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Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD)

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Summary We clarified the clinical features of NICCD (neonatal intrahepatic cholestasis caused by citrin deficiency) by retrospective review of symptoms, management and long-term outcome of 75 patients. The data were generated from questionnaires to paediatricians in charge of the patients. Thirty of the patients were referred to hospitals before 1 month of age because of positive results in newborn screening (hypergalactosaemia, hypermethioninaemia, and hyperphenylalaninaemia). The other 45, the screen-negative patients, were referred to hospitals with suspected neonatal hepatitis or biliary atresia because of jaundice or discoloured stool. Most of the screen-negative patients presented before 4 months of age, and 11 had failure to thrive. Laboratory data showed elevated serum bile acid concentrations, hypoproteinaemia, low levels of vitamin K-dependent coagulation factors and hypergalactosaemia. Hypoglycaemia was detected in 18 patients. Serum amino acid analyses showed significant elevation of citrulline and methionine concentrations. Most of the patients were given a lactose-free and/or medium-chain triglyceride-enriched formula and fat-soluble vitamins. Symptoms resolved in all but two of the patients by 12 months of age. The two patients with unresolved symptoms suffered from progressive liver failure and underwent liver transplantation before their first birthday. Another patient developed citrullinaemia type II (CTLN2) at age 16 years. It is important to recognize that NICCD is not always a benign condition.

Abbreviations

CTLN2	citrullinaemia type 2
NICCD	neonatal intrahepatic cholestasis caused by citrin deficiency

Introduction

SLC25A13, the gene newly implicated as the cause of adult-onset type II citrullinaemia (CTLN2, OMIM #603471), encodes an aspartate–glutamate carrier called citrin (Kobayashi et al 1999; Palmieri et al 2001). CTLN2 is characterized by late onset (11–79 years), frequent loss of consciousness with hyperammonaemia, and ultimately death within a few years of onset (Imamura et al 2003; Kobayashi et al 1993, 1997, 1999; Saheki et al 1987; Yasuda et al 2000). Until recently, there was little information about the manifestations of CTLN2 in the neonatal/infantile period. However, *SLC25A13* mutations have been detected in patients with neonatal hepatitis syndrome (Ohura et al 2001; Tazawa et al 2001; Tomomasa et al 2001) and the clinical features of neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD, OMIM #605814) have been revealed (Ben-Shalom et al 2002; Hachisu et al 2005; Lee et al 2002; Naito et al 2002; Ohura et al 2001, 2003; Tamamori et al 2002; Tanaka et al 2002; Tazawa et al 2001, 2004; Tomomasa et al 2001; Yamaguchi et al 2002). It is now apparent that citrin

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deficiency causes two age-dependent phenotypes, namely CTLN2 in adults and NICCD in infants. While CTLN2 patients have been diagnosed on the basis of well-established criteria (Imamura et al 2003; Kobayashi et al 1993, 1997, 1999; Saheki et al 1987; Yasuda et al 2000), NICCD is less clearly defined. Patients manifest various and transient symptoms, and the criteria for clinical and biochemical diagnosis are still being established. In this study we review clinical data on 75 patients who have been diagnosed with NICCD by DNA analysis.

Patients and methods

Patients

We collected data on the clinical and biochemical features in 75 patients with NICCD by sending out a questionnaire to their paediatricians. All patients had undergone newborn screening for phenylketonuria, homocystinuria, maple syrup urine disease, galactosaemia, hypothyroidism and congenital adrenal hyperplasia at the age of 4–6 days using standard methods. Symptoms, laboratory data, management and long-term outcome of these patients were reviewed retrospectively.

DNA diagnosis of mutations of the *SLC25A13* gene

Genetic analyses for the *SLC25A13* mutations were performed with the consent of the patients' families. The Ethics Committees of the Tohoku University School of Medicine and the Kagoshima University Graduate School of Medical and Dental Sciences approved the study. The procedure for the DNA diagnosis of *SLC25A13* mutations has been reported in detail previously (Kobayashi et al 1999; Tomomasa et al 2001; Yasuda et al 2000).

Results

Patients and mutation types

The 75 patients were classified into two groups, screen-positive and screen-negative (Table 1). Five of the patients were siblings. The female-to-male ratios of the screen-positive and screen-negative patients were 2:1 (30 patients) and 1:1 (42 patients), respectively. The sex of 3 of the screen-negative patients was undetermined. There was no significant difference in the gestational age or birth weight between the two groups, but the birth weight of the NICCD patients overall was lower than the standard birth weight in Japan (3050 g). All patients were confirmed to have citrin deficiency by genetic analysis. The two most frequently detected mutations were 851del4 and IVS11+1G>A (99/140, or 71% of the total alleles). These are the same mutations as those reported most frequently in earlier reviews (Kobayashi et al 2003; Saheki and Kobayashi, 2002; Yamaguchi et al 2002).

Table 1 Perinatal history and *SLC25A13* mutations in 75 patients

	Newborn screening	
	Positive	Negative
Number	30[a]	45[b]
Sex (female: male)	20:10	21:21[c]
Gestational age (wk)	38.5 ± 1.6	39.1 ± 1.4
Birth weight (g)	2533 ± 301	2598 ± 317
Range of birth weight	(1930–3235)	(1988–3202)
Mutation	No. of mutated alleles	
851del4	19	25
IVS11+1G>A	22	33
1638ins23	2	3
S225X	2	1
IVS13+1G>A	3	8
1800ins1	1	4
R605X	0	1
E601X	1	3
Other mutations	1	3
Not determined	3	5

[a]including three siblings; [b]including two siblings; [c]three were gender undetermined

Results of newborn screening

Thirty of the 75 patients were detected through newborn screening (Table 2). All of the screen-positive patients were referred to hospital for further evaluation before the age of 1 month. Thorough metabolic evaluation revealed intrahepatic cholestatic liver disease. Hypergalactosaemia was detected in 8 of the 30 screen-positive patients, hypermethioninaemia was detected in 4, and hyperphenylalaninaemia in 5. Surprisingly, 11 of the screen-positive patients were positive for both methionine and galactose, 1 was positive for both methionine and phenylalanine, and 1 was positive for all three.

Table 2 Results of newborn screening from 30 patients

Positive tests	No. of patients
Gal	8
Met	4
Phe	5
Met & Gal	11
Met & Phe	1
Met, Gal & Phe	1

Gal, galactose; Met, methionine; Phe, phenylalanine

Table 3 Age of the screen-negative patients at the initial visit

Age (mo)	<1	1	2	3	4	5
No. of patients	1	11	16	8	7	2

Table 4 Chief complaints of the 45 screen-negative patients at the initial visit

	No. of patients
Jaundice and/or acholic stools	39
Failure to thrive	11
Increased prothrombin time	2
Hepatomegaly	2
Other symptoms (one patient each):	
Subcutaneous bleeding, Hemolytic anemia, Ascites, Hypoglycemic convulsion, Watery diarrhea, Lethargy	

Some patients had more than one manifestation.

Ages and chief complaints of the screen-negative patients at their first visit

Table 3 shows the age at which the screen-negative patients were first admitted to hospital. All were seen before age 5 months, and more than half were first seen before 2 months.

The chief complaints of the 45 screen-negative patients are presented in Table 4. Presenting features included prolonged jaundice, acholic stool and poor weight gain. Most were referred to hospital as suspected cases of neonatal hepatitis or biliary atresia. Failure to thrive was reported in 11 of these 45 patients, increased prothrombin time in 2, and hepatomegaly in 2. Other manifestations included subcutaneous bleeding, haemolytic anaemia, ascites due to hypoproteinaemia, hypoglycaemic convulsion, watery diarrhoea, and lethargy.

Laboratory data

Laboratory data in the two groups showed similar evidence of liver damage and severe intrahepatic cholestasis (Table 5). Serum total bile acids and conjugated bilirubin were elevated in most patients. Serum transaminase concentrations were mildly elevated, with AST levels usually higher than ALT levels. Total serum protein was measured in 64 of the patients and in 39 it was less than 50 g/L. Levels of vitamin K-dependent coagulation factors were low (<50% of the normal range) in 34 of 49 patients screened. Serum galactose concentration was greater than 1.1 mmol/L in 20 patients out of the 33 in whom it was measured. Three of 54 patients tested for blood ammonia were confirmed to have hyperammonaemia (>110 μmol/L) but none was symptomatic.

Table 5 Laboratory data (mean ± SD)

	NBS-pos. (*n*)	NBS-neg. (*n*)	Reference range
T. Bil (mg/dl)	6.26 ± 2.7 (30)	7.72 ± 3.2 (43)	0.2–1.2
C. Bil (mg/dl)	3.15 ± 1.6 (26)	3.81 ± 1.7 (43)	0–0.7
γ-GTP (IU/L)	301 ± 164 (26)	183 ± 88 (40)	8–57
AST (IU/L)	90.0 ± 88 (29)	147 ± 81 (43)	12–30
ALT (IU/L)	45.9 ± 34 (30)	63.2 ± 40 (43)	8–35
TBA (μmol/L)	254 ± 111 (26)	246 ± 67 (38)	11–28
TP (g/L)	46.5 ± 5.1 (25)	50.5 ± 8 (39)	51–68
PT (%)	37.5 ± 18.1 (19)	43.1 ± 20 (30)	>70
Gal (mmol/L)	2.29 ± 1.76 (22)	2.31 ± 2.54 (11)	<0.2
NH_3 (μmol/L)	69 ± 24 (24)	67 ± 37 (30)	<44

NBS, newborn Screening; pos, positive; neg, negative; *n*, number of patients examined; T. Bil, total bilirubin; C. Bil, conjugated bilirubin; γ-GTP, γ-glutamyl transpeptidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; PT, prothrombin time; Gal, galactose; NH_3, ammonia.

Amino acid levels

The most characteristic feature of the patients was an abnormal amino acid pattern, with significant elevation of citrulline and methionine concentrations (Table 6). Threonine, tyrosine, lysine and arginine were also 2–4 times higher than the control range. Other amino acids were within or near their normal ranges. Hypercitrullinaemia was detected in all of the screen-positive patients. However, the citrulline concentration was normal in 6 of the patients who were negative on newborn screening.

Treatments

Treatments are shown in Table 7. Neonatal hepatitis syndrome or hypergalactosaemia was suspected before the DNA diagnosis of *SLC25A13* in most of the patients. As a result, many received lactose-free or medium-chain triglyceride-enriched formula. Phenylalanine–tyrosine-free formula, protein-free formula and low-methionine formula were used for patients with suspected tyrosinaemia,

Table 6 Amino acid analysis (μmol/L, mean ± SD)

	NBS-pos. (*n*)	NBS-neg. (*n*)	Reference range
Threonine	794 ± 294 (25)	463 ± 235 (37)	65–153
Citrulline	582 ± 360 (27)	314 ± 257 (38)	5.14–37.1
Methionine	346 ± 261 (27)	268 ± 349 (37)	13.4–32.2
Tyrosine	266 ± 143 (26)	203 ± 171 (36)	34.2–93.8
Phenylalanine	114 ± 133 (24)	61.7 ± 33.3 (37)	41.8–112
Lysine	429 ± 150 (24)	360 ± 178 (35)	102–203
Arginine	263 ± 115 (26)	208 ± 121 (37)	28.1–98.7

NBS, Newborn Screening; pos, positive; neg, negative
n, number of patients examined.

Table 7 Treatments

	No. of patients	
	NBS-pos. ($n = 30$)	NBS-neg. ($n = 45$)
Special milk formula		
Lactose-free	19	9
MCT-enriched	7	19
Other formulas	8	15
None	2	16
Medicine		
Fat-soluble vitamins	14	37
Ursodeoxycholic acid	4	27
Phenobarbital	1	12
Fresh frozen plasma	2	3
Glucagon-insulin	0	4
Gamma globulin	0	4

NBS, Newborn Screening; pos, positive; neg, negative
MCT, medium chain triglyceride
Other formulas included phenylalanine-tyrosine-free formula, protein-free formula and low-methionine formula.
Some patients received more than one formula.

hyperammonaemia and hypermethioninaemia, respectively. On the other hand, 18 patients received no special milk formula whatsoever. Two-thirds of the patients were treated with fat-soluble vitamins to prevent the consequences of prolonged cholestasis. Ursodeoxycholic acid and phenobarbital were administered to enhance biliary excretion and control pruritus. Gamma-globulin was administered intravenously in 4 patients with suspected viral hepatitis. Four patients with severe liver damage required treatment with fresh frozen plasma or glucagon-insulin therapy.

Complications and prognosis

Complications are shown in Table 8. Cataracts were reported in 6 patients with hypergalactosaemia. Hypoglycaemia was detected in 18 patients and may be a common symptom in patients with NICCD during infancy. Mild developmental delay was reported in 2 cases, but we could not confirm whether this was the result of the citrin deficiency.

Table 8 Complications & prognosis

	No. of patients
Complications	
Cataracts	6
Hypoglycemia	18
Mild mental retardation	2
Prognosis	
Symptoms resolved	73
Progressive liver failure	2
Development of CTLN2	1

Symptoms resolved by 12 months in all but two of the patients (Table 8). The two unresolved patients reportedly suffered from progressive liver failure and underwent liver transplantation before their first birthday. Another patient was reported to have developed citrullinaemia type II at age 16 years.

Discussion

We have demonstrated that neonatal screening provides an important opportunity for the diagnosis of NICCD (Ohura et al 2001, 2003; Tazawa et al 2004). Thirty of the 75 patients in this survey were detected by elevated concentrations of galactose, methionine and/or phenylalanine. Tamamori and colleagues (Tamamori et al 2004) report that blood citrulline levels in NICCD neonates begin to increase immediately after birth and this is followed by rises in other amino acids and galactose and cholestasis due to hepatic dysfunction. A more efficient method to detect NICCD in infants would be to measure citrulline by tandem mass spectrometry during newborn screening. Shigematsu and colleagues (Shigematsu et al 2002) detected 3 patients with NICCD among 102 200 newborns screened by electrospray tandem mass spectrometry. The incidence of NICCD according to their calculations was ~1 in 34 000. The frequency of homozygotes with *SLC25A13* mutation is considerably higher, however, at an estimated 1 in 19 000 (Kobayashi et al 2003; Lu et al 2005). We speculate that newborn screenings can only detect NICCD patients if the condition manifests during the neonatal period (neonatal onset). The screen-negative patients most likely developed the condition at a later time (infantile onset). We conjecture that some of the NICCD patients might have remained apparently healthy throughout infancy.

All of the screen-positive patients manifested hypercitrullinaemia at their first hospital visits, whereas the amino acid concentrations in 6 screen-negative patients were normal. Hyperaminoacidaemia was transient and usually persisted only for couple of months in the early stage of the disease. It may be that the amino acid abnormality remains undetectable in some screen-negative patients.

An abnormal prothrombin time was a common finding in NICCD (Ohura et al 2003; Tamamori et al 2002; Tazawa et al 2004). Our data revealed that levels of vitamin K-dependent coagulation factors were low (<50% of the normal range) in 34 patients. Among these, two patients developed a critical decrease of coagulation factors (<10% of the normal range), which was probably due not only to malabsorption of vitamin K but also to severe liver damage. The decrease of vitamin K-dependent coagulation factors presents an immediate and constant risk of bleeding. The prothrombin time should be checked repeatedly while cholestatic jaundice persists, and vitamin K should be given if the prothrombin time is

lengthened. Five of the patients required fresh frozen plasma to supplement their coagulation factors after vitamin K failed to normalize the prothrombin time.

Hypergalactosaemia is usually accompanied by cataracts and should be treated with lactose-free formula as soon as it is detected. The screen-negative patients were free from hypergalactosaemia at newborn screening, but developed it subsequently (Tamamori et al 2004; Tazawa et al 2004). Galactose concentration should be checked whenever a citrin deficiency is suspected.

Hypoglycaemia was present in 18 patients. We speculate that this is 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 (Hachisu et al 2005; Saheki and Kobayashi 2002; Tamamori et al 2002). Hypoglycaemia may be a common feature in patients with citrin deficiency.

Most of the patients with NICCD were initially diagnosed with neonatal hepatitis syndrome before the DNA diagnosis, and were thus treated according to protocols for this condition (Balistreri 1985). MCT-enriched formula, fat-soluble vitamins, ursodeoxycholic acid, and phenobarbital were widely used. Lactose-free formula was used for the patients with hypergalactosaemia. When Naito and colleagues (Naito et al 2002) performed lactose challenge tests on an infant with NICCD, the first challenge at 56 days led to a worsening in the liver function tests and a return to hypergalactosaemia, whereas the re-challenge at 152 days did not worsen the laboratory findings. These data suggest that lactose may be toxic to patients with NICCD while cholestasis persists. In our study, however, 18 patients improved without the use of special milk formula.

Two of the screen-negative patients suffered liver failure and underwent liver transplantation before their first birthdays. The first patient (case 1 in Tamamori et al 2002) initially presented at 2 months of age because of poor weight gain and jaundice. Thereafter she was followed carefully, but her liver function tests worsened from age 6 months and liver failure ensued. She underwent living-related donor liver transplantation at age 10 months. The second patient was referred to hospital at age 3 months because of jaundice. His liver function progressively worsened and he finally developed hepatic dysfunction. He underwent living-related donor liver transplantation at 5 months (unpublished data). Interestingly, the provisional diagnosis was tyrosinaemia type I in both cases, even though succinylacetone was not detected in their urine. Patients with NICCD should be followed closely to identify the rare cases with a poor prognosis. One patient who recovered from NICCD before his first birthday developed CTLN2 and required liver transplantation at age 16 (Tomomasa et al 2001). It is important to collect more clinical data on patients with NICCD and CTLN2 to elucidate genetic and environmental factors which determine the age of onset, course and prognosis in patients with CTLN2.

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