Case Report

Two Siblings With Valproate-Related Hyperammonemia and Novel Mutations in Glutamine Synthetase (GLUL) Treated With Carglumic Acid

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Abstract

This case report describes 2 siblings with myoclonic epilepsy who had novel mutations in the glutamine synthetase (GLUL) gene: c.316C>T, p.(Arg106*) and c.42G>C, p.(Lys14Asn). Valproic acid improved seizure control, but was associated with hyperammonemic encephalopathy. Addition of carglumic acid reduced ammonia levels but drug coverage was declined. We therefore designed a protocol to measure the reduction in plasma ammonia in response to carglumic acid therapy. After the first dose of carglumic acid, Patient 1 showed a reduction in plasma ammonia levels within 3 hours, from 114 umol/L to 68 umol/L (reference 12-47 umol/L), and Patient 2 from 108 umol/L to 80 umol/L, which was sustained over a 2 week period. Overall, there was a strong negative correlation between plasma ammonia levels and carglumic acid levels ($r = -0.86$, $p = 0.0013$), and recurrence of hyperammonemic encephalopathy was not observed while the patients were taking carglumic acid.

Keywords

glutamine synthetase, GLUL, hyperammonemia, carglumic acid, valproic acid, epilepsy

Introduction

Ammonia is produced from the breakdown of proteins (whether endogenous or ingested) through deamination of amino acids, and by microorganisms in the gastrointestinal tract.[1,2] Hyperammonemia typically occurs when plasma ammonia levels are above 50 umol/L; the exposure of the central nervous system to such an environment can lead to encephalopathy at levels double that.[3] Changes in neuronal pH, neuronal membrane potentials, astrocytic swelling, altered neurotransmitter response, and changes in cerebral energy metabolism and reduced ATP are considered some of the mechanisms of ammonia neurotoxicity.[4,5] Hyperammonemic disorders can be due to liver dysfunction, side-effects from certain medications (valproic acid, topiramate, carbamazepine, thiazides, isoniazid, or chemotherapies), impaired circulation where portal vein flow to the liver is reduced (because of obstruction or surgical bypass), or inborn errors of metabolism.[6,7] Due to its neurotoxicity, mechanisms have evolved to remove ammonia before it reaches systemic circulation—approximately 90% of ammonia is converted to urea via the urea cycle in periportal hepatocytes.[8] The other 10% is removed by glutamine synthetase (GS) as part of the synthesis of glutamine in perivenous hepatocytes, astrocytes, the kidneys, and skeletal muscles.

GS is an enzyme that synthesizes the amino acid glutamine from a condensation reaction between glutamate and ammonia.[9] Glutamate itself contains ammonia, so producing one molecule of glutamine in effect removes 2 molecules of ammonia.[10] Pathogenic mutations in the GLUL gene, which encodes GS, results in a rare genetic disorder that is characterized by global development delay and epilepsy, in which the anti-convulsant valproic acid (VPA) has demonstrated efficacy.[11] However, adverse reactions

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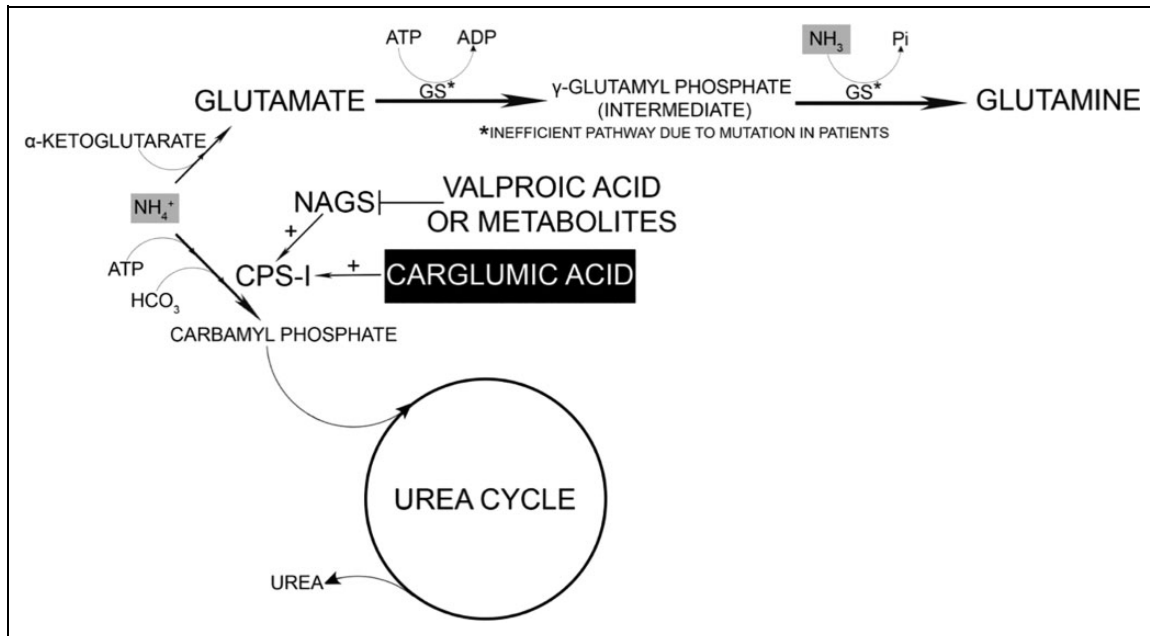Email: khaa@ucalgary.ca

Figure 1. Ammonia removal pathways and the mechanism of action of carglumic acid. Metabolites of VPA inhibit the initiation of the urea cycle. Carglumic acid circumvents this inhibition by directly stimulating CPS-I. Due to the inefficiency of GS in our patients, they need an active urea cycle to effectively remove a sufficient amount of ammonia to prevent encephalopathy.
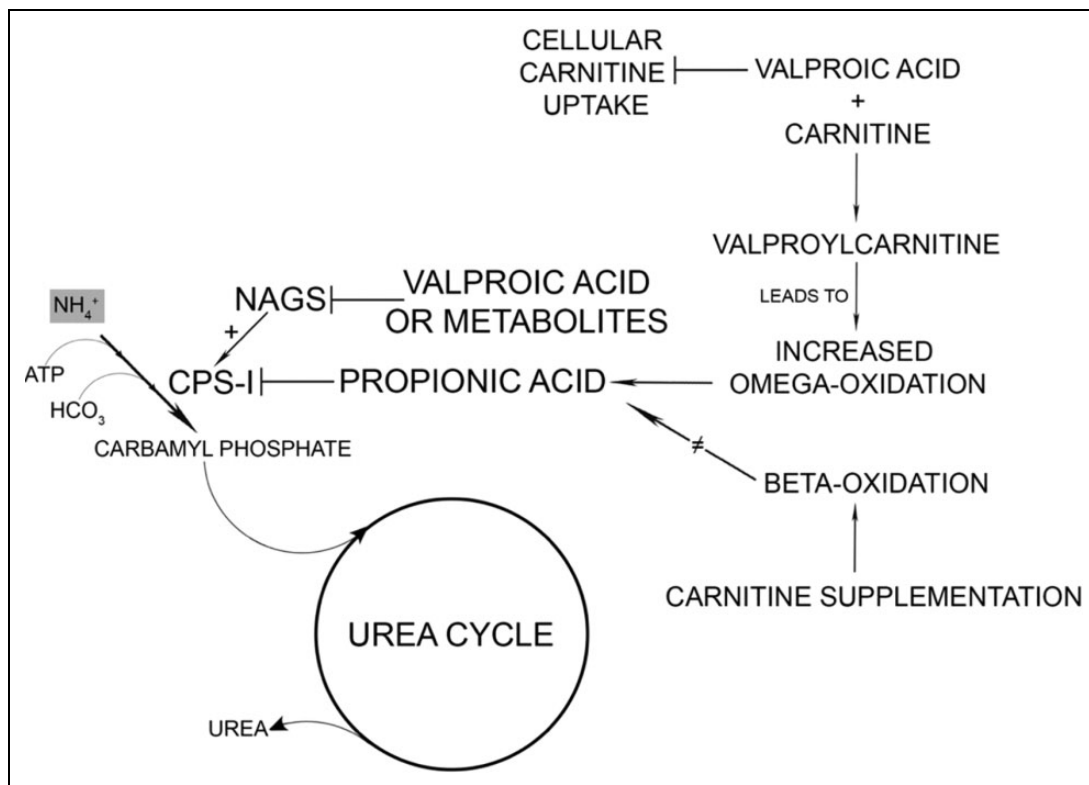

Figure 2. Carnitine mechanism of action. VPA decreases cellular carnitine through the formation of valproylcarnitine and through the inhibition of cellular carnitine uptake. Decreased carnitine levels leads to increased beta-oxidation, which leads to the increased production of the toxic metabolite, propionic acid. Propionic acid inhibits CPS-I. Carnitine supplementation increases cellular carnitine levels, allowing for beta-oxidation, which does not lead to propionic acid production.

Table 1. The First Patient's Ammonia Levels as a Product of Her Treatment Regimen.[a]

Carnitine	Valproic acid	Carglumic acid	Ammonia levels (umol/L)
-	-	-	39
-	+	-	103, 128[b]
+	+	-	103, 99
-	+	+	80

[a]When she was not on carnitine, valproic acid, or carglumic acid, her ammonia level was 39umol/L, but seizures were not controlled. When her ammonia levels increased as a result of valproic acid treatment (103umol/L), she was treated with one dose of carnitine 100mg/kg/dose (weight: 46.5 kg). This was ineffective as ammonia levels remained around 103umol/L, with a low of 99umol/L. During the carglumic acid trial, her ammonia levels decreased to 80umol/L.

[b]Peak ammonia level patient experienced.

related to VPA can also inhibit the urea cycle and induce hyperammonemia. Although the mechanism underlying VPA inhibition of the urea cycle has not been fully elucidated,[12] VPA's reactive metabolite, valproyl-CoA is considered to inhibit N-acetylglutamate synthase (NAGS)—an activator of the urea cycle. The activator NAGS synthesizes N-acetylglutamate (NAG), which activates carbamyl phosphatase synthetase I (CPS-I); CPS-I catalyzes the important first step of ammonia detoxification (Figure 1), which is the rate-limiting step in the urea cycle in individuals who do not have a urea cycle disease. Therefore, valproyl-CoA's inhibition of urea cycle initiation has the potential to result in hyperammonemia in patients who have no urea cycle disease. Chicharro, de Marinis, and Kanner (2007) conducted a meta-analysis that suggested 16%-100% of patients receiving VPA have hyperammonemia as an adverse effect.[13] However, many of these patients are described as asymptomatic (not showing signs of ammonia toxicity).

Carnitine supplementation has demonstrated efficacy in treating some patients with VPA-induced hyperammonemia. These patients have decreased carnitine levels due to valproyl-carnitine formation and VPA inhibition of cellular carnitine uptake.[14] Mitochondrial beta-oxidation requires carnitine as a co-factor; when carnitine's levels are reduced, omega-oxidation becomes dominant.[15] This can lead to the increased production of toxic metabolites, including propionic acid, which inhibits CPS-I. Therefore, by supplementing patients with carnitine, and conjugation and removal of the toxic inhibitory substances, the inhibition of CPS-I can be relieved (Figure 2).[15] However, treatment with carnitine does not always reduce ammonia levels; factors other than accumulation of valprolylcarnitine may cause inhibition of ammonia detoxication. Further, the dose of carnitine required to remove the toxic metabolite may not be tolerable to patients, especially when taken orally, because it can lead to gastrointestinal symptoms. In patients where the discontinuation of VPA is not a favorable option, and carnitine treatment is not successful, an alternate method to reduce ammonia levels is required.

Carglumic acid supplementation is typically used to treat NAGS deficiency. Carglumic acid is a synthetic structural analog of NAG, and thus, also has the ability to activate CPS-I. In VPA-induced hyperammonemia, carglumic acid can be used to reduce the inhibition of NAGS and stimulate CPS-I directly (Figure 1). For example, a case of VPA-induced hyperammonemia, with failed carnitine but successful carglumic acid treatment, is described by Sattar et al. (2018).[16]

Patients and Methods

Two sisters of Filipino ancestry were referred to our clinic with a seizure disorder. The presentation of both affected siblings followed a similar clinical course, with developmental delay and onset of myoclonic epilepsy as early as 9 months of age; they were 21 and 12 years of age, respectively, when the carglumic acid treatment was started. Brain neuroimaging showed no specific abnormal findings. Patient 2 was initially referred to the Metabolic Clinic at Alberta Children's Hospital with suspicion of an underlying metabolic disease. She underwent a muscle biopsy through our standard mitochondrial protocol with negative results for a mitochondrial disease, and no diagnostic findings on blood and urine metabolite analysis (plasma amino acids, plasma acyl-carnitine profile, urine organic acids, and urine orotic acid). She was also enrolled in our whole exome sequencing study called MITO-FIND (Mitochondrial Functional and Integrative Next Generation Diagnostics), which identified 2 heterozygous variants in *GLUL* using techniques that have been previously described.[17] Based on this initial result, clinical verification was performed through family testing (both sisters and both parents), through Blueprint Genetics (Finland), confirmed 2 heterozygous variants in *GLUL*, signifying a compound heterozygous genotype: a c.316C>T, p.(Arg106*) non-sense variant leading to a premature stop codon, and a missense variant c.42G>C, p.(Lys14Asn). Both siblings had the same variants and they were bi-parentally inherited consistent with the clinical features of glutamine synthetase deficiency.

There were several anticonvulsant medications that were implemented to improve seizure control prior to exome sequencing which included lamotrigine, levetiracetam, clonazepam, and topiramate, but the best response (reduced severity and frequency of seizures) was achieved with VPA. Unfortunately, both patients had to discontinue VPA several times due to VPA-induced symptomatic encephalopathy, with the ammonia threshold for symptoms determined to be greater than 100 umol/L (peak levels were 128 umol/L in Patient 1, and 156 umol/L in Patient 2). Hyperammonemic symptoms included tremors, fatigue, behavioral outbursts, disorientation, and the inability to walk. Levocarnitine was tried, with one patient responding to a high dose (200 mg/kg per dose; patient weight: 21.3 kg) of intravenous levocarnitine; however, this was not sustainable as an outpatient. Neither patient would tolerate even 50 mg/kg/day of levocarnitine due vomiting and gastrointestinal discomfort, and subsequently, ammonia levels would rebound. On one admission with Patient 2, once the initial bolus of 200 mg/kg/dose of levocarnitine intravenous infusion was completed and plasma ammonia levels rebounded, the patient subsequently was treated with carglumic acid (Tables 1 and 2). This led to a reduction of plasma ammonia levels, but a supply for outpatient use of carglumic acid was declined because the patient did not meet the criteria of a primary urea cycle disorder. The only other choice was to discontinue VPA, but this led to an increase in seizure frequency, decreased responsiveness during the day, and reduced appetite and weight loss in both patients. Several months following VPA discontinuation, Patient 1 presented to the hospital with status epilepticus and VPA was restarted. This resulted in improved seizure control, but ammonia levels increased again. Shortly thereafter, Patient 2 was also

Table 2. The Second Patient's Ammonia Levels as a Product of Her Treatment Regimen.[a]

Carnitine	Valproic acid	Carglumic acid	Ammonia levels (umol/L)
-	-	-	47
-	+	-	130, 156[b]
+	+	-	61, 100
-	+	+	76

[a] When she not on carnitine, valproic acid, or carglumic acid, her ammonia level was 47umol/L, but seizures were not controlled. When ammonia levels increased as a result of valproic acid treatment (130umol/L), she was treated with 200mg/kg IV carnitine (weight: 21.3 kg), then was put on 700 mg IV Q8 H. Ammonia levels decreased to 61umol/L, but then rebounded to 100umol/L when measured 17 hours later, on a maintenance dose. During the carglumic acid trial, her ammonia decreased to 76 umol/L.

[b] Peak ammonia level patient experienced.

restarted on VPA, which improved seizure control, but resulted in a rise in ammonia as well.

Because of Patient 2's success using carglumic acid to reduce ammonia levels while on valproic acid, we made an application for compassionate use of carglumic acid through Health Canada's special access program. Using this data, medication reimbursement could be provided for long-term use if there was demonstrated efficacy directly related to the use of carglumic acid.

Within these regulatory requirements, we designed a protocol to treat both patients with carglumic acid over 14 days to try to reduce plasma ammonia levels without discontinuing valproic acid. The objective was to determine the degree of ammonia reduction, whether this could be sustained on an outpatient basis, and if the drug could be tolerated. The carglumic acid was dosed at target of 250 mg/kg per day, in 2 divided doses over the day. Patient 1 (46 kg) was given 29 tablets (29 x 200 mg = 5800 mg; 252 mg/kg/day) and Patient 2 (36 kg) was given 22 tablets (22 x 200 mg = 4400 mg; 244 mg/kg/day) of carglumic acid per day. Blood levels of ammonia and carglumic acid were collected as follows: Day 0, 9:00 am (fasting) followed by first carglumic acid dose and repeat bloodwork at 12:30 pm (3 hours post first dose carglumic acid and after the first morning meal), Day 3: 9:00 am (before feeds and carglumic acid morning dose), Day 7: 9:00 am (before feeds and carglumic acid morning dose) and Day 14: 9:00 am (before feeds and carglumic acid morning dose). Only a 2 week supply of carglumic acid was available and repeat laboratory tests were drawn 4 weeks after carglumic acid was stopped. Testing of carglumic acid levels was performed by Biopharma Services Inc., Toronto, Ontario using standard protocols. Ammonia levels were tested at Alberta Precision Laboratories using standardized protocols in clinical care.[18] The patients were not on a protein-restricted diet (because previous attempts to try this were not successful and they lost weight) and we advised the family to continue with the usual diet, and they informed us that no changes in diet were required over the duration of this protocol.

Results

Fasting ammonia levels were measured before carglumic acid treatment was initiated, and 4 times throughout the 14 day trial. The carglumic acid was stopped after day 14, in order to check rebound ammonia levels 28 days after stopping carglumic acid (day 42) (Figure 3A and B). After the first dose of carglumic acid, the first patient showed a reduction in plasma ammonia

levels within 3 hours, from 114 umol/L to 68 umol/L (reference 12-47 umol/L), and the second patient from 108 umol/L to 80 umol/L, following with a rise in carglumic acid levels. Patient 1 continued to have a sustained reduction in ammonia levels with a strong negative correlation (r = −0.86; p = 0.0013) over the course of the trial (Figure 4). Similarly, in Patient 2, there was a strong negative correlation (r = −0.86; p = 0.0013), suggesting a linear dose-dependent association between the carglumic acid level and plasma ammonia level. This implies that higher carglumic acid levels are associated with decreased ammonia levels. Both patients tolerated the dosing of carglumic acid well without gastrointestinal upset. Patient 1 had a rebound hyperammonemia of 103 umol/L at the 4 week post-carglumic acid follow-up, and Patient 2's levels rebounded to 133 umol/L over the same time period. We compared the association between ammonia and carglumic acid levels in both patients. One of the patients took longer to consume their diet on the first day, so the immediate post-drug levels on the first day were therefore excluded for both patients; only first morning blood levels for both compounds, prior to any food intake or the morning carglumic acid doses, were compared for linear regression. The linear regression showed a strong inverse association between blood carglumic acid levels and ammonia levels (Figure 4) that was statistically significant, showing that the reduction in ammonia levels was commensurate with rising blood carglumic acid levels following first order kinetics.

Discussion

The literature describes 3 previous cases of GS deficiency, of which all the patients are deceased.[12,19] The first patient was a boy in Turkey born to consanguineous parents, who was resuscitated at birth. He had epileptic encephalopathy and multiple organ failure, resulting in his death at 2 days of age.[19] This patient was homozygous for the c.970C>T, p.(Arg324Cys) mutation in the *GLUL* gene. The second patient was a girl in Turkey, born to consanguineous parents, who experienced convulsions and respiratory failure on the first day of life.[12] She was diagnosed with epileptic encephalopathy, characterized by multifocal seizures, and developed a blistering erythematous rash after 2 weeks. At 28 days of life she passed away as a result of multiple organ failure. She was found to be homozygous for the c.1021C>T, p.(Arg341Cys) mutation in *GLUL*. The third patient was a Sudanese boy born to consanguineous parents. He presented slightly differently than the first 2 patients; he survived until 6 years of age, at which point he died of acute respiratory decompensation.[12] At 13 days of age, he developed tonic-clonic seizures, and had repeated hospitalizations for uncontrolled seizures, apnea, upper and lower airway infections, and necrolytic erythema. He was found to be homozygous for the mutation c.970C>A, p.(Arg324Ser) in *GLUL*, which affects the same codon as the first patient, but demonstrated a different clinical course.

Frieg et al. (2016) conducted molecular dynamics simulations comparing the enzymatic activity of Arg324Cys, Arg341Cys, and Arg324Ser.[20] Residual enzyme activity was
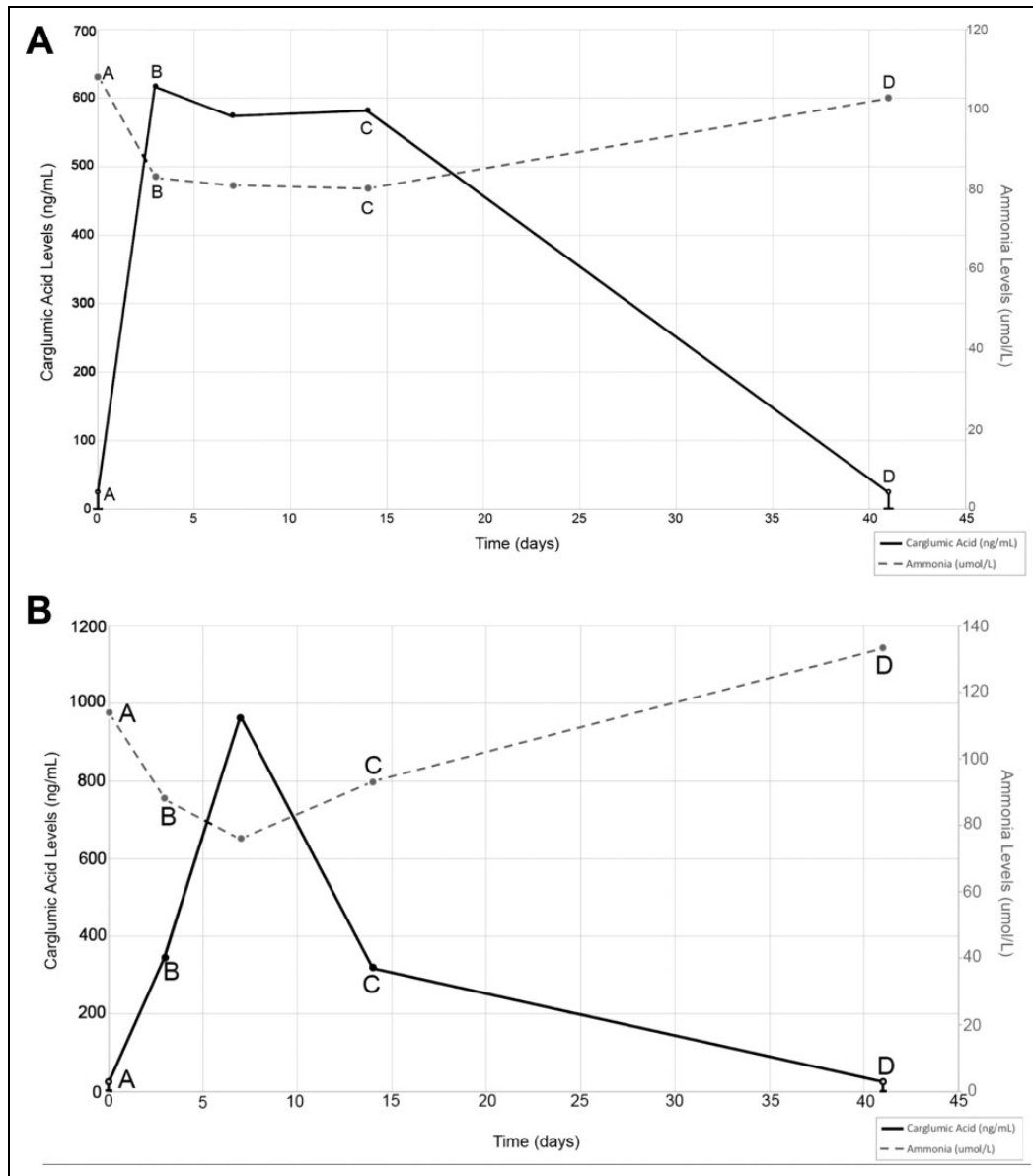
Figure 3. A, The first patient's ammonia levels over the 42 day trial. (A) Baseline ammonia and carglumic acid levels before treatment initiation. (B) First blood draw following carglumic acid initiation. Ammonia levels stabilized near 80 umol/L. (C) Carglumic acid treatment was stopped. (D) Ammonia and carglumic acid levels returned near baseline, 28 days after treatment ceased. B, The second patient's ammonia levels over the 42 day trial. (A) Baseline ammonia and carglumic acid levels before treatment initiation. (B) First blood draw following carglumic acid initiation. Ammonia levels were reduced to 88 umol/L at this point, despite the fact she vomited. Ammonia levels continued to decrease to 76 umol/L on day seven. (C) Carglumic acid treatment was stopped. (D) Ammonia levels rebounded above baseline to 133 umol/L 28 days after treatment ceased.

seen in the Arg324Ser variant, which corresponds to the milder phenotype observed in that patient. However, the variants Arg324Cys and Arg341Cys result in structurally defective proteins leading to a complete loss of enzyme activity.

There are several differences and similarities between these 3 patients and our patients. Our patients' mutation is compound heterozygous, with a non-sense variant and a missense variant, which may have produced a non-lethal phenotype. Therefore, our patients' may have higher level of residual activity in GS in the liver compared to the other 3 variants. The patients reported

by Häberle et al. (2005, 2011) all had low levels of glutamine and high levels of ammonia, but our patients had high ammonia despite normal glutamine levels.[12,19] Brain MRIs on those patients were similar, including cerebral and cerebellar atrophy, agyria, immature white matter, hypomyelination, paraventricular cysts, subependymal cysts, and enlarged lateral ventricles.[21] Our patients have significant developmental delay and difficult to control seizures, but without major structural abnormalities on brain neuroimaging. This suggests that whole exome sequencing, and not biochemical screening, may be more effective in detecting more cases.
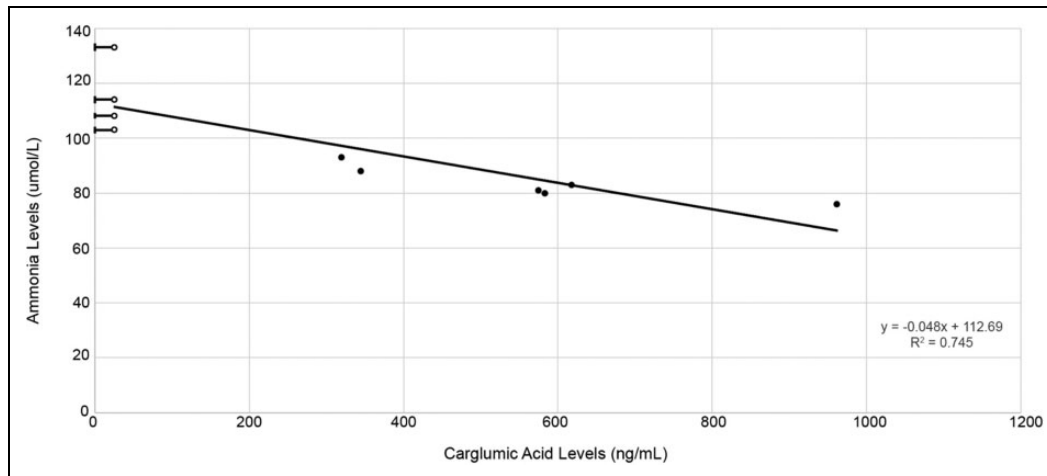
Figure 4. The linear relationship between ammonia levels and carglumic acid levels in both patients. This shows a strong negative correlation ($r = -0.86$, $p = 0.0013$).

The use of VPA was associated with improved seizure control, but symptomatic hyperammonemia in our patients with *GLUL* deficiency. The VPA levels stayed in the therapeutic range during our period of monitoring (819 umol/L in Patient 1, 602 umol/L in Patient 2; reference 350-700 umol/L). One limitation of this trial was our inability to monitor valproic acid metabolites to see if any changes occurred with carglumic acid therapy.[22] We were unable to find laboratories that could test VPA metabolites, and therefore, we do not know if using carglumic acid reduces metabolites of VPA, or whether it increases the detoxification of ammonia using the urea cycle. With stable and therapeutic blood valproic acid levels, and normal urea cycle function (no mutations were identified in the urea cycle enzymes), we hypothesize that carglumic acid likely removes the inhibition of valproic acid on residual glutamine synthetase activity or on carbamoyl phosphate synthetase 1. In an experimental animal model of glutamine synthetase, protein restriction was not helpful,[23] and when we tried protein restriction in our 2 patients, they both lost weight and were actually less interactive. The optimal clinical solution in our patients was unrestricted protein, valproic acid to treat the seizures, and carglumic acid to control ammonia levels. It is not clear whether other patients who have valproate-induced hyperammonemia may have mutations in *GLUL* or may benefit from carglumic acid, but only a wider study in a larger number of patients would be able to provide further insight on this.

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Declaration of Conflicting Interests

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