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Case Report

Two novel mutations in the *CPS1* gene of a newborn with carbamoyl phosphate synthetase 1 deficiency identified by next-generation sequencing

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Abstract: Carbamoyl phosphate synthetase 1 deficiency (CPS1D) is a rare autosomal recessive hereditary disease which usually presents as lethal hyperammonemia. Here we report the case of a newborn infant with lethal hyperammonemia. Blood liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis showed increased concentrations of alanine, glutamine and histidine. Urine gas chromatography-mass spectrometry (GC/MS) showed increased levels of organic acids, while uracil and whey acid were not detected. Subsequent new generation sequencing (NGS) identified two heterozygous mutations in *CPS1* gene. Both of them were identified in his parents. One was c.446T>C; p.Leu149Ser, in the 4 exon of *CPS1*. The other was C.2023delT; p.Cys675fs, in the 17 exon of *CPS1*. These two novel heterozygous mutations had not been reported previously. Early diagnosis and timely intervention are very important to improve clinical outcome. The new *CPS1* gene mutation broadens the spectrum of *CPS1* phenotypes. NGS is a credible way in complex diseases without a specific phenotype.

Keywords: Carbamoyl phosphate synthetase 1 deficiency, hyperammonemia, gene mutation, neonate

Introduction

Carbamoyl phosphate synthetase 1 deficiency (CPS1D) is a rare autosomal recessive hereditary disease caused by mutations of *CPS1* gene. The incidence of Carbamyl phosphate synthetase 1 deficiency (CPS1D) was reported to be 1/62000 in the United States [1], 1/800000 in Japan [2], 1/539000 in Finland [3]. Nakamura et al retrospectively analyzed 177 patients with urea cycle disorders from 1999 to 2009 in Japan and found that the proportion of CPS1D excesses 10% [4].

On the basis of age of onset, clinical manifestations and the activity level of the CPS1 enzyme, CPS1D has been classified into two clinical phenotypes: neonatal onset and later onset. Neonatal onset CPS1D manifests clinically within the first few days of life. Affected infant present with refusal to feed, lethargy, hypothermia, vomiting, hypotonia, convulsions, coma and even death. In contrast, later onset CPS1D is associated with less severe clinical manifestations. Kurokawa [5] conducted clinical research and genetic test of 18 patients with CPS1D, including 15 cases of neonatal onset, most of these cases with severe high blood ammonia.

Materials and methods

Subjects

The case was a 3420-g term male newborn delivered by a healthy primigravida mother in a level II birth center. No family history of urea cycle defect. Apgar scores were 10/10/10. The newborn physical examination was normal at birth. He was formula-fed. On the second day after birth, he was admitted to a level II Neonatal Intensive Care Unit (NICU) for hypothermia and refusal to feed. His condition became worse and he had a seizure on the fourth day and was transferred to our level III NICU. On admission his vital signs were as follows: temperature 35.4°C, heart rate 140/min, respiratory rate 38/min and blood pressure 66/41 mmHg. The

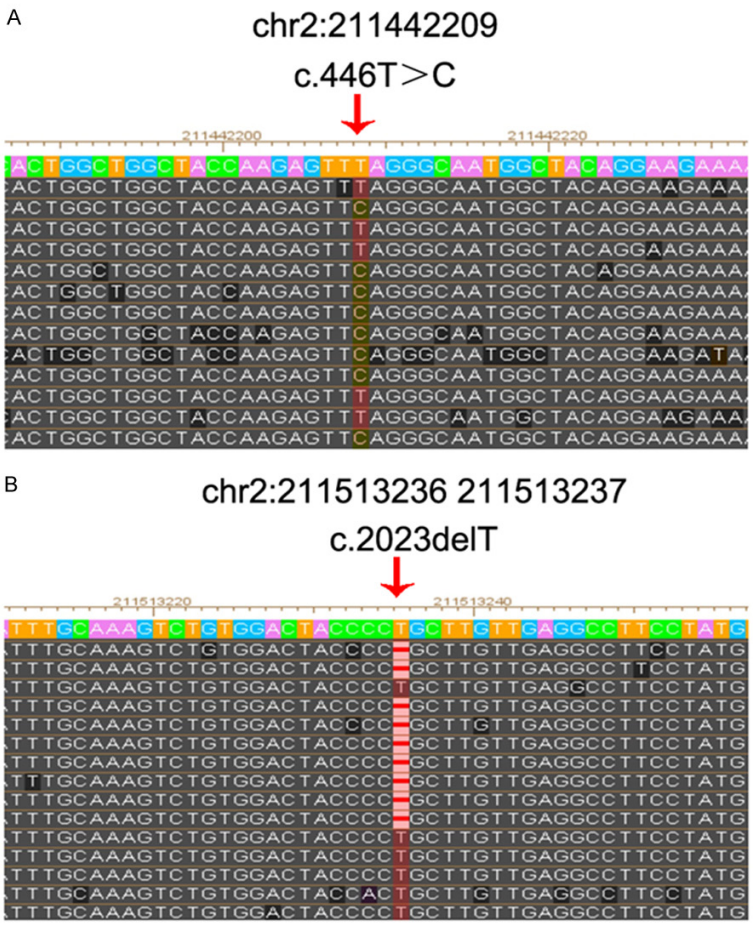

Figure 1. Two *CPS1* gene pathogenic variants of CPS1D. Two *CPS1* gene pathogenic variants were identified in a patient with carbamoyl phosphate synthetase 1 deficiency by whole exome sequencing.

remainder of the physical examination was unremarkable. He had a severe metabolic acidosis on arterial blood gas (pH 7.244, $PaCO_2$ 23.8 mmHg, base excess -16 mmol/L, lactic acid 4.90 mmol/L). Liver and kidney functions were normal. He had hyperammonemia (586.00 μmol/L) and his blood ammonia quickly ascended to 1320.00 μmol/L. After a comprehensive physical examination and careful inspection, treatment was started.

Clinical and laboratory data

Clinical assessment was undertaken in Xin Hua Hospital. The blood ammonia level was analyzed repeatedly during hospitalization. The newborn presented with an overwhelming course with refractory hyperammonemia. Blood liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis showed an alanine concentration of 380.89 μmol/L (normal range 70-350 μmol/L), glutamine 377.07 μmol/L (normal range 2-35 μmol/L), histidine 686.18 μmol/L (normal range 13-250 μmol/L), urea/citrulline was 13.03 (normal range 1.5-11), and citrulline/arginine ratio 0.2 (normal range 0.3-6.5). Urine gas chromatography-mass spectrometry (GC/MS) showed organic acids were increased in concentration and uracil and whey acid were not detected.

Genetic analyses

Informed consent was obtained from the guardian of the infant following institutional guidelines. Genomic DNA from peripheral blood leukocytes derived from the newborn and his parents was extracted using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). The exon regions of 505 genes including *CPS1* were specifically enriched using biotinylated capture probe (MyGenostics, Baltimore, MD, USA) as described previously [6, 7]. Sanger sequencing was used to confirm the mutations. The PCR product was purified using SPRI beads (Beckman Coulter, Inc.) according to manufacturer's protocol. The enrichment libraries were sequenced using an Illumina HiSeq 2000 sequencer. Short read mapping and alignment were performed using BWA software (Burrows Wheeler Aligner).

Results

Clinical treatment and outcome

The therapeutic measures concluded correcting acidosis, antispasmodic, reducing intracranial pressure and maintaining the main visceral function. There was no evidence of clinical improvement. Nevertheless, he gradually developed deep comatose, no urine, electrolyte disorder (hypocalcemia, hyperkalemia). Eventually he died when he was 7 days old.

A thorough history, physical examination, and laboratory workup failed to identify a clear etiology of hyperammonemia, so prompt genetic investigation was carried out.

Molecular diagnosis

Targeted sequence capture and next-generation sequencing has been employed for genetic analysis.The targeted genes were specifically enriched using a biotinylated capture probe (MyGenostics, Baltimore, MD, USA). Sanger sequencing was used to confirm the mutations. We recognized two novel mutations of *CPS1* gene. One mutation is a T to C substitution at nucleotide 446 of the *CPS1* gene (GenBank accession No. BC140943), which leads to an amino acid change from leucine to serine at codon 149. The mutation of c.446T>C; p. Leu149Ser was also detected in his asymptomatic father (**Figures 1A** and **2A**). Another mutation c.2023delT (GenBank accession No. AC008172) leading to the frame shift of reading code box, encoded proteins from 675th cysteine frame-shift mutations (p.C675fs). It was also detected in his asymptomatic mother (**Figures 1B** and **2B**). None of these two genetic variants have been previously reported. The parents gave permission to publish the paper in an anonymous manner. The case did not have autopsy.

Discussion

Carbamoyl phosphate synthetase 1 (CPS1), which catalyzes the first step of the urea cycle, is a mitochondrial matrix enzyme and it is expressed in the hepatocytes and epithelial cells of the intestinal mucosa. The human *CPS1* gene is located on chromosome 2q35 and spans nearly 120 KB. It is composed of 38 exons and 37 introns [8-10]. It has an open reading frame of 4503 coding nucleotides and encodes a polypeptide of 1500 amino acids residues [11-13]. Until now, more than 220 genetic variants associated with CPS1D have been reported [14].

The two mutations we report herein are located on exon 4 (c.446T>C; p.Leu149Ser) and exon 17 (C.2023delT; p.Cys675fs). These two novel heterozygous mutations were inherited individually from our patient's parents. Based on function, the human *CPS1* gene could be divided to four sections, including N-terminal domain, bicarbonate phosphorylation domain, unknown function (oligomerization) domain and carbonate phosphorylation domain [15]. Exons 4 and 17 belong to bicarbonate phosphorylation domain. But the exact mechanism of dysfunction of human CPS1 caused by these two mutations remains unclear.

CPS1D is a severe type of urea cycle disorder that can cause lethal hyperammonemia. Clinically, severe CPS1D can present with hypothermia, anorexia, vomiting, convulsions, coma, and other signs. Irreversible damage to the central nervous system is the most serious complication of CPS1D, which is closely related to the long-term prognosis of neonates. Early identification of these signs is important. Treatment of CPS1D could be summarized as follows: First, early detection and thorough careful clinical observation, preventing the occurrence of metabolic imbalance. Second, it is important to reduce blood ammonia in the acute phase. Sufficient intravenous infusion of glucose electrolyte solution is paramount in order to inhibit protein catabolism. Third, reducing toxic metabolite accumulations using arginine and L-carnitine is necessary. Sodium benzoate and sodium phenyl butyrate should also be used to enhance excretion of excess nitrogen. If hyperammonemia persists in spite of these measures, peritoneal dialysis or hemodialysis or hemofiltration dialysis should be started instantly.

The neonatal onset type usually exhibits severe hyperammonemia and patients often die shortly after birth. We emphasize the importance of collecting and storing of biological samples. Blood LC/MS-MS and urine GC-MS are helpful for early diagnosis of genetic metabolic disease and now widely used in neonatal screening. Genetic analysis of chorionic villi or amniotic fluid cells is feasible, fast, and specific [16].

Conclusions

CPS1D is a severe type of urea cycle disorder that can cause lethal hyperammonemia. Clinically, severe CPS1D can present with hypothermia, anorexia, vomiting, convulsions, coma, and other signs. Early identification of these signs is important. When suspicion for inherited metabolic defect is high, these tests may need to be repeated in the acute phase of disease or metabolic crisis period when the ini-

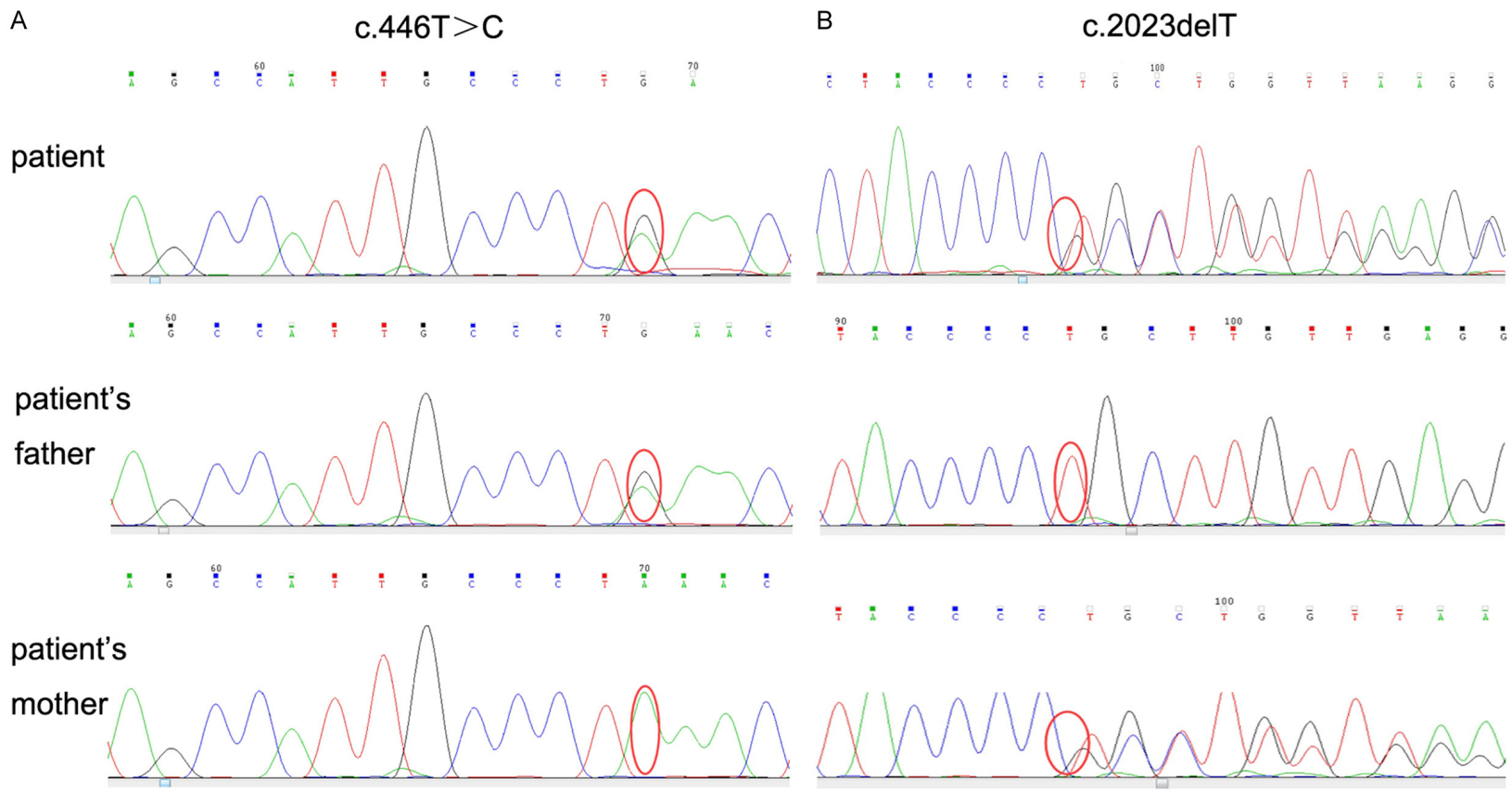

Figure 2. Two *CPS1* pathogenic variants were also identified in his parents. The mutation of c.446T>C (A) was detected in his asymptomatic father and another mutation c.2023delT (B) was detected in his mother, both were confirmed by Sanger sequencing.

tial test is negative. Biochemical assays and gene mutation analysis are helpful in making the diagnosis conveniently. Prenatal diagnosis in urea cycle disorders is also available.

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Disclosure of conflict of interest

None.

Abbreviations

CPS1D, Carbamoyl phosphate synthetase 1 deficiency; NGS, new generation sequencing; NICU, Neonatal Intensive Care Unit.

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