증 례

동종 접합자 CPS1 돌연 변이를 동반한 신생아 발병형 Carbamoyl Phosphate Synthetase 1 결핍증의 치명적 사례

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A Fatal Case of Neonatal Onset Carbamoyl Phosphate Synthetase I Deficiency with Homozygous CPS1 Mutation

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Carbamoyl phosphate synthetase I (CPS1) deficiency is a rare autosomal recessive urea cycle disorder that causes hyperammonemic crisis. CPS1 is the first enzyme encoded by the CPS1 gene, which catalyzes the first step of the urea cycle. In CPS1 deficiency, ammonia, the toxic metabolite produced by the interruption of the urea cycle, is accumulated in the blood and brain, leading to hyperammonemic encephalopathy and irreversible brain damage. Here, we report a fatal case of neonatal-onset CPS1 deficiency in a 4-day-old girl presenting with recurrent seizures, who was revealed to be homozygous for c.1529delG (p.Gly510Alafs*5).

Key words: Carbamoyl phosphate synthetase I deficiency, Newborn, Urea cycle disorder, Hyperammonemia

Introduction

Carbamoyl phosphate synthetase I deficiency (CPS1D; MIM #237300) is a rare autosomal recessive urea cycle disorder (UCD) characterized mainly by hyperammonemia. CPS1 is a mitochondrial enzyme encoded by the gene CPS1, which catalyzes the first step of the urea cycle, the formation of carbamoyl phosphate from ammonia, bicarbonate and adenosine triphosphate (ATP) using an allosteric activator N-acetylglutamate (NAG)[1]. In CPS1D, the normal urea cycle is interrupted because of low or absent CPS1, and toxic ammonia accumulates in the blood and brain, leading to neurologic sequelle[2].

Unless promptly treated, hyperammonemia, accumulation of excessive amounts of the toxic metabolite ammonia, caused by CPS1D can lead to metabolic encephalopathy, coma, death or severe brain damage despite the patient being kept alive through intensive medical care. The time of onset and severity of the clinical presentation seem to be related to the amount of residual activity of CPS1. CPS1D has two main forms based on the age of onset: a fatal neonatal type and a less severe, delayed-onset type. The prognosis of

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patients with the neonatal type of CPS1D is generally poor, with high serum ammonia levels at initial presentation (known as severe hyperammonemic crisis in neonates), which most often leads to irreversible brain damage within the first few days of life[3].

The CPS1 gene (MIM #608307) spans approximately 120 kb on chromosome 2q35, comprising 38 exons and 37 introns. More than 240 pathogenic variations have been identified in CPS1 according to the Leiden Open Variation Database (LOVD, http://www.LOVD.nl/CPS1) and the Human Gene Mutation Database (HGMD, http://www.hgmd.org/)[4].

A 4-day-old girl presented at our hospital with hyperammonemic crisis and was diagnosed with CPS1D, homozygous for c.1529delG (p.Gly510Alafs*5) in exon 14. Here, we report her clinical course and the results of gene assay.

Case Report

A 4-day-old girl was referred to Seoul National University Children's Hospital (SNUCH) because of recurrent seizures and severe hyperammonemia. She was delivered by normal vaginal spontaneous delivery at 40 weeks of gestation and weighed 3,170 g at birth. The Apgar scores were 9 and 10 at 1 and 5 min, respectively. It was the fourth pregnancy of unrelated healthy parents, and the patient had a healthy 4-year-old sister. The first baby died on day 3 after seizure occurred on day 2, and a miscarriage occurred early in the second pregnancy (Fig. 1).

Seizures started on the second day after birth, and she appeared lethargic, tachypneic, and diaphoretic. Seizures lasted for less than 1 minute without any treatment. On the fourth day after birth, recurrent generalized tonic-clonic seizures

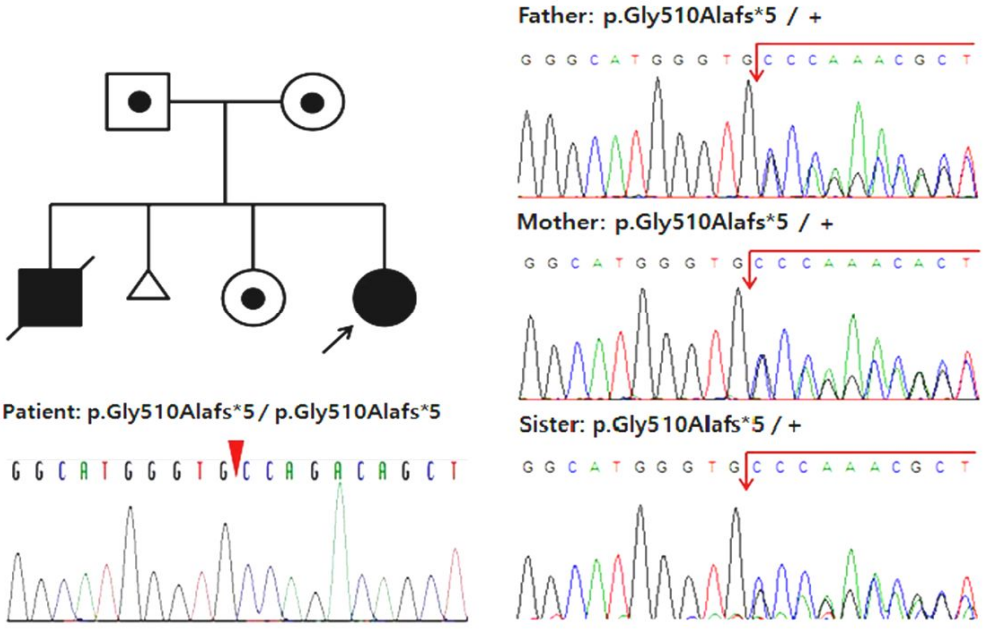

Fig. 1. The pedigree of the family carrying CPS1 pathogenic variants identified in a patient with CPS1D and her family members. The mother, father, and living sister were all heterozygous (p.Gly510Alafs*5/+) carriers, and the patient was homozygous (p.Gly510Alafs*5/ p.Gly510Alafs*5) for the gene.

developed, requiring intravenous lorazepam and phenobarbital treatment. Light reflex, brain stem reflex, and self-respiration became absent, and hypotension developed. She was intubated and mechanically ventilated. Hyperammonemia (>587.2 umol/L) was documented, and she was transferred to SNUCH for continuous renal replacement therapy (CRRT) at 4th day after birth.

On admission, she had hyperammonemia (554.9 μmol/L; reference range, 8.81–29.95 μmol/L) without metabolic or lactic acidosis. Her heart rate was 166 beats per minute (bpm), and blood pressure was 61/33 mmHg while on continuous infusion of dobutamine. Serum aspartate aminotransferase and alanine aminotransferase levels were 110 IU/L and 38 IU/L respectively (reference range, 1–40 IU/L). Newborn screening tests using tandem mass spectrometry showed markedly elevated alanine levels (1,575.98 μmol/L, cutoff: 340.97 μmol/L). Plasma amino acid analysis showed elevated alanine (8,284 μmol/L, cutoff: 421 μmol/L), glutamine (1893 μmol/L, cutoff: 776 μmol/L), lysine (1,631 μmol/L, cutoff: 200 μmol/L), glycine (583 μmol/L, cutoff: 338 μmol/L), and proline (699 μmol/L, cutoff: 254 μmol/L) levels. Citrulline (6 μmol/L, cutoff: 8 μmol/L) was decreased; however, orotic acid in urine was within the reference range (5.08 mmol/mol of creatinine; reference range, 0.2–6.0), suggesting CPS1D or N-acetylglutamate (NAG) synthase deficiency rather than distal UCDs. CRRT was performed for rapid removal of toxic ammonia immediately after admission (on the fourth day after birth), and intravenous sodium benzoate and sodium phenylbutyrate were administered. Two days later, plasma ammonia decreased to 117.44 μmol/L, and CRRT was discontinued (Fig. 2). However, her electroencephalogram suggested severe diffuse cerebral dysfunction, and brain sonography revealed bilateral and symmetric increased echogenicity in the deep gray matter with diffuse increased echogenicity in the brain, suggesting brain edema on the sixth day after birth. The patient was still flaccid, and reflexes, such as light and brain stem reflexes, were absent. The parents decided to discontinue further medical management. Following uncontrolled metabolic acidosis, hypotension became refractory, and the patient died on the 19th day after birth.

Her DNA was extracted from peripheral blood leukocytes and was amplified via polymerase chain reaction (PCR). Direct Sanger sequencing was subsequently performed using amplified PCR products and revealed a homozygous mutation in the *CPS1* gene (Fig. 1): c.1529delG (p.Gly510Alafs*5) in exon 14. Family screening of this identified mutation showed that both of her parents and living sister were heterozygous carriers of the same mutation found in the patient (Fig. 1).

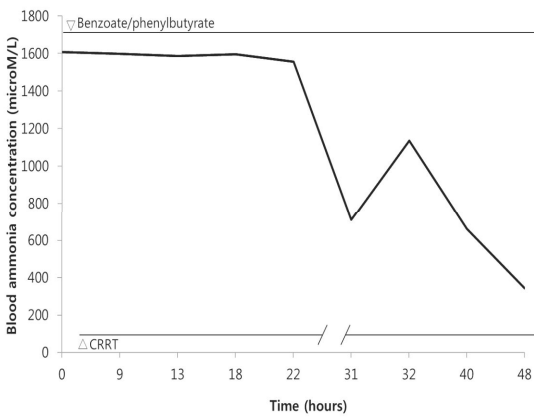

Fig. 2. Graph of blood ammonia concentrations as a function of time in the patient. Benzoate and phenylbutyrate were administered immediately after admission, followed by continuous renal replacement therapy (CRRT). CRRT was interrupted because of intractable hypotension. Note that blood ammonia level was controlled within 48 h after admission. Benzoate and phenylacetate infusions are indicated by the horizontal line at the top of the chart. CRRT is shown by a horizontal line at the bottom (breaks in the line indicate interruption of CRRT).

Discussion

In the present study, we report a case of neonatal-onset CPS1D, resulting from a homozygous mutation of CPS1 located on chromosome 2q35 independently segregated from parents of heterozygous carriers. The patient experienced severe clinical deterioration immediately after birth, which led to severe brain edema, coma, and neonatal death at the age of 19 days. The prevalence of UCD is 1/35,000 in the United States[5]. The incidence of CPS1D is much rarer and reported to be 1/1,300,000 in the United States, 1/800,000 in Japan, and 1/539,000 in Finland[6]. Nakamura et al retrospectively analyzed 177 patients with UCDs from 1999 to 2009 in Japan and found that CPS1D comprised approximately 10% of patients[7].

To date, more than 240 CPS1 mutations have been identified among the splicing mutations. These pathogenic variants include missense changes (~59%), deletions (~13%), small insertions or duplications (~6%), indels (~2%), nonsense (~7 %), gross deletions, and splicing pathogenic variants (~13%), but in most cases those pathogenic variants have not been confirmed[3]. The mutation identified in our patient, c.1529delG (p. Gly510Alafs*5) in exon 14, has been reported in the Japanese population[2]. Of cases with mutation of c.1528delG in Japanese infants with CPS1 deficiency, two infants died before 1 week, and the other patient who had 17% enzyme activity was alive without mental retardation until 13 years of age. The present case is reportedly the first case of c.1529delG mutation in Korea[8].

CPS1 is a proximal and rate-limiting enzyme in the urea cycle, and CPS1D is the most severe form of UCDs. Patients with complete deficiency rapidly develop hyperammonemia in the newborn period. Most patients with residual enzyme activity (10-25% of normal) have the late-onset type of the condition and are successfully rescued from crisis, but they are at risk for recurrent hyperammonemic episodes in their life[9]. The onset and duration of neonatal hyperammonemic crisis is the most important determinant for severe brain injury. Hyperammonemia and its rapid correction should be promptly detected to minimize neurological consequences because pre-existing brain injury is mostly irreversible and may persist even if hyperammonemia is corrected[10]. The patient in the present case might have experienced hyperammonemic crisis at least for 72 h, which was a significant risk factor for severe neurologic impairment.

Therefore, although genetic analysis has been clinically applicable for the diagnosis of UCDs, a high index of suspicion is required for early and appropriate management if newborn infants show neonatal seizure and hyperammonemia without acidosis. Amino acid analysis should be performed, and CPS1D must be considered if low plasma levels of citrulline and arginine, high plasma levels of glutamine, increased transaminases, and low or normal levels of orotic acid in the urine are noted. Newborn screening tests, including amino acid and acylcarnitine analysis, using tandem mass spectrometry could be helpful to rule-out distal UCDs with elevated citrulline levels. In addition, genetic analysis of chorionic villi or amniotic fluid cells would be valuable for prenatal screening of the next pregnancy.

The present case indicated an autosomal recessive inheritance pattern, and genetic analysis identified identical variants in her sister and their parents. The first baby who died on day 3 after seizure could be presumed to have the same genotype as that of our patient, who had a homozygous mutation in the CPS1 gene and had signi-

ficant CPS1D, leading to rapid development of hyperammonemia.

Herein, we report a case of an infant with homozygous for c.1529delG (p.Gly510Alafs*5) who was born to heterozygous carrier parents, resulting in neonatal-onset CPS1D with rapid clinical deterioration leading to death. Neonatal-onset CPS1D is a rare disease that could present with nonspecific symptoms (vomiting, poor feeding, tachypnea, seizure), and other UCDs and organic acidurias with hyperammonemia also present similar symptoms. Various efforts should be immediately made to lower ammonia level, and rapid amino acid analysis of plasma and urine should be guaranteed. Comprehensive and rapid approach for diagnosis and treatment is essential in patients with CPS1D.

요 약

Carbamoyl phosphate synthetase I (CPS1) 결핍은 상염색체 열성 유전을 하는 드문 요소회로 대사 이상 질환으로, 요소회로의 첫번째 단계 효소인 CPS1 결핍에 의해 고암모니아혈증이 발생하여 신경학적 이상을 초래하게 되는 질환이다. CPS1 결핍은 신생아기부터 성인까지 여러 시기에 발현될 수 있으나, 주로 신생아기에 증상이 발현하고, 신생아기에 증상이 발생할 경우 치명적인 고암모니아혈증이 발생하여 예후가 불량하여 사망에 이를 수 있다. 본 증례는 고암모니아혈증이 발견되어 투석 및 집중 치료하였음에도 심한 뇌손상이 빠르게 진행되어 사망한 신생아로, CPS1 유전자의 동종 접합자 변이를 확인하여 확진 되었으며 이후 시행한 가족 검사에서 부모와 생존한 자매가 모두 이종 접합자 보인자로 확인되어 보고하는 바이다.

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