Novel Compound Heterozygote Mutations of the SLC25A13 Gene in an Infant with Neonatal-onset Type II Citrullinemia Detected by Newborn Mass Screening

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Case report

A female neonate was referred at the age of 16 days because of a high serum citrulline level on a tandem mass screening test. She was born at 40 weeks gestational age by normal vaginal delivery, with a body weight of 2,300 g and was being fed breast milk. No specific family history was noted.

Citrin deficiency caused by the SLC25A13 gene mutations is associated with both neonatal-onset type II citrullinemia (CTLN2), also known as neonatal intrahepatic cholestasis caused by citrin deficiency and adult-onset CTLN2. Neonatal-onset CTLN2 is an autosomal recessive disorder characterized by poor growth, intrahepatic cholestasis, and increased serum citrulline. A 16-days old infant with hyperammonemia was referred for evaluation of increased plasma citrulline diagnosed using tandem mass spectrometry. Blood amino acid analysis showed significant elevation of citrulline. Mild elevation in serum galactose levels had been found. DNA analysis of the SLC25A13 gene in this patient showed two novel compound heterozygous mutations, c.221C>T in exon4 and c.1645C in exon16 (p.[Ser74Phe]+[Gln549X]). We suggest that infants with a high serum citrulline level on a tandem mass screening test are candidates for gene analysis and blood amino acid analysis for neonatal-onset CTLN2.

Key Words: Citrullinemia, Citrin, Mutation

Introduction

Citrullinemia (CTLN) has been classified into two forms according to the pathogenesis. Type I CTLN (CTLN1) has either neonatal or infantile onset, with an argininosuccinate synthetase (ASS) enzyme defect in all tissues arising from mutations in the ASS gene on chromosome 9q34. Type II CTLN (CTLN2) is characterized by decreased liver-specific ASS activity due to citrin deficiency without any abnormalities in hepatic ASS messenger RNA or the ASS gene. The two phenotypes of CTLN2 are neonatal-onset CTLN2, also known as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) and adult-onset CTLN2.

We report an infant with neonatal-onset CTLN2 caused by novel compound heterozygous mutations in the SLC25A13 gene.

Case report

A female neonate was referred at the age of 16 days because of a high serum citrulline level on a tandem mass screening test. She was born at 40 weeks gestational age by normal vaginal delivery, with a body weight of 2,300 g and was being fed breast milk. No specific family history was noted.
She had mild jaundice, but hepatomegaly was absent by physical examination. Her height and weight were in the 25–50th and <3th percentile, respectively, and head circumference was in the 25th percentile. She did not present with neurological symptoms. Findings on neurologic examination were normal.

Biochemical data examined on admission were aspartate transferase/alanine transaminase 74/24 IU/L (normal range: 5–45/15–55 IU/L), total/direct bilirubin 8.50/1.98 mg/dL (normal range: <10.0/0–0.2 mg/dL), ammonia 160 ug/dL (normal range: 29–70 ug/dL), and galactose 11.0 mg/dL (normal range: 0–8mg/dL). Blood amino acid analysis revealed citrulline 567 μmol/L (normal range: 10–45 μmol/L), alanine 327 μmol/L (normal range: 131–710 μmol/L), methionine 74 μmol/L (normal range: 10–60 μmol/L), threonine 656 μmol/L (normal range: 10–60 μmol/L), tyrosine 190 μmol/L (normal range: 55–147 μmol/L), and arginine 294 μmol/L (normal range: 6–140 μmol/L). The levels of 4-hydroxyphenyl lactic acid and 4-hydroxyphenylpyruvic acid were increased in urine organic acid analysis. Mild elevation of galactose was noted in an initial galactosemia test. DNA analysis showed p.Ser74Phe mutation in exon 4 and p.Gln549X mutation in exon 16 in the SLC25A13 gene (Fig. 1).

All abnormal laboratory findings were resolved by six months of age without specific treatment (Fig. 2). She is now aged 14 months and developing normally with a general infant formula and being transitioned to food. Her height and weight are within the normal range. She has been doing well, without symptoms of hyperammonemia and neurologic abnormalities.

**Discussion**

Citrin, encoded by the SLC25A13 gene on chromosome 7q21.3, is a liver-type mitochondrial aspartate–glutamate carrier. The function of citrin is to participate in proteins and urea synthesis and translocate the cytosolic nicotinamide adenine dinucleotide (NADH) reducing equivalent into mitochondria. The various symptoms of neonatal-onset CTLN2 and adult-onset CTLN2 may be caused by defective aspartate export from the mitochondria to the cytosol and defects in the malate aspartate shuttle. Most neonatal-onset CTLN2 patients show spontaneous im-
Neonatal-onset CTLN2 patients showed cholestasis and multiple aminoacidemia, involving elevated levels of citrulline, threonine, methionine, and tyrosine. Liver biopsies from neonatal-onset CTLN2 patients showed fatty change, round cell infiltration, and mild fibrosis. The clinical phenotypes of neonatal-onset CTLN2 are diverse, ranging from asymptomatic elevation of citrulline levels to severe tyrosinemia-like features. Laboratory findings showed mild hyperammonemia, hypoproteinemia, and galactosemia.

Our case showed that citrulline as well as methionine, tyrosine, alanine, and threonine levels were elevated in blood amino acid analysis. And our patient showed mild jaundice, mild hyperammonemia and galactosemia without neurological symptoms.

Lu et al. estimated the frequencies of SLC25A13 homozygotes to be 1 in 19,000 in Japan, 1 in 50,000 in Korea, and 1 in 17,000 in China. In the Ko et al. study, six cases of citrin deficiency were reported based on biochemical and molecular findings in Korea. Four neonatal-onset CTLN2 patients presented with high citrulline levels on a newborn screening test or neonatal cholestasis and two adult onset CTLN2 patients were identified.

Only a few patients can be diagnosed by a newborn screening test, because not all neonatal-onset CTLN2 patients show highly elevated plasma citrulline levels. Several studies have sought to develop methods for early diagnosis and neonatal screening of neonatal-onset CTLN2. Screening could be performed by Western blotting using an anti-citrin antibody and tandem mass screening. Additional findings such as high methionine, arginine, phenylalanine, and galactose levels support the diagnosis and enhance the positive predictive value of the test.

Kobayashi et al. determined that SLC25A13 contains 18 exons and encompasses approximately 200 kb of DNA. At least 31 types of SLC25A13 gene mutations have been found in patients with citrin deficiency. In our case, a novel compound heterozygous mutations (c.221C>T in exon4 and c.1645C in exon16 (p.[Ser74Phel]+[Gln549X]) in the SLC25A13 gene were identified.

According to a recent report by Saheki et al., low-carbohydrate and high-protein diets seem to be effective for CTLN2. However, since a high protein diet also induces hyperammonemia, it is important to determine the appropriate amount of protein intake. Liver transplantation is very effective, but issues are donor and cost. Sodium benzoate, sodium phenylbutyrate, and arginine, to facilitate the urea cycle have been recommended. Recently, sodium pyruvate was attempted in patients in the dormant stage, but long-term efficacy needs to be determined.

Drinking alcohol and taking medications such as rabeprazole, acetaminophen and other anti-inflammatory and analgesic drugs should be avoided, as they can trigger adult-onset CTLN2. Nutritional management with appropriate intake of proteins and carbohydrates should be considered in order to avoid an accumulation of nitrogen and NADH-reducing equivalents.

In conclusion, we suggest that infants with a high serum citrulline level on a tandem mass screening test are candidates for gene analysis and blood amino acid analysis for neonatal-onset CTLN2.
References


