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Carbamoly-phosphate synthetase 1 (CPS1) deficiency: A tertiary center retrospective cohort study and literature review

Mahmood Noori [a], Omar Jarrah [a], Aisha Al Shamsi [b,*]

[a] Pediatrics Department, Tawam Hospital, Al Ain, United Arab Emirates
[b] Genetic Metabolic Division, Pediatrics Department, Tawam Hospital, Al Ain, United Arab Emirates

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ABSTRACT

Background: Protein metabolism and urea production maintain protein and amino acid homeostasis in normal status. Ammonia results from amino acid turnover and is produced by intestinal urease-positive bacteria. Ammonia must be detoxified, and the urea cycle converts ammonia into urea. CPS1 is an enzyme in the urea cycle that catalyzes ammonia and bicarbonate condensation. CPS1 deficiency presents in the neonatal period with hyperammonemia, resulting in death or neurological sequelae if patients survive.
Objectives/aims: To share the experience of patients with CPS1 deficiency from Tawam Hospital and to shed light on the spectrum of variants found in those patients.
Methods: A retrospective chart review was done. All patients with CPS1 deficiency admitted to Tawam Hospital from 2010 to 2023 were included. Collected data included age and ammonia level at presentation, the time needed to drop ammonia level below 100 μmol/L, acute management modality provided, long-term neurological sequelae, sequence variants, severity, and duration of hyperammonemia encephalopathy, age at last follow-up, and, if applicable, survival for at least six months.
Results: Only five patients with CPS1 deficiency over 13 years were found; two males and three females. Three patients are doing relatively well at 18 months, 7, and 9 years of age. The presented age was in the neonatal period except in one patient. One patient was found to have frameshift, resulting in a premature stop codon in the *CPS1* gene, had a devastating course that ended with death. One patient had recurrent hyperammonemia episodes in her first year of life, which led to microcephaly and global developmental delay. One patient underwent hemodialysis, and one patient underwent peritoneal dialysis. All patients except one were on Carglumic acid which could contribute to their survival and disease control. All variants reported here are novel except one.
Conclusion: Although the presentation was different in severity, three patients are doing relatively well and approaching their developmental milestones. Thus, early recognition, prompt actions to drop high ammonia level, and good follow-up plans are emphasized. Further studies are needed to correlate the genotype-phenotype of reported variants here, which can help predict the severity of CPS1 deficiency.

1. Introduction

Protein synthesis, protein degradation, amino acid oxidation, and urea production are ways of maintaining protein and amino acid homeostasis in normal status. Proteins from nutrition or endogenous sources are broken down into amino acids and utilized to reconstitute proteins with very little loss (<10 %). The daily turnover rate of protein in humans (250–400 g/day) vastly exceeds the level of protein intake (50–80 g/day) [1]. Amino acid turnover in the body results in excessive ammonia production. In addition, ammonia is produced by intestinal urease-positive bacteria and is constantly produced during amino acid metabolism. Thus, ammonia needs to be detoxified appropriately, and the urea cycle performs the critical function of converting ammonia into urea. If this cycle is defective, patients develop diseases presenting with severe hyperammonemia, typically in the neonatal period, caused by congenital or secondary defects in the enzymes or transporters that comprise the urea cycle [2]. Other than the urea cycle pathway, the following enzymes and pathways generate ammonia: ammonia is produced through the catabolism of glutamine by glutaminase (mucosal epithelial cells) [3], glutamate dehydrogenase (GDH; small intestine)

* Corresponding author.
E-mail addresses: mabdulkadeer@seha.ae (M. Noori), ojarrah@seha.ae (O. Jarrah), aishamsi@seha.ae (A. Al Shamsi).

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and bacterial deaminase (large intestine) [4]. In addition, urea is metabolized to ammonia by bacterial urease (large intestine) [5]. It is worth knowing that ammonia produced in the gastrointestinal tract accumulates in the portal vein, where the concentration is 2–5 times higher than in the systemic circulation, which explains the hyperammonemia caused by portosystemic shunts [6]. The other route of ammonia production is through nitrogen metabolism associated with amino acid catabolism (mainly in the brain and skeletal muscle) [6].

The ammonia produced in various body tissues is transported as glutamine to the liver, where this amino acid is converted into glutamate via transamination. This process releases ammonia, which is metabolized to urea by the urea cycle. Furthermore, glutamine is broken down into ammonia and glutamate by glutaminase, especially in the liver and kidneys. If catabolism is disturbed by liver dysfunctions due to cirrhosis or urea cycle defects (UCDs), for example, then ammonia can rise to levels capable of causing brain damage. Hyperammonemia leads to cerebral edema, lethargy, anorexia, vomiting, hyperventilation (or hypoventilation), hypothermia, neurologic posturing, and coma. Hence, the mainstay of treatment for UCD patients is to prevent catabolism and control the plasma glutamine level. This can be used as an essential biomarker for total nitrogen metabolism and, if elevated, as an indicator of too high protein intake [2].

Carbamoyl-phosphate synthetase 1 (CPS1: EC 6.3.4.16) is the urea cycle's first and rate-limiting enzyme. CPS1 catalyzes the condensation of ammonia and bicarbonate into carbamoyl-phosphate in the mitochondrial matrix, which requires *N*-acetylglutamate (NAG), magnesium, and ATP. CPS1 deficiency (OMIM# 237300) is an autosomal recessive disorder and is one of the most severe forms of UCDs. It is caused by pathogenic/likely pathogenic variants in the *CPS1* gene located in 2q34. The estimated Incidence of CPS1 deficiency is 1:1,300,000 [7], and the estimated prevalence is 1:975,000 in the US [8], 1:800,000 in Japan [9], and 1:539,000 in Finland [10]. Unfortunately, no CPS1 deficiency statistics exist in the Arab or Gulf countries.

Based on the age of onset, clinical manifestations, and whether CPS1 enzyme activity is absent or residual, two distinct phenotypes of CPS1 deficiency are identified: neonatal and late-onset. Patients with neonatal onset are healthy at birth and may do well for several days. Subsequently, they refuse feeding and become lethargic. The clinical picture is characterized by hypothermia, vomiting, hypotonia, convulsions, and coma, sometimes resulting in death or neurological sequelae if patients survive [11]. On the other hand, patients with late-onset disease demonstrate a more heterogeneous clinical picture, such as feeding difficulties, failure to thrive, psychomotor retardation, and late-onset acute metabolic decompensation with hyperammonemia and therefore making the diagnosis difficult [12].

Biochemical findings reveal severe hyperammonemia, low levels of citrulline and high levels of glutamine in plasma amino acid analysis, and low levels of orotic acid in urine. In the case of a suggestive biochemical profile, molecular testing is required to distinguish CPS1 deficiency from NAG synthase (NAGS) deficiency [2].

Treatment modalities include targeting either ammonia generation or absorption, including reducing dietary protein, ammonia removal through dialysis (either hemodialysis or, less efficiently, peritoneal dialysis), and pharmacological drugs such as Sodium Phenylacetate, Sodium Benzoate, L-arginine, and Carglumic acid [13]. It has been reported that early liver transplantation prevents hyperammonemia events and leads to a complete metabolic correction, but pre-existing neurological damage is not reversed [11].

In this study, we aimed to review the medical records of all patients with CPS1 deficiency followed by the Tawam Metabolic team to share the experience of patients with CPS1 deficiency presented between 2010 and 2023 and to shed light on the variants found in those patients.

2. Methods

2.1. Study design

Retrospective data collection was done by reviewing the patient's electronic medical records. All pediatric patients admitted to Tawam Hospital and diagnosed with CPS1 deficiency from 2010 to 2023 were included. Cases were identified by tracking the following ICD codes: ICD-10-CM E72.29 and E72.20.

This study was approved by the Tawam Human Research Ethics Committee (Ref. No.: KD/AJ/1025).

2.2. Study location

The study was done in Tawam Hospital, a tertiary hospital in Al Ain, United Arab Emirates (UAE).

2.3. Data collection

Collected data included age and ammonia level at presentation, the time needed to drop the ammonia level to below 100 μmol/L, the modality of acute management provided, long-term neurological sequelae, sequence variants, family history, the number, severity, and duration of hyperammonemia encephalopathy, age at last follow-up, and, if applicable, survival for at least six months.

3. Results

Our cohort study revealed five patients with CPS1 deficiency, two males and three females. Detailed description is as follows:

3.1. Patient 1

A 30-day-old male infant was born to consanguineous parents and presented with lethargy and poor feeding without fever. Sepsis work-up was done, and it was reassuring. Due to progressive lethargy, ammonia level was obtained and found to be 400 μmol/L. Metabolic screening was initiated. The amino acid profile showed critically low levels of both Arginine and citrulline (i.e., Arginine level of 5 μmol/L (normal level: 50–76 μmol/L) and Citrulline level of 2 μmol/L (normal level: 9–21 μmol/L)). Urine orotic acid was 2.1 mmol/mol Cr (normal range: 1.0–3.2 mmol/mol). He neither required dialysis nor developed encephalopathy. His high ammonia level was managed only with ammonia scavengers and essential amino acid formula. During his follow-up, he was noted to have some speech difficulties by 14 months of age with acceptable other developmental milestones. He is nine years old, has acceptable school performance, and has resolved his speech delay. Sequence analysis of the UCD panel showed a previously unreported homozygous variant in exon 26 of the *CPS1:* NM_001122633.2: c.3095 A > T p.(Glu1032Val). This variant is located at a highly conserved nucleotide and amino acid position, with moderate physicochemical differences between the amino acids glutamic acid and valine. Software analyses showed inconsistent predictions: MutationTaster predicts this variant is probably damaging, whereas SIFT and Align-GVGD predict toleration. This variant is classified as class 3 (a variant of uncertain significance) according to the ACMG recommendations. No pathogenic variants were identified in *ARG1, ASS1*, *NAGS*, and *OTC* genes.

3.2. Patient 2

This is a sibling of Patient 1. She presented at 34 h of age with poor feeding, hypotonia, and lethargy. The first ammonia level was 369 μmol/L. With maximum medical support, she continued to have an elevation in ammonia level, reaching 618 μmol/L on the third day of life. Hemodialysis was initiated, and the ammonia level dropped to 27 μmol/L within 6 h. The amino acid profile (which was collected after starting

Intravenous Arginine) showed an Arginine level of 485 μmol/L (normal level: 50–76 μmol/L) and Citrulline level of 29 μmol/L (normal level: 9–21 μmol/L). She is now seven years old and has no significant concerns regarding developmental milestones. A genetic test was not done due to financial issues with the family, and the diagnosis was made based on family history being the sibling of patient 1 with a homozygous c.3095 A > T (p.Glu1032Val) in *CPS1* gene.

3.3. Patient 3

A 6-day-old female neonate was born to consanguineous parents and presented with fussiness, poor feeding, and lethargy with no fever. Her initial sepsis screening was negative. Due to the worsening of her lethargy, ammonia level was obtained and was 400 μmol/L with evidence of metabolic alkalosis and lactate level of 27.9 mg/dL. She was referred to Tawam Hospital, where ammonia level was repeated and found to be 202 μmol/L. Her amino acid profile showed an Arginine level of 32 μmol/L (normal level: 50–76 μmol/L) and a Citrulline level of 3 μmol/L (normal level: 9–21 μmol/L). Urine orotic acid was 2.1 mmol/mol Cr (normal range: 1.0–3.2 mmol/mol). Ammonia scavengers and essential amino acid formula managed to drop her high ammonia level. During her last follow-up at 18 months of age, she had normal developmental milestones with no recurrence of metabolic crisis. Sequence analysis of the UCD panel revealed a homozygous variant in the *CPS1*: NM_001122633.2: c.2909G > T p.(Gly970Val). This variant is located in a highly conserved nucleotide and amino acid position, with moderate physicochemical differences between the exchanged amino acids (Alamut v.2.9). Software analyses show inconsistent predictions: PolyPhen-2, SIFT, and MutationTaster indicate this variant is probably damaging, whereas Align-GVGD predicts toleration. This variant is reported in the Exome Aggregation Consortium with a frequency of 0.000009, i.e., in 1 among 113,858 alleles (ExAC database). This variant is classified as class 2, according to the ACMG recommendations. No pathogenic variants were identified in *ARG1, ASS1*, *NAGS*, and *OTC* genes.

3.4. Patient 4

A 3-day-old female neonate was born to consanguineous parents and presented with a history of lethargy for one day without fever. Lethargy was progressive; thus, ammonia level was obtained and found to be 1396 μmol/L, with evidence of respiratory and metabolic acidosis with a lactic acid level of 10 mmol/L. Infectious, cardiac, or hydration causes did not explain the acid-base imbalance. She required emergency peritoneal dialysis, which the family refused, so medical management was initiated. She developed a clinical picture of encephalopathy with no encephalopathy changes in brain MRI. The amino acid profile showed an Arginine level of 30 μmol/L (normal level: 50–76 μmol/L) and a Citrulline level of 18 μmol/L (normal level: 9–21 μmol/L). Urine orotic acid was 2.7 mmol/mol Cr (normal range: 1.0–3.2 mmol/mol). Unfortunately, she died at 36 days of age due to complications related to ventilation dependency and respiratory failure. Her latest ammonia level was 170 μmol/L. She had two siblings who died in the neonatal period for no apparent reason and were not investigated. Sequence analysis of the UCD panel revealed a homozygous duplication of 1 nucleotide in exon 15 of the *CPS1*: NM_001875.2: c.1590dupT p.(Val531CysfsX9). This variant is predicted to cause a frameshift, resulting in a premature stop codon (p.Val531CysfsX9). This variant is novel and was previously described in the same patient [14], and due to its truncation nature, it is most likely disease-causing. This variant is classified as pathogenic according to the ACMG recommendations. No pathogenic variants were identified in *ARG1*, *ASS1*, *NAGS*, and *OTC* genes. So, she was confirmed to have CPS1 deficiency.

3.5. Patient 5

A 3-day-old female neonate was born to non-consanguineous parents and presented with poor feeding, rapid breathing, and lethargy, which progressed to encephalopathy. She was afebrile with no focus of infection and with negative sepsis screening. Due to the worsening of her neurological status, her ammonia level was obtained and was >1400 μmol/L. Emergency peritoneal dialysis was initiated along with adjunctive medical management. The amino acid profile revealed an Arginine level of 80 μmol/L (normal level: 50–76 μmol/L) and a Citrulline level of 53 μmol/L (normal level: 9–21 μmol/L). Urine orotic acid was 6.5 mmol/mol Cr (normal range: 1.4–5.3 mmol/mol). After her initial hyperammonemia episode, she remained well. She had no crisis till she reached nine months of age, when she required repeated admissions with mild to moderate hyperammonemia levels managed by Ammonia scavengers only. She is three years old with microcephaly and global developmental delay (bedridden but has head control, smiling, and babbling). Sequence analysis of the UCD panel showed a homozygous in exon 27 of the *CPS1*: NM_001122633.2: c.3355-1G > T p.(Asn1119Trpfs*10). This variant is predicted to disrupt the highly conserved acceptor splice site, according to HGMD Professional 2020.3. This variant has previously been described as disease-causing CPS1 deficiency [15], and is classified as likely pathogenic according to the recommendations of ACMG. No pathogenic variants were identified in *ARG1, ASS1*, *NAGS*, and *OTC* genes.

Table 1 summarizes all the studied patients' clinical features and molecular results.

4. Discussion

CPS1 deficiency is a rare genetic disorder that causes disruptions in the urea cycle and elevated ammonia levels in the body. Due to its rarity, we had only five patients with CPS1 deficiency over 13 years in Tawam Hospital (from 2010 to 2023). Despite the poor prognosis of CPS1 deficiency in terms of 6 months survival and long-term neurological sequelae, it doesn't seem to be the case here, provided that early management of hyperammonemia was established. Three of our five patients are doing relatively well at 18 months, 7, and 9 years of age. All the patients described here had the neonatal early onset phenotype. Patient 1 in this cohort presented at 30 days of age with a relatively high ammonia level (400 μmol/L). Of interest is that this patient had a benign course with no recurrent hyperammonemia crisis and a minimal developmental delay of speech, which was resolved later. Patient 3 behaved similarly to Patient 1 in terms of the benign course of the disease and no recurrent hyperammonemia crisis.

On the other hand, Patient 4 had a devastating course that ended with death. She had a total of 72 h with a high ammonia level of more than 1500 μmol/L as the parents declined dialysis. Of note, this patient's genetic sequence variant showed frameshift, resulting in a premature stop codon in the *CPS1* gene compared to other patients, which can also contribute to disease severity.

The fifth patient, who presented with lethargy and an altered level of consciousness, had a dramatic change in neurological status once the ammonia level was controlled. Peritoneal dialysis was very helpful in dropping ammonia from unrecordable levels (>1400 μmol/L) to below 500 μmol/L within almost 60 h. The drop in ammonia level significantly improved this patient's consciousness level over the days. Due to her recurrent hyperammonemia episodes, she had microcephaly and global developmental delay, which could also be due to severe hyperammonemia episodes in her first days of life.

In this cohort study, only one patient (Patient 2) underwent hemodialysis for an ammonia level of 600 μmol/L, which significantly dropped within 6 h. This patient is seven years old and doing generally well, with no concerns about her developmental milestones.

The existing literature does not reveal any obvious genotype-phenotype correlations among patients with CPS1 deficiency. As enzyme activity analysis was not done for any of the studied patients in this study, we attempted to analyze genotype-phenotype correlations based on the clinical manifestations of our patients. Most variants

Table 1
The studied patients' clinical, biochemical features, and genetic variants.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Ethnicity	Syrian	Syrian	Pakistani	UAE/Oman	Philippine
Gender	Male	Female	Female	Female	Female
Consanguinity	Yes	Yes	Yes	Yes	No
Age at Initial Presentation	30 days	1.5 days	6 days	3 days	3 days
Age at last follow-up	9 years	7 years	18 months	Died at 4 weeks of age	3 years
Initial Presenting Symptoms	Lethargy, poor feeding	Lethargy, hypotonia	excessive crying, poor sucking, lethargy	lethargy hypoglycemia	Poor feeding, Lethargy, increased work of breathing
Initial Ammonia level	400 μmol/L	369 μmol/L	400 μmol/L	1396 μmol/L	> 1400 μmol/L
Maximum Ammonia level	806 μmol/L	618.1 μmol/L	202 μmol/L	1962 μmol/L	>1400 μmol/L
Arginine Level at presentation (normal level: 50–76 μmol/L)	5 μmol/L	485 μmol/L*	32 μmol/L	30 μmol/L	80 μmol/L
Citrulline Level presentation (normal level: 9–21 μmol/L)	2 μmol/L	29 μmol/L*	3 μmol/L	18 μmol/L	53 μmol/L
Dialysis (needed & type)	None	Yes, hemodialysis	None	No, family declined dialysis	Yes, Peritoneal
Current Medications Given	Citrulline, Sodium benzoate	Citrulline, Sodium benzoate	Carbaglu and Citrulline	Sodium benzoate, Sodium phenylbutyrate, and Arginine	Sodium benzoate, Sodium phenylbutyrate, Carbaglu, Citrulline and Arginine
Duration to drop ammonia level to < 100 μmol/L	7 days	1 day	1 day	6 days	3 days
Neurological sequels	Mild speech delay, which resolved.	None	None	Not applicable	Microcephaly, global developmental delay
Neurology image (Brain MRI)	Not done	Not done	Not done	Showed no gyral malformation	Not done
Number of hyperammonemia episodes	3	2	1	1 and was severe	12
Current Developmental milestones	Appropriate with resolved speech delay	Appropriate for age	Appropriate for age	Not applicable	Globally delayed, head control, smiling, babbling
Variant in *CPS1* gene	NM_001122633.2: c.3095A > T p.(Glu1032Val)	Not done	NM_001122633.2: c.2909G > T p.(Gly970Val)	NM_001875.2: c.1590dupT p.(Val531CysfsX9).	NM_001122633.2: c.3355-1G > T p.Asn1119Trpfs*10

* Amino acid profile was collected after the patient was started on IV Arginine.

identified in this study were located in the CPS1 domain, indicating that the CPS1 domain plays a vital role in enzyme activity and clinical presentation. All variants reported here are novel except variant c.3355-1G > T in the *CPS1* gene. Further studies are needed to correlate the genotype-phenotype of such variants, which can help predict the severity of CPS1 deficiency.

Carglumic acid, or N-carbamoyl-L-glutamate, is a synthetic analogue of *N*-acetyl-L-glutamate (NAG), an allosteric activator of CPS1. Previous reports have demonstrated variable clinical responsiveness to Carglumic acid in patients with CPS1 deficiency, suggesting that the effect of Carglumic acid may be variant-dependent. CPS1 gene variants may decrease the stability of the CPS1 protein or lower the affinity of the enzyme for NAG. Therefore, Carglumic acid supplementation may improve CPS1 function in some patients through saturation of NAG sites in partial CPS1 deficiency, maximizing CPS1 activation and protecting CPS1 from thermal and proteolytic inactivation. However, in other CPS1 variants, Carglumic acid may compete with NAG binding instead and decrease residual ureagenesis [16–18].

In this cohort, all the patients except Patient 4 were started on Carglumic acid, which could contribute to their survival and disease control. At the same time, it should be noted that Carglumic acid is not suitable for treating patients 4 and 5 as they have loss of function variants, i.e. functional CPS1 protein is not produced in these two patients.

Early discovered nitrogen scavengers (sodium benzoate, phenylacetate, and phenylbutyrate).

are effective in lowering ammonia and commonly used methods for managing CPS1 deficiency. However, they have side effects and administration issues that can reduce compliance with therapy and limit optimal outcomes. Glycerol phenylbutyrate, a pro-drug of phenylbutyrate, was developed to improve therapy for patients with urea cycle disorders. It is better tolerated than sodium phenylbutyrate, enabling patients with urea cycle disorders to reach ammonia and glutamine targets. Maintenance of target ammonia levels allows better long-term control in patients with urea cycle defects with reduced neurological sequelae [19].

Although the presentation was different in severity, three out of five reported patients here are doing relatively well and approaching their developmental milestones. Thus, early recognition, prompt action to drop high ammonia levels, and good follow-up plans are emphasized.

The main limitations of our study include the use of single-center data, which contributed to the small sample size, and the study's retrospective nature, which may result in missing reported clinical features or missed follow-ups. Therefore, further prospective multi-center studies of larger size are recommended for proper assessment of manifestations and longer follow-ups for complications surveillance and management based on different causing variants.

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Ethical approval

This article contains no studies with human participants or animals performed by authors. This study is approved by the Tawam Human Research Ethics Committee, Ref. No.: KD/AJ/1025).

Informed consent was obtained during clinical evaluations to proceed with genetic tests.

CRediT authorship contribution statement

Mahmood Noori: Writing – original draft, Data curation. **Omar Jarrah:** Writing – original draft, Data curation. **Aisha Al Shamsi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data is openly available in a public repository that issues datasets with DOIs.

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