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Genetic Metabolic Series

A Newborn with Hypothermia and Hyperammonemia

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A full-term small gestational age female infant was transferred to the NICU for hypothermia and seizure-like activity. In the first 10 h of life, the infant had difficulty feeding and developed mild hypothermia, which resolved after placing her on a radiant warmer. At 47 h of life, she again developed hypothermia, with an axillary temperature of 34.4 °C. At that time, a physical and neurological examination was notable for an altered level of consciousness, irregular breathing, hypertonia, central rigidity, and exaggerated Moro reflex, as well as jaundiced skin. Her serum direct bilirubin (1.1 mg/dL, reference interval 0–0.3 mg/dL) and aspartate transaminase (58 U/L, reference interval 10–35 U/L) were increased, whereas her alanine transaminase and total bilirubin were within normal limits. Abdominal examination was unremarkable with no evidence of hepatosplenomegaly. At 55 h of life, she developed severe hyperammonemia (plasma ammonia 1906 μmol/L, reference interval 56–92 μmol/L) and respiratory alkalosis (pH 7.6, reference interval 7.35–7.45; pCO_2 25 mmHg, reference interval 41–54 mmHg; and bicarbonate 24 mEq/L, reference interval 17–24 mEq/L). The increased ammonia raised suspicion for an inborn error of metabolism. A urine organic acid and serum amino acid analysis was performed. The results are shown in Fig. 1.

DIAGNOSIS AND SUMMARY

This patient has citrullinemia type I (CTLN1)[2] [OMIM # 215700], a urea cycle disorder (UCD) resulting from a deficiency of the enzyme argininosuccinate synthetase (ASS) [EC 6.3.4.5]. ASS is a urea cycle enzyme that catalyzes the synthesis of argininosuccinate from citrulline and aspartate. The urea cycle (Fig. 2) is a metabolic pathway occurring primarily in the liver and is responsible for the removal of nitrogenous waste by converting ammonia to urea. It is composed of 5 catalytic enzymes [carbamoyl phosphate synthetase 1 (CPS1); ornithine transcarbamoylase (OTC); argininosuccinate synthetase (ASS); argininosuccinate lyase (ASL), and arginase (ARG1)], one cofactor producing enzyme [N-acetylglutamate synthase (NAGS)]), and 2 amino acid transporters [ornithine translocase (ORNT1) and citrin]. There are 8 distinct urea cycle disorders that may result from deficiencies in any one of these enzymes or transporters. Hyperammonemia, a nonspecific marker of inadequate nitrogen detoxification, is the biochemical hallmark of most UCDs; therefore, a plasma ammonia concentration is the first laboratory test indicated for the workup of a UCD. As noted in this patient, respiratory alkalosis is a common symptom in UCDs caused by hyperventilation secondary to hyperammonemia and cerebral edema.

In addition to plasma ammonia, urine organic acid and serum/plasma amino acid analyses are necessary for the diagnosis of UCDs. In this case, urine organic acid analysis revealed a large orotic acid peak. Increased urine orotic acid is suggestive of a deficiency in 1 of 4 catalytic enzymes: OTC, ASS, ASL, or ARG1. In OTC, ASS, or ASL deficiency, carbamyl phosphate is shunted into the pyrimidine synthetic pathway, leading to the accumulation of orotic acid. However, in ARG1 deficiency, the accumulation of arginine, which activates NAGS, results in increased production of orotic acid. It is worth mentioning that qualitative methods for urine organic acid analysis may have variable analytical sensitivity for orotic acid and more sensitive quantitative methods are available. Serum amino acid analysis aids in pinpointing the biochemical defect by determining which of the 4 enzymes is involved. This patient's serum amino acid analysis revealed a large increase in citrulline (1481 μmol/L, reference interval 3–55 μmol/L) and the absence of

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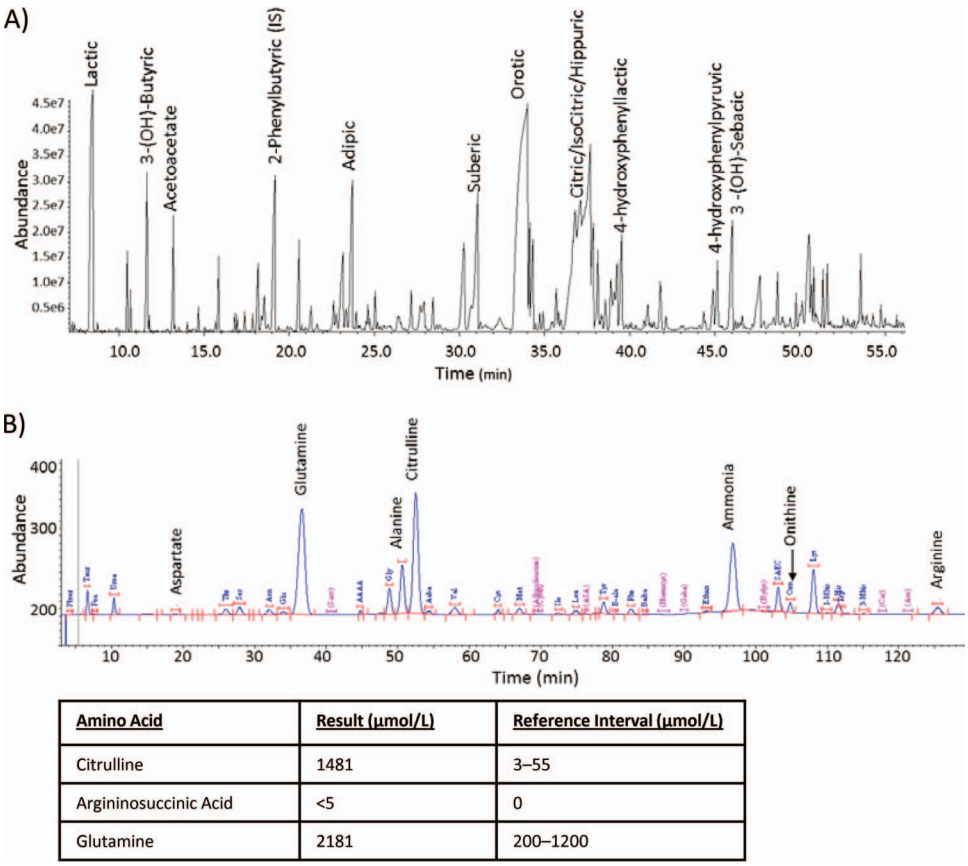
[2] Nonstandard abbreviations: CTLN1, citrullinemia type I; UCD, urea cycle disorder; ASS, argininosuccinate synthetase; CPS1, carbamoyl phosphate synthetase 1; OTC, ornithine transcarbamoylase; ASL, argininosuccinate lyase; ARG1, arginase; NAGS, N-acetylglutamate synthase; ORNT1, ornithine translocase.



Amino Acid	Result (μmol/L)	Reference Interval (μmol/L)
Citrulline	1481	3–55
Argininosuccinic Acid	<5	0
Glutamine	2181	200–1200

Fig. 1. Organic acid and amino acid analysis.

Urine organic acids were extracted into ethyl acetate/ether and converted to trimethylsilyl derivatives prior to analysis using a GC 7890B/MS 5977A system equipped with an agilent HP-5MS column (A). Amino acid analysis of the patient's serum by HPLC (B). Serum amino acids were separated on an ion exchange column followed by post-column reaction with ninhydrin using the Biochrom 30+ amino acid analyzer.

argininosuccinic acid, indicating a deficiency in ASS. Increased citrulline can also be found in patients with ASL and citrin deficiency; however, a citrulline concentration of >1000 μmol/L is highly suggestive of ASS deficiency. Glutamine increases are often observed in patients with UCDs due to excess nitrogenous waste. Of note, this patient's newborn screening results showed an increase of citrulline, consistent with the diagnosis of a UCD. Furthermore, ASS1 sequence analysis revealed that the patient was homozygous for the pathogenic variant, c.470G>A (p.R157H), which is associated with an acute neonatal (classical) form of citrullinemia.

CTLN1 is a rare autosomal recessive disorder with an incidence of 1 in 50 000 births. It has overlapping clinical features with other UCDs, which include seizures, increasing lethargy and somnolence, diminished oral intake, hypothermia, and cerebral edema-associated respiratory changes (hyper or hypoventilation). Presentation is also difficult to differentiate from neonatal sepsis or birth hypoxia. Diagnosis of CTLN1 is based on clinical suspicion and laboratory evaluation (plasma ammonia >150 μmol/L, plasma citrulline >1000 μmol/L, and absence of arginosuccinic acid in the amino acid profile). The classical form of CTLN1 often presents within 42–72 h of life. A nonclassical form or delayed-onset form of CTLN, which can present just outside the newborn period to adulthood, has also been reported. Severe hyperammonemic episodes can occur in these patients; however, neurologic findings may be subtle. Presenting symptoms may include migraine-like episodes, lethargy, somnolence, ataxia, and slurred speech. Of note, pathogenic variants associated with this nonclassical form of CTNL1 have been reported. Prompt diagnosis and treatment is critical to prevent coma or death in both forms of CTNL1. Treatment includes the removal of ammonia by hemofiltration, caloric supplementation

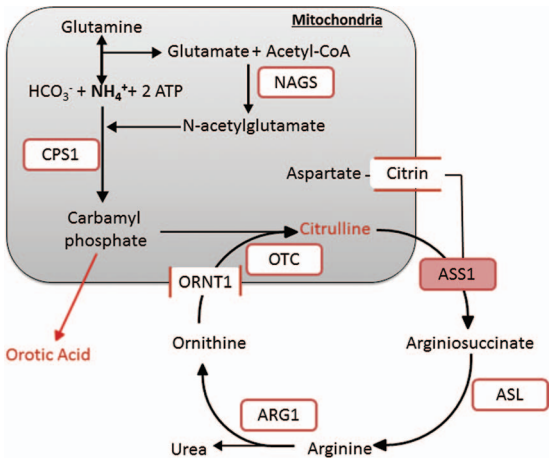


Fig. 2. Urea cycle.

The enzymes and transport proteins involved in the urea cycle are illustrated in boxes with a red outline. The filled red box indicates the enzyme deficiency seen in citrullinemia type I.

with a protein-restricted diet, and pharmacologic scavenging of excess nitrogen. The goal of long-term management includes prevention of catabolic decompensation. Clinical and laboratory monitoring is essential to ensure adequate protein intake and adequate metabolic control. In this patient's case, hemodialysis was initiated at day 3 of life and her plasma ammonia fell rapidly to normal concentrations. To aid in nitrogen clearance, she was started on arginine supplementation (150 mg/kg every 6 h) and phenylbutyrate (150 mg/kg every 6 h), a nitrogen scavenger that aids in the excretion of glutamine. Arginine supplementation in patients with CTLN1 drives the urea cycle forward by replenishing ornithine and maximizing excretion of citrulline. The patient's seizures were corrected upon the resolution of hyperammonemia. Fortunately, imaging studies showed no evidence of permanent neurological sequelae. She was started on a high-calorie, protein-restricted formula upon resumption of oral feeds. After 3 weeks of hospital stay, she was discharged home.

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