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Short Communication

Compound heterozygote variants: c.848A > G; p.Glu283Gly and c.890C > T; p.Ala297Val, of Isovaleric acid-CoA dehydrogenase *(IVD)* gene causing severe Isovaleric acidemia with hyperammonemia

Anne Chun-Hui Tsai *, Hsin-Ti Lin, Maxwell Chou, Jessica Bolen, Chelsea Zimmerman, Danielle DeMarzo, Yazmin Enchautegui-Colon

Section of Genetics, Department of Pediatrics, University of Oklahoma Health sciences Center, Oklahoma Children's Hospital, Oklahoma City, OK, United States of America

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ABSTRACT

With the execution of expanded newborn screen (NBS) program nationwide, it is uncommon to see severe hyperammonemia associated with isovaleric acidemia (IVA). We present a seven-day-old boy with severe IVA complicated by hyperammonemia. This child was flagged by NBS at 4 days old, but confirmatory testing was delayed due to COVID19 pandemic and parental skepticism. His parents did not adhere to the leucine-restricted diet as recommended. On day 7, the patient presented to the ER with ammonia of 588 μg/dL. Ammonia subsequently rose to >1000 μg/dL. This child received carnitine, 1 dose of Ammonul (sodium benzoate and sodium phenylacetate), arginine, carglumic acid (Carbaglu) and CRRT. Plasma amino acid assay revealed a glutamine level of 256 μmol/L, which is below the lower limit of normal upon arrival to ER and PICU. The hyperammonemia was corrected in 15 h and with the continued use of carglumic acid for 3 days, there was no rebound of hyperammonemia. However, the patient suffered from bone marrow suppression associated with the organic acidemia and required frequent platelet transfusions, as well as G-CSF for neutropenia. The management of this patient provides supporting evidence of the many theoretic metabolic "facts" including why Ammonul is not helpful in organic acidemias.

1. Introduction

Isovaleric acidemia (IVA) is a rare autosomal recessive metabolic disorder which disrupts or prevents normal metabolism of the branched-chain amino acid leucine [1,2]. The enzyme encoded by *IVD*, isovaleryl-CoA dehydrogenase (EC 1.3.8.4), plays an essential role in breaking down proteins from the diet. Specifically, the enzyme is responsible for the third step in processing leucine, an essential amino acid [3]. If a mutation in the *IVD* gene reduces or eliminates the activity of this enzyme, the body is unable to break down leucine properly [1]. As a result, free isovaleric acid may accumulate and damage the brain and nervous system [4]. With restriction of leucine intake, supplementation of glycine and carnitine, and being keen and responsive during intercurrent illness, affected children usually do well [5].

2. Case report

The patient was ascertained as a seven-day-old boy with severe IVA complicated by hyperammonemia. He was born vaginally at 39 1/7 weeks of gestation to a 27-year-old G4P4 mother and 39-year-old father. At birth, weight was 6 lb. 9 oz., length was 20.9 in, and occipitofrontal circumference was 34 cm. Apgar scores were 8 and 9. The patient was admitted to the NICU due to maternal GBS without proper antibiotics prophylaxis. He was discharged after 2 days with no concerns.

This child was flagged by NBS on day 4 of life, but confirmatory testing was delayed due to COVID19 pandemic and because the parents were skeptical and hesitated to bring the child to the NBS follow up clinic. While waiting for the confirmatory results, the child's mother did not adhere to the leucine-restricted diet recommended by the clinic. On day 7 of birth, the patient presented to the emergency room with ammonia of 588 μg/dL. Ammonia subsequently rose up to >1000 μg/dL.

* Corresponding author at: Pediatrics/Genetics, University of Oklahoma Health Science Center, Children's Hospital, Oklahoma, 1200 Children's Ave., Suite 12103, Oklahoma City, OK 73104, United States of America.
E-mail address: Anne-Tsai@ouhsc.edu (A.C.-H. Tsai).

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Table 1
Ammonia and plasma amino acid levels 7–24 days after birth.

Days after Birth	7	8	8	9	10	24	Reference Range
Ammonia ug/dL	13:30: 588 16:30: >1000 20:00: 809 22:00: > 1000	1:00: 588 3:00: 291 5:00236	7:00: 146 9:00: 101 12:00: 79 CRRT stopped at 2:00 pm	50–92	NA	NA	96–157
Glutamine (μmol/L)	230 (13:30)	231 (3:00)	214 (15:00)	259	237	870	376–709
Glycine (μmol/L)	170	155	159	614	469	990	232–740
Alanine (μmol/L)	216	214	226	315	230	644	131–710
Leucine (μmol/L)	70	68	70	123	88	51	48–160

Ammonul (sodium benzoate and sodium phenylacetate) was intentionally withheld due to concern that sodium benzoate would bind to glycine which is needed for renal elimination of isovaleric acid as isovalerylglycine. Initiation of CRRT was delayed due to lack of blood product to prime the machine. The patient was administered D10 plus salts, arginine, carnitine, and one dose of carglumic acid (6:10 pm), and his ammonia decreased to 809 μg/dL (on the 8:00 pm check). Ammonul was given while waiting for the blood product (around 8:40 pm) and repeat ammonia level increased to greater than 1000 μg/dL (on the 10:00 pm check). Plasma amino acid assay came out the next day, which revealed a glutamine level of 256 μmol/L, which is below the lower limit of normal. The hyperammonemia was corrected in 15 h by CRRT and with the continued used of carglumic acid, there was no rebound of hyperammonemia. However, the patient suffered from bone marrow suppression associated with the organic acidemia and required frequent platelet transfusions as well as G-CSF for neutropenia. The patient continued follow-up in the inborn errors of metabolism clinic with restricted protein diet supplemented by glycine 200 mg/kg/day and carnitine 100 mg/kg/day. He is now 14 months old, doing well and seems developmentally normal.

2.1. Molecular genetics

Next generation sequencing of the *IVD* gene was performed due to abnormal newborn screening results and clinical concern for isovaleric acidemia (IVA). The test results revealed two heterozygous variants, c.848A > G (p.Glu283Gly) and c.890C > T (p.Ala297Val), in this individual. Parental testing confirmed the two alleles were in trans phase.

The c.848A > G variant in exon 8 of the *IVD* gene replaces glutamic acid with glycine at codon 283 (p.Glu283Gly), a highly conserved amino acid. This variant has not been reported in the literature. It is absent from the general population data bases [6], indicating it is unlikely benign. In silico analysis predicts this change to be deleterious to protein function or structure. The lab classified this variant as of uncertain significance but based on the physical presentation, biochemical profiles and history, we believe it to be pathogenic. The second variant, c.890C > T, is located in exon 9 of *IVD*. It results in a missense substitution of alanine with valine at codon 297 (p.Ala297Val), a highly conserved amino acid. This variant has been identified in patients with IVA diagnosed by clinical manifestations and abnormal biochemical profiles [7,8]. The c.890C > T variant is documented in the population database [6] with a relatively low frequency of 0.00080%, indicating it is unlikely benign. Multiple in silico analysis tools predict this variant is probably damaging to protein function or structure. Taken together, the lab interpreted this variant as likely pathogenic; clinically, we believe it to be pathogenic.

His brother was born 13 months later. He also carries the same pathogenic variants and is clinically affected.

2.2. Relevant plasma amino acid levels corelate with ammonia levels

See Table 1

2.3. Medical management timeline with ammonia levels

See Fig. 1

3. Discussion

It has been observed and suggested by multiple biochemical geneticists that Ammonul (sodium benzoate and sodium phenobutyrate) is not helpful in hyperammonemia secondary to organic acidemia [9,10]. However, this medicine is still commonly used in the medical community in hyperammonemia patients. Our patient demonstrated that Ammonul is not helpful. Carglumic acid, however, is a rather effective treatment for hyperammonemia secondary to IVA which was

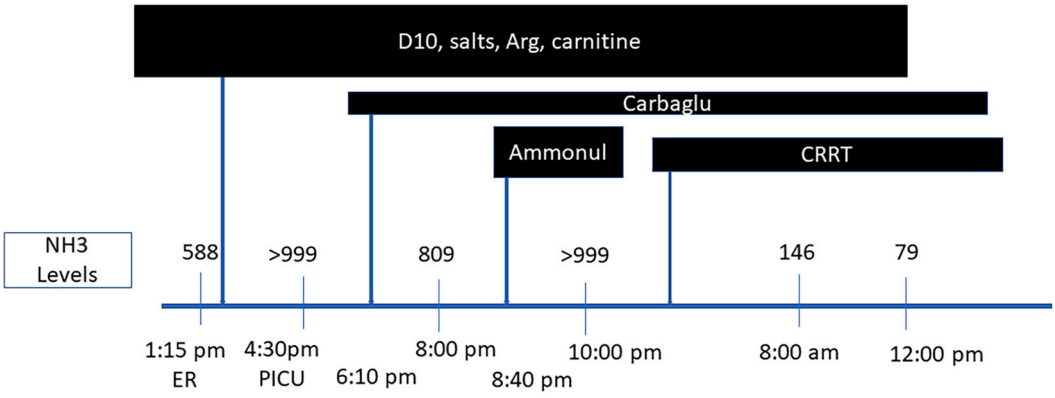

Fig. 1. Timeline of treatments and corresponding ammonia levels during the first 24 h of hospitalization.

demonstrated 2 h after the initiation of the medicine and subsequently in the brother who was born 13 months later. Although GCSF has been used in MMA/PA, this patient proved that CGSF is also helpful in IVA-induced bone marrow suppression.

We caution the usage of Ammonul to treat IVA induced hyperammonemia. Ammonul has two major components, sodium benzoate and sodium phenylacetate [11]. Sodium benzoate functions by binding ammonia with glycine to make hippuric acid and remove ammonia, which further decreases the available glycine in the patient [5,11]. Glycine is beneficial in patient with isovaleric acidemia because it can be enzymatically conjugated to isovaleryl-CoA to become isovalerylglycine, which is less toxic and can be readily excreted through the urine [12]. Sodium phenylacetate is supposed to bind to glutamine to remove ammonia [11]; however, as demonstrated in this patient's plasma amino acid assay, the glutamine level was 256 μmol/L which was lower than normal range. Thus, Ammonul was not only of no use, but also could harm the patient by bringing the levels down further. As shown in Fig. 1, after administration of one dose of Ammonul, the patient's ammonia level went higher than 1000 μg/dL.

Carglumic acid is a carbamoyl phosphate synthetase 1 (CPS 1) activator used to treat both acute and chronic hyperammonemia in N-acetylglutamate (NAG) deficient patients [2]. In organic acidemia, excessive acid inhibits the NAG enzyme. Carglumic acid acts as a replacement for NAG, which is an essential allosteric activator of CPS 1 [2]. CPS 1 is the first enzyme of the urea cycle and helps convert ammonia to urea. In IVA-induced urea cycle dysfunction, carglumic acid supplements the NAG enzyme, converting excess amounts of ammonia into urea. Previous reports have suggested that carglumic acid is an effective treatment for hyperammonemia secondary to IVA [13–16]. Carglumic acid, in conjunction with continued administration of IV glucose, arginine, and carnitine, may have been helpful for this patient. It was given at 6:15 pm, and an ammonia level decreased from >1000 μg/dL at 4:30 pm to 809 μg/dL at 8:00 pm. After discontinuation of CVVH, with the continuation of carglumic acid for three days, there was no rebound. Although in the literature, there are patients with ammonia over 1600 μmol/L who responded to carglumic acid alone without dialysis [13], we believe removing excessive organic acid by CVVH is beneficial to the body.

Granulocyte-colony stimulating factor (G-CSF), which stimulates the bone marrow to make more granulocytes [17], has not been used in IVA before in literature. The patient presented with anemia, neutropenia and thrombocytopenia, which are common complications in traditional cases of organic acidemia [18]. G-CSF has been previously used in patients with neutropenia and organic acidemia [19,20]. This patient received recombinant GCSF for 2 days (day 5–7 of admission) and neutropenia improved and white counts returned to normal. As there is no fast-acting medication to increase platelet counts, the patient received almost q8-hour platelet transfusions to maintain platelet account above 50 K and prevent intraventricular hemorrhage. Bone marrow suppression lasted about a week, consistent with previous reports in the literature [19,20].

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CRediT authorship contribution statement

Anne Chun-Hui Tsai: Conceptualization, Writing – original draft, Visualization. **Hsin-Ti Lin:** Writing – original draft. **Maxwell Chou:** Writing – original draft. **Jessica Bolen:** Writing – review & editing. **Chelsea Zimmerman:** Investigation. **Danielle DeMarzo:** Writing – review & editing. **Yazmin Enchautegui-Colon:** Writing – review & editing.

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