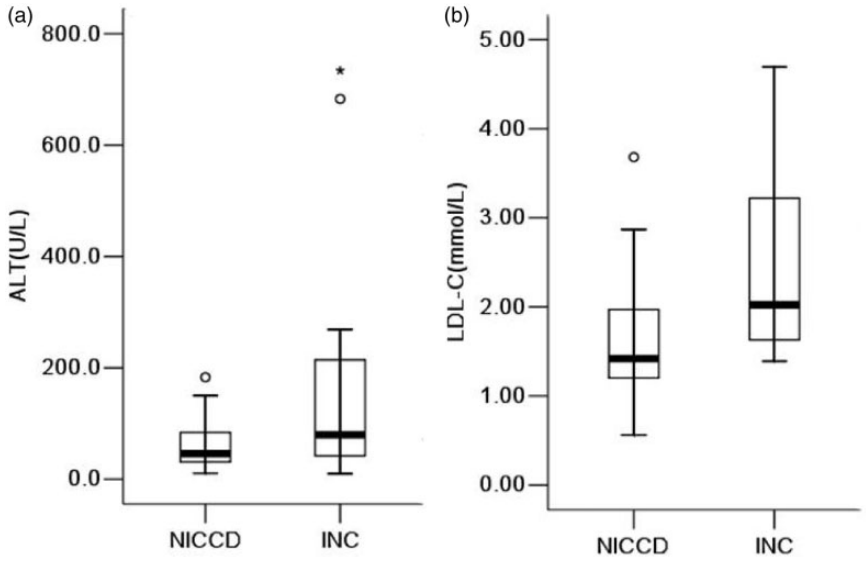

Figure 1 The serum levels of ALT (a) and LDL-C (b) in the patients with NICCD and INC
○ and ⋆ represented the outlier and extreme value, respectively

indices: TG, HDL-C and LDL-C were also tested, respectively. *T*-test statistics showed that there was significant difference in LDL-C ($P < 0.05$) and non-HDL-C ($P < 0.05$) levels between NICCD and INC groups.

In Figure 1, ALT and LDL-C distributions between two groups were shown, the median of serum ALT (46.5:80) and the mean of LDL-C (1.62:2.51) and Non-HDL-C (2:24:3.01) in NICCD group were lower in comparison to INC group, and these differences were significant ($P < 0.05$).

Variations of *SLC25A13* genetic mutations in different genders and ages

In this study, 10 types of *SLC25A13* mutations were detected in 36 NICCD infants. Among them, four high-frequency mutations including *851del4*, *IVS16ins3kb*, *IVS6 + 5 G > A* and *1638ins23* were accounted for 61%, 11%, 6% and 4%, respectively, with the sum of 82%; The other six mutations were *IVSins6kb, R360X, C.2 T > C, C.415 G > A, Q259X* and *IVS11 + 1 G > A*, all have been reported in other studies. The characteristics of mutation in another five alleles were unclear (Table 3). $\chi 2$ test showed that there was no significant difference between distribution of *SLC25A13* genetic mutations and genders in NICCD group ($\chi 2 = 2.27$, $P = 0.687$). NICCD infants were divided into four groups according to age in months, and $\chi 2$ test showed there was also no significant difference between *SLC25A13* genetic mutations and ages in NICCD group ($\chi 2 = 12.03$, $P = 0.443$).

Correlations between *SLC25A13* genetic mutations and biochemical indices

$\chi 2$ test was used in correlation analysis of *SLC15A13* genetic mutations and biochemical changes (Table 3). Test results in

Table 3 Correlation analysis of *SLC25A13* mutated alleles with biochemical indices in NICCD patients

		No. of *SLC25A13* gene mutated alleles						
Indices	**Range**	***851del4***	***IVS16ins3kb***	***IVS6+5G>A***	***1638ins23***	**Others[a]**	χ^2	***P***
FBG (mmol/L)	<2.80	2	0	0	0	0	6.74	0.565
	2.80–5.60	33	4	3	2	12		
	>5.60	9	4	1	1	1		
FINS (mU/L)	0.5–4.72	20	4	2	1	9	10.48	0.233
	4.73–7.12	15	1	1	0	1		
	>7.12	9	3	1	2	1		
C–P (ng/ml)	0.1–0.87	15	4	0	0	3	15.00	0.059
	0.88–1.84	21	0	3	1	7		
	>1.84	8	4	1	2	1		
HOMA–IR	<1.13	18	4	2	1	9	1.94	0.163
	1.13–1.79	17	1	1	0	1		
	>1.79	9	3	1	2	1		
FBCI	<0.90	21	4	2	1	8	0.64	0.422
	0.91–1.80	14	1	1	0	2		
	>1.80	9	3	1	2	1		
HBCI	<57.13	22	4	1	1	8	0.02	0.901
	57.14–128.88	15	0	2	0	1		
	>128.88	7	4	1	2	2		
ALT (U/L)	<30	9	1	1	0	3	3.91	0.866
	30–65	18	4	2	2	8		
	>65	17	3	1	1	2		
AST (U/L)	<37	6	1	1	0	2	5.90	0.659
	37–68	14	4	2	0	6		
	>68	24	3	1	3	5		
GGT (U/L)	12–123	18	6	4	0	8	11.15	0.025*
	>123	26	2	0	3	5		
ALP (U/L)	185–555	31	5	3	0	11	8.49	0.075
	>555	13	3	1	3	2		
AFP (ng/mL)	<88	10	3	2	0	4	7.83	0.451
	88–2576.2	10	1	1	0	4		
	>2576.2	24	4	1	3	3		
TG (mmol/L)	<0.56 0.56–1.7	5	2	0	1	2	6.15	0.631
		29	6	3	2	10		
	>1.7	10	0	1	0	1		
HDL–C (mmol/L)	<1.0	21	4	2	3	6	5.88	0.661
	1–1.5	15	2	2	0	3		
	>1.5	8	2	0	0	4		
LDL–C (mmol/L)	<1.62	23	6	4	0	11	13.35	0.100
	1.62–3.68	21	2	0	3	2		

*$P < 0.05$

[a]The mutations except *851del4*, *IVS16ins3kb*, *IVS6 + 5 G > A* and *1638ins23*.

Table 3 showed that there were no association between *SLC25A13* mutations' distributions and FBG, FINS, C-P, HOMA-IR, FBCI, HBCI, ALT, AST, ALP, TG, HDL-C and LDL-C. However, SLC15A13 genetic mutations were associated with GGT level ($\chi 2 = 11.15$, $P = 0.025$). Further analysis of the association between *SLC25A13* mutations and GGT level showed the Pearson coefficient was 0.366 ($P = 0.025$).

Discussion

Citrin is a mitochondrial inner membrane aspartate-glutamate carrier[35] that plays an important role not only in gluconeogenesis, glycolysis, metabolism of lipid, but also synthesis of nucleotide and protein, as well as urea cycle. Mutations in citrin encoding gene-*SLC25A13* can cause NICCD. NICCD is an autosomal recessive

hereditary disease. Southeast Asia is a high prevalence area for this hereditary metabolic disease and its distribution is wide.[16,29,35–39] Clinical manifestations characterized with metabolic disorders such as hypoglycemia, liver function abnormalities, and hypoalbuminemia are presented in the first few months of life in NICCD patients.[17,40] Currently, there is no clinical diagnostic criteria for NICCD due to nonspecific clinical manifestations and biochemical findings. The most reliable diagnostic evidence is based on the analysis of *SLC25A13* genetic defects.[17,36,41] The underlying mechanisms of metabolic abnormalities leading by citrin defect have not been fully understood yet.[42] In clinical, relationships between *SLC25A13* genetic mutations and biochemical changes, especially insulin and glucose level remain unclear.

Therefore, this study analyzed the characteristics and meanings of biochemical changes including FBG and FINS through collecting the data from NICCD infants, along with the genetic diagnostic results of *SLC25A13*.

General condition of subjects

Infant cholestatic jaundice affects approximately 1 in every 2500 infants worldwide.[1] The most common causes are biliary atresia, infection and hereditary metabolic abnormalities. One of the common etiologies in infant cholestatic jaundice is NICCD, while infant cholestatic jaundice with unknown etiology is called INC. In this study, 36 infants were gathered into NICCD group, while 36 infants into INC group according to the inclusion and exclusion criteria. NICCD is primarily neonatal or infantile onset, mostly within two months of age regardless of the gender. Infants may present lower birth weight, which may be due to the impact of citrin defects-during fetal development.[22]

Characteristics of FBG, FINS and C-P in NICCD infants

It has been reported that NICCD is related to hypoglycemia. Some scholars believed that citrin defect may cause malate aspartate shuttle obstruction, subsequently causing intracellular NADH/NAD^+ratio to increase, inhibiting oxidative phosphorylation of NADH, and resulting in disruption of glycolysis and gluconeogenesis.[40,43]

In this study, the mean FBG in the NICCD group was lower than the INC group, but was not statistically significant. The result was inconsistent with other domestic studies,[22] which may be due to the following reasons: (1) in this study, the control group comprised infants with INC instead of healthy infants. Therefore, the result could only show that difference of FBG level between non-NICCD and NICCD patients was not significant; (2) the incidence of hypoglycemia was relatively low in this study, the incidence of hypoglycemia was 3%. In this study, the mean age of NICCD infants was 6.5 months old, and the only case of hypoglycemia was a 4-month-old infant, whereas in Ohura's research,[20] most NICCD infants were less than 5 months old, and the youngest was only 2 months old. In the research mentioned previously, the incidence of hypoglycemia was 24%; this pointed out that hypoglycemia may be common in neonatal-onset NICCD. The incidence of hypoglycemia in NICCD infants could be related to ages, that hypoglycemia has higher incidence in younger patients. In addition, Ohura *et al*.[20] collected a total of 75 NICCD cases in their study, while there were only 36 cases in this study.

In this study, the only hypoglycemic infant's blood glucose level was 1.14 mmol/L, while FINS and C-P levels were 0.5 mU/L and 0.69 ng/ml, respectively. In this patient, whether hypoglycemia was caused by endogenous high insulin has yet to be determined. The result of this study demonstrated that among patients with infant cholestatic jaundice, the statistical analysis showed no significant difference in FINS and C-P levels between NICCD and INC groups. Whether the insulin and C-P levels in NICCD infants are different from normal infants remains unknown.

NICCD infants' other biochemical changes characteristics

Studies have shown that serum ALT level in NICCD group was lower than INC-group,[44] but was higher than normal reference range.[32] The result of this study was consistent with previous researches. ALT mainly distributes in liver. When liver cells are damaged, increased liver cell membrane permeability allowed ALT to be released into peripheral blood. ALT mainly reflects acute liver damage, so for NICCD infants with chronic liver damage, their ALT level was lower than INC group.

Non-HDL-C consists of LDL-C very low-density lipoprotein cholesterol (VLDL-C), intermediate-density lipoprotein cholesterol (IDL-C), lipoprotein (a) and other atherosclerosis causing particles.[45] Therefore, it reflects abnormal lipid metabolism better than LDL cholesterol, leading to a more accurate and comprehensive assessment in regulation of total cholesterol. In this study, NICCD infants manifested low level of non-HDL-C in comparison to INC group ($P < 0.05$), this may due to two different etiologies and pathogenesis. This result was firstly reported. LDL-C is cholesterol-rich lipoproteins mainly synthesized by liver. It plays a role in transporting cholesterol from liver to the body. Low plasma level of LDL-C may be one of the characteristics in NICCD infants.

Correlation between *SLC25A13* genotypes' distributions and biochemical changes

In this study, 10 types of *SLC25A13* mutations were detected in 36 NICCD infants. Among them, four high-frequency mutations including *851del4*, *IVS16ins3kb*, *IVS6 + 5G > A* and *1638ins23* were accounted for 61%, 11%, 6% and 4%, respectively, with the sum of 82%, which were consistent with previous domestic findings.[12,15,24,25,36] The most common type of mutations was *851del4*, which made up to 61% of all mutations. It also accounts for 70% *SLC25A13* mutation carriers in China.[46] Among infants who suffered from cholestatic jaundice, the carrier rate was 1 in every 17 infants, it was higher than in healthy people, which was 1 in every 93 infants.[24] *SLC25A13* mutations did not associate with FBG, FINS, C-P, ALT, AST, ALP, TG, HDL-C and LDL-C changes. This may be due to the effects of various *SLCA25A13* genetic mutations toward citrin function that were not different; this hypothesis

could be verified at the protein level. In addition, the relationship between genotype and phenotype in NICCD infants might be influenced by other factors, such as certain unknown modifier genes, dietary factors, or therapeutic effects of treatment. This study suggested that *SLC15A13* genetic mutations' distributions were associated with GGT level, but the significance of this finding needed further exploration. Whether GGT level in certain *SLC25A13* mutations' carriers is higher than other mutations, this question requires further study.

The treatment and follow-up of NICCD patients

Low-carbohydrate and high-protein diets seem to be effective for NICCD patients.[20] Lactose (galactose)-restricted and medium-chain MCT-supplemented formula has been shown to rapidly improved the clinical condition of NICCD patients.[47] Because the restriction on the intake of lactose (galactose) can avoid further increasing the NADH/NAD ration in the liver cytoplasm, and MCT supplementation can provide energy to hepatic cells by producing an excess of acetyl-CoA in mitochondria. Fat soluble vitamins, ursodeoxycholic acid, and phenobarbital are recommendable. The majority of NICCD patients show recovery within several months by diet therapy. However, there are some NICCD patients who have suffered liver failure and underwent liver transplantation.[20,48,49] In this study, 31 of the 36 NICCD patients were followed up for three months. HC was detected in 1 patient,[32] another 30 cases shown benign prognosis. In order to identify the rare cases with a poor prognosis, it is important to follow up patients with NICCD carefully.

Authors' contributions: QS and LF conducted the experiments; JL provided the technical assistance; ZL reviewed the manuscript; CL wrote the paper. The corresponding author verifies that all individuals who made contributions to this study are included.

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DECLARATION OF CONFLICTING INTERESTS

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