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증 례

# *SLC25A13* 이형접합 유전자 변이와 부합하는 생화학적 소견을 가진 영아 시트린 결핍증 1례

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# An Infant Case of Citrin Deficiency with Corresponding Biochemical Features and a Heterozygous *SLC25A13* Mutation

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Citrin deficiency (OMIN #605814) is an autosomal recessive disorder caused by the *SLC25A13* gene mutation with abnormal biochemical findings, including increased serum ammonia, citrulline, arginine, galactose, serum threonine-to-serine ratio, serum pancreatic secretory trypsin inhibitor, and alpha-fetoprotein. Citrin deficiency can manifest in three ways: in newborns as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), in older children as failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD), and in adults as citrullinemia type 2 (CTLN2) with recurrent hyperammonemia and neuropsychiatric symptoms. We report a 35-day-old asymptomatic patient with citrin deficiency who had abnormal biochemical findings.

**Key words:** Citrin, Neonatal intrahepatic cholestasis caused by citrin deficiency, *SLC25A13*

## Introduction

Citrin deficiency (CD; citrullinemia type 2, OMIN #605814) is classified into three types: neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD), and adult-onset type 2 citrullinemia (CTLN2)[1]. Kobayashi et al. found that CTLN2 is caused by a mutation of the *SLC25A13* gene[2, 3], and Palmieri et al. showed that citrin is housed in the mitochondrial inner membrane[4]. Citrin in liver-type aspartate/glutamate carrier isoform 2 (AGC2) plays important roles in the urea cycle, malate-aspartate NADH shuttle, aerobic glycolysis, gluconeogenesis, and synthesis of proteins and nucleotides[5, 6]. CD leads to abnormal biochemical findings including increased serum ammonia, citrulline, arginine, galactose, serum threonine-to-serine ratio, serum pancreatic secretory trypsin inhibitor, and alpha-fetoprotein. Recently, we encountered a 35-day-old asymptomatic patient with CD who was diagnosed with abnormal biochemical findings consistent with CD. Here we report a rare case of

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CD and literature review.

## Case Report

A 35-day-old male infant was referred to Samsung Changwon Hospital for evaluation of citrullinemia identified on a newborn mass screening (citrulline 246 μM, reference <55 μM). He was born by normal vaginal delivery at 39 weeks' gestation and weighed 2.8 kg. He was the second liveborn child of unrelated parents with no family history of citrullinemia. He had met normal developmental milestones and had no specific symptoms, but he did demonstrate failure to thrive. On the initial physical examination, his weight was 3,500 g (<3$^{rd}$ percentile), height was 54 cm (<3$^{rd}$ percentile), and head circumference was 36.5 cm (<3$^{rd}$ percentile). He had no hepatosplenomegaly or trichorrhexis nodosa, and his neurologic examination was non-specific. The initial tandem mass screening test showed increased methionine of 185.6 (reference <60.1 μM) and citrulline of 332.4 (reference <67.3 μM). Subsequent plasma aminoacid analysis revealed methionine of 275.3 nmol/mL (reference 9-42), citrulline of 503.4 nmol/mL (reference 3-35), arginine of 67.1 nmol/mL (reference 12-133) and threonine/serine ratio of 3.9 (reference <1.10). Urine organic analysis including orotic acid showed non-specific findings. Routine laboratory findings showed ammonia 67 μM/L (reference <47), aspartate aminotransferase (AST) of 140 IU/L (reference 0-40), alanine aminotransferase (ALT) of 50 IU/L (reference 0-40), alkaline phosphatase (ALP) 264 IU/L (reference 4-41), serum protein/albumin 6.1/4.0 g/dL (reference 5.8-8.3/3.1-5.2), bilirubin total/direct of 8.1/2.2 mg/dL (reference 0.2-1.2/0-0.3), and prothrombin time (PT)/activated partial thromboplastin time (aPTT) 14.9 seconds/48.3 seconds (reference 9.5-13.8/23-41). Abdominal ultrasound was non-specific without fatty change. He was diagnosed with abnormal biochemical findings coinciding with CD. We performed DNA sequence analysis of the *SLC25A13* gene, which revealed a heterozygous mutation, c.[1817G>A];[?] (p.[W606*];[?]) (Fig. 1). We did not perform DNA sequence analysis of the parents due to their refusal.

At 3 months of age, the patient underwent follow-up laboratory tests that showed AST/ALT 97/52 IU/L and bilirubin total/direct 5.4/2.9 mg/dL. However, serum galactose was within normal

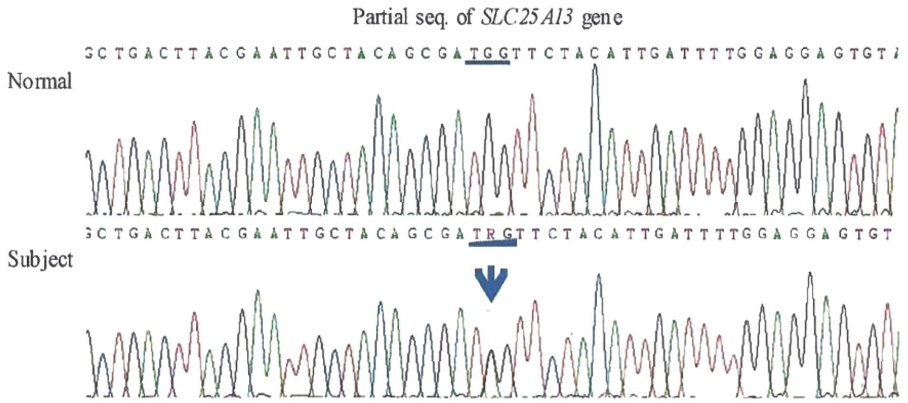


**Fig. 1.** Mutation analysis of SLC25A13 showed a heterozygous mutation, c.[1817G>A];[?] (p.[W606*];[?]).

range. Thus, we decided to defer special treatment such as medium-chain triglyceride oils, fat-soluble vitamins, ursodeoxycholic acid, or vitamin K. The patient is now 5 months old. On physical examination, his weight was 7.3 kg ($10^{th}$ percentile), height was 64 cm ($24^{th}$ percentile), and head circumference was 44 cm ($44^{th}$ percentile). He had no hepatosplenomegaly, normal development for age and neurologic examination was non-specific. Laboratory tests showed AST of 104 IU/L, ALT of 115 IU/L, ammonia 64 μM/L, normal protein/albumin, bilirubin total/direct and PT/aPTT.

## Discussion

CD can lead to failure in the supply of aspartate from the mitochondria to the cytoplasm, which is used in the synthesis of argininosuccinate, resulting in high citrulline and ammonia levels[4, 6]. The *SLC25A13* gene on chromosome 7q21.3 is responsible for adult-onset type II citrullinemia (CTLN2)[3]. The *SLC25A13* gene consists of 18 exons and encodes a liver-type mitochondrial aspartate-glutamate carrier (citrin). Ohura et al. and Tazawa et al. have demonstrated that mutations in the *SLC25A13* gene cause NICCD. CD patients were first described in Japan[6, 7] and are reported mainly in East Asia including Japan, Korea, China, Taiwan, Thai and Malaysian, although cases have been reported worldwide including in the USA, Italy, Spain, United kingdom and Bulgarian[3, 7-14]. The estimated frequency of the 12 common mutations in *SLC25A13* homozygotes reported by Lu et al. was 1/17,000 in China, 1/19,000 in Japan, and 1/50,000 in Korea[15]. According to Kobayashi et al. the frequencies of carrier and homozygotes on nine *SLC25A13* mutations were 1/50 and 1/10,000 in Korea, 1/69 and 1/19,000 in Japan, 1/79 and 1/25,000 in China, and 1/98 and 1/38,000 in Taiwan, respectively[3].

In Korea, c.851_845del and c.1177+1G>A mutations of *SCL25A13* were shown to comprise 85 % of all mutations[15]. Ko et al. reported four NICCD Korean cases of citrin deficiency in 2007, and mutation analysis identified them as compound heterozygotes carrying each of the c.851del14, c.1177+1G>A, c.1230_1G>A, G393S, and IVS16 ins3kb mutant alleles. All of these cases resolved within 5 to 9 months[16]. In 2007, Ko et al. also reported three NICCD Korean cases from 47 patients with neonatal cholestasis. They identified the compound heterozygous 1,638-1,660dup/S225X mutation, compound heterozygous 851del4/S225X mutation, and heterozygous 1,638-1660 dup mutation. All 3 of these patients achieved catch-up growth and normalized liver function with nutritional manipulation[17]. Other Korean cases have reported c.[674C>A];[1638_1660dup] (p.[Ser225*];[Ala554Glyfs*17])[18] and c.[852_855delTATG];[1180+1G>A] mutation[19].

In the present case, a heterozygous mutation, c.[1817G>A];[?] (p.[W606*];[?]), was detected, which has not yet been reported in the literature. Considering the biochemical findings associated with NICCD, our patient most likely has a compound heterozygous mutation with either an unknown mutation or is a normal carrier with mosaicism. In addition, mutiplex ligation-dependent probe amplification (MLPA) assay is required to detect large deletions/duplications or small rearrangements of *SLC25A13* in the present case because a large deletion of the *SLC25A13* gene reported two cases[20, 21]. The clinical picture of patients with NICCD includes variable symptoms such as growth retardation with transient intra-

hepatic cholestasis, prolonged cholestatic jaundice, hepatomegaly, diffuse fatty liver, parenchymal cellular infi1tration associated with hepatic fibrosis, variable liver dysfunction, hypoproteinemia, bleeding diathesis, hemolytic anemia, hypoglycemia[1, 22, 23], and multiple ovarian antral follicles[24]. Biochemical characteristics of NICCD include increased serum ammonia, citrulline, arginine, galactose, serum threonine-to-serine ratio, serum pancreatic secretory trypsin inhibitor, and alpha-fetoprotein[1, 23]. The differential diagnoses of NICCD include citrullinemia type 1 (CTLNl; ASS deficiency), argininosuccinic aciduria (argininosuccinate lyase [ASL] deficiency), lysinuric protein intolerance, pyruvate carboxylase deficiency, renal insufficiency, and classical galactosemia[1].

The treatment of NICCD patients includes a diet supplemented with fat-soluble vitamins and medium-chain triglyceride formula for prolonged jaundice, vitamin K for increased prothrombin time, and the use of lactose-free formula if the patient has an increased serum galactose level[1, 20, 21, 23]. The prognosis of NICCD patients is generally good. Although most patients with NICCD do not have a severe presentation and symptoms often resolve by one year of age with appropriate treatment, some infants experience infection and liver cirrhosis, and others require liver transplantation[26, 27].

Therefore, the patient's natural history and genetic background need to be further investigated in order to provide appropriate genetic counseling and management.

## 한 글 요 약

시트룰린혈증 2형은 *SLC25A13* 유전자 변이에 의한 시트린 결핍증으로 생기는 상염색체 열성 유전질환으로 고암모니아혈증, 시트룰린혈증, 저혈당증, 갈락토즈혈증 등의 생화학적 이상소견이 동반 되는 질환이다. 임상적으로 영아형인 '시트린 결핍증에 의한 신생아 간내 담즙정체(NICCD)', 소아형인 '시트린 결핍에 의한 성장 부진과 이상지방혈증(FTTDCD)', 성인형인 '성인기 발병 시트룰린혈증 2형(CTLN2)'의 세 가지 형태로 나타난다. 그 중 NICCD는 영아기 발생 간내 담즙 정체, 간 기능 장애, 저단백혈증, 저혈당증, 성장부진, 지방간 등의 증상이 나타나고 임상증상, 생화학적 검사 이상을 통해 질환을 의심한 후 *SLC25A13* 유전자 분석 검사를 통해 확진 할 수 있다. 저자들은 생후 35일에 시트룰린혈증으로 방문한 영아에서 NICCD와 부합되는 생화학적 검사 소견과 *SLC25A13* 유전자 염기서열 분석 검사상 c.[1817G>A]과 [?] (p.[W606*];[?]) 이형접합변이로 NICCD를 진단하였기에 보고하는 바이다.

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