

# Neonatal intrahepatic cholestasis caused by citrin deficiency in two Malaysian siblings: outcome at one year of life

Thong M K, Boey C C M, Sheng J S, Ushikai M, Kobayashi K

## ABSTRACT

**We report two Malaysian siblings with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). The younger sibling, a six-month-old Chinese girl, presented with prolonged neonatal jaundice, and was investigated for biliary atresia. Urine metabolic screen showed the presence of urinary-reducing sugars, and she was treated with a lactose-free formula. NICCD was suspected based on the clinical history, examination and the presence of urinary citrulline. Mutation study of the SLC25A13 gene showed the compound heterozygotes, 851del4 and IVS16ins3kb, which confirmed the diagnosis of NICCD in the patient and her three-year-old female sibling, who also had unexplained neonatal cholestasis. Long-term dietary advice, medical surveillance and genetic counselling were provided to the family. The diagnosis of NICCD should be considered in infants with unexplained prolonged jaundice. DNA-based genetic testing of the SLC25A13 gene may be performed to confirm the diagnosis retrospectively. An awareness of this condition may help in early diagnosis using appropriate metabolic and biochemical investigations, thus avoiding invasive investigations in infants with neonatal cholestasis caused by NICCD.**

**Keywords: citrin deficiency, citrullinaemia type 2, metabolic liver disease, molecular genetics, neonatal intrahepatic cholestasis, neonatal jaundice, prolonged jaundice**

*Singapore Med J 2010;51(1):e12-e14*

## INTRODUCTION

Citrin is a mitochondrial inner membrane aspartate-glutamate carrier that functions as part of the malate-aspartate shuttle, transferring cytosolic-reduced nicotinamide adenine dinucleotide into the mitochondria. It plays a role in the synthesis of urea, protein and nucleotide by supplying mitochondrial aspartate to the cytosol in the liver.<sup>(1)</sup> There are two disease entities

attributed to the deficiency of citrin: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD; OMIM#605814) and adult-onset Type II citrullinaemia (CTLN2; OMIM#603471).<sup>(2)</sup> Infants with NICCD often present with transient intrahepatic cholestasis with mild liver dysfunction, hepatomegaly, transient multiple aminoacidaemia (citrulline, methionine, tyrosine) and hypoglycaemia. To the best of our knowledge, these two conditions and their outcomes have not been published in the English medical literature from Southeast Asia.

## CASE REPORT

A six-month-old Malaysian Chinese baby girl of non-consanguineous parents was referred to the University of Malaya Specialist Centre at six weeks of life for investigation of prolonged neonatal jaundice. The antenatal period was uneventful, and she was delivered at term with a birth weight of 2.75 kg. She was initially breastfed but later received additional formula feeds. Her stools were pale yellow in colour. Although she was thriving well, she was noted to be jaundiced from the first month of life and was investigated for prolonged jaundice. On review of the family history, it was found that the patient had an elder sister, aged three years old, who also had jaundice from one to three months of age. Her urinary-reducing sugar was positive. The sister was initially treated in another hospital. Her jaundice had disappeared after lactose-free formula was started so no other investigation was performed.

The physical examination confirmed the presence of jaundice in the patient. She was thriving well and had no dysmorphic features. The abdominal examination revealed a soft palpable liver 2 cm below the right subcostal margin. The rest of the findings were non-contributory. The investigations showed hyperbilirubinaemia 210 (control range 3–17) umol/L with conjugated fraction 126 (control range 0–3) umol/L, serum alkaline phosphatase 9,813 (control range 50–136) IU/L, serum alanine aminotransferase 47 (control range 30–65) IU/L, serum aspartate aminotransferase 168 (control range 15–37) IU/L and gamma glutamyl transferase 285 (control range 5–55) IU/L. The full blood

Genetics and Metabolism Unit, Department of Paediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

Thong MK, MBBS, MPaed, MD  
Clinical Geneticist and Professor of Paediatrics

Gastroenterology, Hepatology and Nutrition Unit

Boey CCM, FRCP, MD, PhD  
Professor of Paediatrics

Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8544, Japan

Sheng JS, MSc  
Scientist

Ushikai M, MSc  
Scientist

Kobayashi K, PhD  
Associate Professor and Molecular Geneticist

**Correspondence to:**  
Dr Meow-Keong Thong  
Tel: (60) 3 7949 2065  
Fax: (60) 3 7955 6114  
Email: thongmk@um.edu.my

count, hypothyroid screen and glucose-6-phosphate dehydrogenase screen were normal. The alpha-lantitrypsin and serum TORCH screens were normal. Ultrasonography of the liver and abdomen was normal, but the isotope study with hepatobiliary iminodiacetic acid scan did not show any excretion. An on-table cholangiogram, however, showed good excretion into the small intestine, excluding the possibility of biliary atresia. The liver biopsy showed marked cholestasis with ballooning degeneration and fibrosis of the portal tracts. No inflammatory cell was noted.

The urine metabolic screen showed the presence of urinary-reducing sugars (positive) and tyrosine (positive) on two occasions. In view of the suspicion of galactosaemia, she was empirically started on a lactose-free formula, and breastfeeding was stopped. The urinary-reducing sugars subsequently became negative. The galactose-1-phosphate uridyl transferase assay and the urinary succinylacetone level were normal, while the urine organic acid showed a marked increase in 4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid, 4-hydroxyphenylpyruvic acid and n-acetyl tyrosine, which was consistent with liver impairment. The urine amino acid showed a marked elevation of urine citrulline and a slight increase in tyrosine, histidine and alanine, with an absence of succinylacetone and argininosuccinic acid, suggesting NICCD. Plasma amino acid tests were not done at that time. Mutation analysis of the SLC25A13 gene was performed at the Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan. The results showed that the patient was a compound heterozygote for two different mutations: a known 851del4<sup>(3)</sup> and a novel IVS16ins3kb that was reported recently.<sup>(4)</sup>

On review at five months of age, the patient had recovered well. The liver function test, serum ammonium, urine and plasma amino acid assays were normal. The caregivers were counselled regarding the condition. Appropriate dietary advice was provided to the parents, who were told to ensure that the patient maintained a high-protein and low-carbohydrate diet, and avoided paracetamol and other anti-inflammatory medications. The patient was well at one year of life. After the diagnosis of NICCD was made in the patient, the older sibling was tested and found to have the identical compound heterozygous mutations, 851del4 and IVS16ins3kb. Following this molecular diagnosis, the parents were counselled that both their daughters have NICCD. The older sister has remained well and has no specific food preference.

## DISCUSSION

While NICCD during infancy is not considered a life-threatening disease, it is vital to note that three affected individuals had undergone liver transplantation.<sup>(5)</sup> The clinical features may include intrahepatic cholestasis, diffuse fatty liver with parenchymal cellular infiltration associated with hepatic fibrosis and variable liver dysfunctions, such as coagulopathy and hypoalbuminaemia. This may lead to extensive investigations in the affected infant to exclude biliary atresia. Other clinical features include ketotic hypoglycaemia, hepatomegaly and haemolytic anaemia in childhood. As awareness of NICCD is generally low, these patients may be empirically treated for galactosaemia with lactose-free formula. After weaning, the patient with NICCD may show an aversion to carbohydrate-rich foods and a fondness for protein-rich foods.

Unfortunately, some individuals with NICCD may develop CTLN2 with neuropsychiatric features during early adulthood.<sup>(2)</sup> CTLN2 is a severe condition characterised by recurrent episodes of hyperammonaemia and neuropsychiatric symptoms, such as nocturnal delirium, irritability, delusions, disorientation, changes in sensorium, convulsions and coma. Death may result from cerebral oedema. The onset is sudden, and the precipitating factors may include alcohol ingestion, medications or surgery. The liver may show fatty infiltration and mild fibrosis, with minimal liver dysfunction. Although the prognosis of CTLN2 is guarded, it is amenable to liver transplantation.<sup>(6)</sup>

The differential diagnoses may include citrullinaemia Type 1 (CTLN1), argininosuccinic aciduria and pyruvate carboxylase deficiency in patients presenting with increased urinary or plasma citrulline. Hyperammonaemia may lead one to suspect urea cycle defect and organic acidemias. The neonate with cholestasis may be investigated for galactosaemia, biliary atresia, Byler disease and other forms of hereditary hyperbilirubinaemia. The diagnosis of NICCD is made based on the history and the above mentioned physical examination findings, and supported by transient aminoacidaemia (citrulline, methionine, tyrosine), transient galactosuria, an increased alpha foetoprotein concentration and the presence of mild liver dysfunction. The symptoms and abnormal biochemical findings disappear by one year of age. Due to the transient nature of the biochemical abnormalities in NICCD, genetic testing is useful when the patient is seen after the first year of life, or when the parents are considering prenatal diagnosis. In addition, the parents of

patients with NICCD confirmed by genetic testing may receive early counselling on the risk of CTLN2 for their children, which will change their long-term management and health surveillance program. For example, as the outcome of NICCD patients is not always benign, the confirmation of their status as NICCD by genetic testing may mean a life-time of dietary modifications and early preparation for liver transplantation, if they develop recurring neuropsychiatric symptoms.<sup>(4)</sup> A patient with “idiopathic” neonatal cholestasis may not be subjected to similar restrictions or concerns. Therefore, continuing research and follow-up of these families are important for future reference.

The frequency carriers for the SLC25A13 mutation is one in 65 and one in 48 in the Japanese and Southern Chinese populations, respectively.<sup>(4,7)</sup> Recently, NICCD was reported in patients from Israel,<sup>(4,8)</sup> the United States of America,<sup>(9)</sup> the United Kingdom<sup>(4,10)</sup> and the Czech Republic.<sup>(4)</sup> Increasingly, this condition is being detected through expanded newborn screening programmes.<sup>(11,12)</sup> There is a need for greater awareness of this disease entity in this part of Southeast Asia. This is to avoid unnecessary and potentially hazardous investigations in infants. As such, it would be reasonable to consider this diagnosis in the workup for patients with neonatal cholestasis. This condition is also amenable to dietary management, which consists of a high-protein and low-carbohydrate diet, and avoidance of risk factors, such as alcohol and certain drugs. As this an autosomal recessive condition, genetic counselling should be provided to the affected family. Mutation study of the SLC25A13 gene may be used when the diagnosis is uncertain, or when prenatal diagnosis is being considered. In our case, the molecular diagnostic approach had confirmed the diagnosis of NICCD in both patients. Long-term health and medical surveillance has been provided for the family. Therefore, the diagnosis of NICCD in infants with unexplained prolonged jaundice should be considered, with a DNA-based genetic testing of the SLC25A13 gene performed to confirm the diagnosis.

The detailed methods for the identification and diagnosis of the novel mutation, IVS16ins3kb, which was first found and characterised in a Japanese CTLN2 patient, have been described in the literature.<sup>(4)</sup> However, the clinical and biochemical data have previously been reported mainly in Japanese,<sup>(13,14)</sup> Korean<sup>(15,16)</sup> and Chinese<sup>(4)</sup> NICCD patients with the IVS16ins3kb mutation, and it is well known that many patients

and carriers of the mutation 851del4 are found widely in East Asia.<sup>(4,7)</sup> Therefore, the presence of both these mutations in Malaysian Chinese patients, as reported in this case, suggests that the IVS16ins3kb mutation, like the 851del4 mutation, may also be widely distributed in East Asia.<sup>(3)</sup>

## REFERENCES

1. Saheki T, Kobayashi K. Physiological role of citrin, a liver-type mitochondrial aspartate-glutamate carrier, and pathophysiology of citrin deficiency. *Recent Res Devel Life Sci* 2005; 3:1-15.
2. Saheki T, Kobayashi K. Mitochondrial aspartate glutamate carrier (citrin) deficiency as the cause of adult-onset type II citrullinemia (CTLN2) and idiopathic neonatal hepatitis (NICCD). *J Hum Genet* 2002; 47:333-41.
3. Kobayashi K, Sinasac DS, Iijima M, et al. The gene mutated in adult-onset type II citrullinaemia encodes a putative mitochondrial carrier protein. *Nat Genet* 1999; 22:159-63.
4. Tabata A, Sheng JS, Ushikai M, et al. Identification of 13 novel mutations including a retrotransposal insertion in SLC25A13 gene and frequency of 30 mutations found in patients with citrin deficiency. *J Hum Genet* 2008; 53:534-45.
5. Tamamori A, Okano Y, Ozaki H, et al. Neonatal intrahepatic cholestasis caused by citrin deficiency: severe hepatic dysfunction in an infant requiring liver transplantation. *Eur J Pediatr* 2002; 161:609-13.
6. Saheki T, Kobayashi K, Iijima M, et al. Adult-onset type II citrullinemia and idiopathic neonatal hepatitis caused by citrin deficiency: involvement of the aspartate-glutamate carrier for urea synthesis and maintenance of the urea cycle. *Mol Genet Metab* 2004; 81 Suppl 1:S20-6.
7. Lu YB, Kobayashi K, Ushikai M, et al. Frequency and distribution in East Asia of 12 mutations identified in the SLC25A13 gene of Japanese patients with citrin deficiency. *J Hum Genet* 2005; 50:338-46.
8. Ben-Shalom E, Kobayashi K, Shaag A, et al. Infantile citrullinemia caused by citrin deficiency with increased dibasic amino acids. *Mol Genet Metab* 2002; 77:202-8.
9. Dimmock D, Kobayashi K, Iijima M, et al. Citrin deficiency: a novel cause of failure to thrive that responds to a high protein, low carbohydrate diet. *Pediatrics* 2007; 119:e773-7.
10. Hutchin T, Preece MA, Hendriksz C, et al. Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) as a cause of liver disease in infants in the UK. *J Inher Metab Dis* 2009, June 11[E-pub ahead of print].
11. Lee NC, Chien YH, Kobayashi K, et al. Time course of acylcarnitine elevation in neonatal intrahepatic cholestasis caused by citrin deficiency. *J Inher Metab Dis* 2006; 29:551-5.
12. Ohura T, Kobayashi K, Tazawa Y, et al. Clinical pictures of 75 patients with neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). *J Inher Metab Dis* 2007; 30:139-44.
13. Tazawa Y, Kobayashi K, Abukawa D, et al. Clinical heterogeneity of neonatal intrahepatic cholestasis caused by citrin deficiency: case reports from 16 patients. *Mol Genet Metab* 2004; 83:213-9.
14. Tokuhara D, Iijima M, Tamamori A, et al. Novel diagnostic approach to citrin deficiency: analysis of citrin protein in lymphocytes. *Mol Genet Metab* 2007; 90:30-6.
15. Kim SZ, Jeon YM, Song WJ, et al. Two cases of citrin deficiency detected by newborn screening in Korea. *J Inher Metab Dis* 2006; 29 Suppl 1:84.
16. Ko JM, Kim GH, Kim JH, et al. Six cases of citrin deficiency in Korea. *Int J Mol Med* 2007; 20:809-15.