Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) in three Malay children

NGU Lock Hock MRCP*, ZABEDAH Md Yunus MPath**, Keiko KOBAYASHI, PhD***

*Genetics Department, Kuala Lumpur Hospital, **Biochemistry Unit, Specialized Diagnostic Centre, Institute for Medical Research, and ***Department of Molecular Metabolism and Biochemical Genetics, Kagoshima University Graduate School of Medicine and Dental Sciences, Kagoshima, Japan.

Abstract

Citrin deficiency is an autosomal recessive disorder caused by mutation in the SLC25A13 gene. It has two major phenotypes: adult-onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestatic caused by citrin deficiency (NICCD). NICCD is characterized by neonatal/infantile-onset cholestatic hepatitis syndrome associated with multiple amino acidemia and hypergalactosemia. NICCD is self-limiting in most patients. However, some patients may develop CTLN2 years later, which manifests as fatal hyperammonemia coma. We report three unrelated Malay children with genetically confirmed NICCD characterised by an insertion mutation IVS16ins3kb in SLC25A13 gene. All 3 patients presented with prolonged neonatal jaundice which resolved without specific treatment between 5 to 10 months. Of note was the manifestation of a peculiar dislike of sweet foods and drinks. Elevated plasma citrulline was an important biochemical marker. NICCD should be considered in the differential diagnosis of cholestatic jaundice in Malaysian infants regardless of ethnic origin.

Key words: citrin deficiency, neonatal intrahepatic cholestasis, SLC25A13 gene

INTRODUCTION

Citrin is a mitochondrial membrane aspartate–glutamate carrier with important physiological role in syntheses of urea, protein, nucleotide and gluconeogenesis. Citrin deficiency is an autosomal recessive disorder caused by mutations in the SLC25A13 gene on chromosome 7q21.3. It has two major phenotypes: adult-onset type II citrullinemia (CTLN2; MIM #603471) and neonatal intrahepatic cholestatic caused by citrin deficiency (NICCD; MIM#605814). CTLN2 is characterized by late-onset recurring encephalopathy associated with hyperammonemia and hypercitrullinemia, and various hepatopancreatic pathology such as non-alcoholic steatohepatitis, hepatoma and chronic pancreatitis are often found. NICCD is characterized by neonatal/infantile-onset cholestatic hepatitis syndrome associated with multiple amino acidemia (involving citrulline, threonine, methionine, arginine, and tyrosine), hypergalactosemia, hypoproteinemia, hypoglycemia, cholestasis, and fatty liver. Most symptoms in patients with NICCD will resolve within 1 year of age and require no further special treatment. However, several decades later some patients may develop CTLN2. Peculiar fondness for protein and lipid-rich foods but aversion to carbohydrate-rich foods has been recognized in many of them.

Until recently, citrin deficiency had been thought to be found mostly in individuals of Japanese, Korean, Chinese or Vietnamese ancestry. The incidence of citrin deficiency in Malaysia is unknown. There are previously reported cases among Chinese infants presenting with cholestatic jaundice. However, citrin deficiency is yet to be reported among other local ethnic groups. In this case series, we report three Malay children presenting with NICCD phenotype, all with proven mutations in SLC25A13.
CASE REPORTS

Case 1
A female, was born small for gestational age at full term, weighing 2.3kg at birth. She was the second child of unrelated parents with no family history. She had a mild neonatal jaundice which resolved spontaneously. She became jaundice again at 2 months old. When she was referred to exclude biliary atresia at 4 months old, she was still jaundiced with pale-coloured stools and had a mild hepatosplenomegaly.

Liver function test revealed conjugated hyperbilirubinemia and evidence of liver dysfunction. The coagulation profile was marginally prolonged. Her serum α-fetoprotein (αFP) level was elevated. However, she had no hypoglycemia. Screening for infectious hepatitis was negative. Sonographic examination did not show abnormalities of the biliary tract. Plasma amino acids analysis showed mildly elevated plasma citrulline, threonine, methionine, arginine, and tyrosine, but was interpreted as non-specific findings. There was no liver biopsy performed.

She received no specific treatment. Her growth was slow during the first year but subsequently caught up. Jaundice resolved at 10 months. The liver function test and plasma amino acids profile normalized at 16 months of age. Her developmental milestones were normal. When she was reviewed at 6 years old, she remained healthy but found to have peculiar fondness for protein and lipid rich foods such as peanuts and seafood. She disliked carbohydrate-rich food including rice and sugary drinks. At this time, NICCD was suspected and mutation study of SLC25A13 showed that she was homozygous for IVS16ins3kb mutation (Figure 1).

Case 2
A female, was delivered at full term with low birth weight of 2.1kg. She was the 4th child of a consanguineous couple. One of her siblings had a history of prolonged neonatal jaundice of unknown cause. At 2 months old, she presented with prolonged neonatal jaundice. Laboratory tests revealed conjugated hyperbilirubinemia. Metabolic screening was performed when Hepatobiliary Iminodiacetic Acid (HIDA) scan had ruled out biliary atresia and screening tests for infections were normal. His plasma citrulline, methionine and tyrosine were elevated, and NICCD was suspected. Total blood galactose was elevated seven times from normal but normalized at the age of 3 months. Mutation study showed that he was compound heterozygous for the IVS16ins3kb and c.851_854delGTAT mutations (Figure 1). During his last review at 5 months old, the jaundice had resolved.

DISCUSSION
This is the first report of genetically confirmed NICCD in a Malay cohort. The clinical and laboratory findings are summarised in Table 1. Interestingly, all three patients (5 out of 6 mutated alleles) have an insertion mutation IVS16ins3kb in SLC25A13 gene, which has been recently reported among patients from East Asia. It...
FIG. 1: The insertion mutation IVS16ins3kb of SLC25A13 gene was identified using agarose gel electrophoresis method. DNA was amplified using LA-Taq polymerase. Two primer sets were used: Ex16F: 5’-GTATGCCCTGACGCATC TTAG-3’ and Ex18-3'R:5’-GCTTCATTCCCAGGAGGA-3’. Patient 1 (a) and Patient 2 (b) were homozygous; whereas Patient 3 and his parents (c) were heterozygous for this mutation.

has been suggested that this unique mutation occurred in an ancestor during very early historical period in some region of East Asia. This is a retrotransposonal insertion mutation with the inserted sequence showed an antisense strand of processed complementary DNA from a gene on chromosome 6. This insertion mutation caused a frame shift leading to premature termination at codon 585 in citrin protein.1

Our report suggests that NICCD should be considered in the differential diagnosis of cholestatic jaundice in Malaysian infants regardless of their ethnic origin. Plasma amino acids analysis should be included in the initial
Table 1. Clinical, laboratory and genetic findings of the 3 NICCD patients

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Birth weight (g)</td>
<td>2,300</td>
<td>2,100</td>
<td>2,500</td>
</tr>
<tr>
<td>Onset of cholestatic jaundice (month)</td>
<td>2</td>
<td>&lt;1</td>
<td>&lt;2</td>
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<tr>
<td>Associated clinical findings</td>
<td>a, b, c</td>
<td>a, b, c, d</td>
<td>b</td>
</tr>
<tr>
<td>Jaundice clinically resolved (month)</td>
<td>10</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Aversion to carbohydrate-rich foods</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Biochemical normalization (month)</td>
<td>16</td>
<td>27</td>
<td>?</td>
</tr>
</tbody>
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<thead>
<tr>
<th>Age (month)</th>
<th>Total bilirubin (μmol/L)</th>
<th>Direct bilirubin (μmol/L)</th>
<th>Total protein (g/L)</th>
<th>Albumin (g/L)</th>
<th>Aspartate aminotransferase (IU/L)</th>
<th>Alanine aminotransferase (IU/L)</th>
<th>Alkaline phosphatase (IU/L)</th>
<th>γ-glutamyl transpeptidase (IU/L)</th>
<th>Prothrombin time (second)</th>
<th>α-fetoprotein (ng/ml)</th>
<th>Blood ammonia (μmol/L)</th>
<th>Plasma citrulline (μmol/L)</th>
<th>Plasma methionine (μmol/L)</th>
<th>Plasma tyrosine (μmol/L)</th>
<th>Plasma threonine (μmol/L)</th>
<th>Plasma arginine (μmol/L)</th>
<th>Blood galactose (μmol/L)</th>
<th>Urine reducing sugar</th>
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<tr>
<td>4</td>
<td>164</td>
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<td>73</td>
<td>45</td>
<td>153</td>
<td>49</td>
<td>611</td>
<td>312</td>
<td>15.3</td>
<td>27480</td>
<td>62.7</td>
<td>59</td>
<td>149</td>
<td>130</td>
<td>472</td>
<td>164</td>
<td>n.d.</td>
<td>-ve</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>39</td>
<td>80</td>
<td>50</td>
<td>138</td>
<td>71</td>
<td>469</td>
<td>179</td>
<td>11.7</td>
<td>4253</td>
<td>73.0</td>
<td>25</td>
<td>46</td>
<td>112</td>
<td>221</td>
<td>61</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

**Reference range**
- Total bilirubin: 0-21 μmol/L
- Direct bilirubin: 0-7 μmol/L
- Total protein: 66-87 g/L
- Albumin: 45-60 g/L
- Aspartate aminotransferase: 0-50 IU/L
- Alanine aminotransferase: 0-50 IU/L
- Alkaline phosphatase: 117-390 IU/L
- γ-glutamyl transpeptidase: 7-32 IU/L
- Prothrombin time: 12.0-13.8 seconds
- α-fetoprotein: <8.2 ng/ml
- Blood ammonia: 14.7-55.3 μmol/L
- Plasma citrulline: 8-55.0 μmol/L
- Plasma methionine: 5-34 μmol/L
- Plasma tyrosine: 24-105 μmol/L
- Plasma threonine: 40-139 μmol/L
- Plasma arginine: 32-142 μmol/L
- Blood galactose: <540 μmol/L

**SLC25A13 mutation**
- Patient 1: Homozygous IVS16ins3kb
- Patient 2: Homozygous IVS16ins3kb
- Patient 3: c.851_854delGTAT

a: failure to thrive; b: hepatomegaly; c: splenomegaly; d: renal tubulopathy; n.d.: not determined; ?: not known yet; +ve: positive; -ve: negative
evaluation of any infant with cholestatic jaundice. Elevated plasma citrulline is the most important biochemical marker for NICCD but this biochemical abnormality is only transient. Unless plasma amino acids analysis is done early, hypercitrullinemia may not be detected. Molecular testing will provide the definitive diagnosis of NICCD. We believe that in Malaysia NICCD is substantially under diagnosed.

The management of NICCD is directed toward treating the consequences of cholestasis and secondary galactosemia. Lactose may be toxic to NICCD patients and therefore lactose-free formula is recommended until cholestasis has resolved.¹ The amelioration of NICCD symptoms after infancy suggests hepatocyte maturation and possible compensation by other mitochondrial carriers. However, patients with NICCD should be followed closely as some of them might develop CTLN2, which could be saved by liver transplantation.⁴

REFERENCES