A neonate with ornithine aminotransferase deficiency; insights on the hyperammonemia-associated biochemical phenotype of gyrate atrophy

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ABSTRACT

Gyrate atrophy of the choroid and retina (GACR) secondary to deficiency of ornithine aminotransferase (OAT) is a rare autosomal recessive metabolic disorder usually diagnosed in childhood when patients develop myopia and a characteristic retinal degeneration accompanied by hyperornithinemia. Plasma ammonia is normal or sub-normal after the neonatal period. A few GACR patients present in early infancy with hyperammonemia, encephalopathy and a biochemical profile of low plasma ornithine, citrulline and arginine, with increased urinary excretion of homocitrulline and orotic acid, resembling a primary urea cycle disorder. In these patients, ornithine levels do not increase until late infancy or following arginine or citrulline supplementation. We describe a patient with OAT deficiency who presented in the first month of life with episodes of lethargy, vomiting, and hypo-thermia. He had two episodes of hyperammonemia associated with subnormal levels of plasma ornithine, citrulline and arginine as well as elevated urinary excretion of homocitrulline and orotic acid. Unlike previously reported cases, intermittent hyperornithinemia was observed prior to the first hyperammonemic episode and citrulline supplementation. The latter alleviated the symptoms, normalized ammonia level, and led to increased plasma ornithine concentration. Furthermore, despite a protein restricted diet and ammonia scavenger treatment, continued supplemental citrulline was necessary to prevent hyperammonemia. Molecular analysis confirmed OAT deficiency, differentiating it from proximal urea cycle disorders and deficiency of the mitochondrial ornithine transporter, ORC1, (Hyperammonemia-Hyperornithinemia-Homocitrullinuria syndrome).

Synopsis: Hyperornithinemia alternating with hypoornithinemia and hyperammonemia in a neonatal-onset case of gyrate atrophy with ornithine aminotransferase deficiency.

1. Introduction

Gyrate atrophy of the choroid and retina (GACR, MIM# 258870) is a rare autosomal recessive disorder caused by deficiency of ornithine aminotransferase (OAT, EC 2.6.1.13) [1,2]. GACR typically presents in childhood with myopia, reduced night vision, and a characteristic chorioretinal degeneration accompanied by hyperornithinemia. Progressive loss of visual fields and central acuity leads to blindness in the fourth to sixth decade of life. Ophthalmoscopic examination reveals chorioretinal atrophy beginning in the mid-periphery of the retina. Posterior sub-capsular cataracts typically develop late in the second decade. In many patients, additional mild systemic effects have been observed with white matter changes and EEG abnormalities in the central nervous system [3], creatine deficiency in the central nervous system and skeletal muscle [4–6], atrophy of type 2 muscle fibers with tubular aggregates, and mild abnormalities in electromyogram [7]. No alterations in
the life span have been noted in GACR. At the biochemical level, OAT deficiency is characterized by persistent hyperornithinemia (10–15 fold normal levels), consistent with the major OAT function as an ornithine degrading enzyme [2]. A distinct phenotype of OAT deficiency manifests in the neonatal period, typically in premature infants, with episodes of acute hyperammonemia, vomiting and encephalopathy [8–11]. In these infants plasma ornithine levels are reduced, rather than elevated, until later in infancy and/or following arginine or citrulline supplementation.

In contrast to humans, mice homozygous for an Oat knockout allele uniformly develop lethal hyperammonemia associated with subnormal levels of ornithine and arginine on the second to third day of life [12]. Here, we describe an unusual case of neonatal OAT deficiency found to have transient hyperornithinemia prior to developing hyperammonemia. Supplementation with citrulline prevented hyperammonemia, while ammonia scavenger therapy with dietary protein restriction was not sufficient. Diagnosis was complicated by fluctuations of plasma ornithine and other intermediates of the urea cycle combined with transient homocitrullinuria and orotic aciduria and ultimately required confirmation with molecular methods.

2. Case description

A premature male (34 weeks and 6 days gestational age) was born to a 35-year-old G6 P5 mother via C-section due to maternal preeclampsia. Birth weight was 3160 g (95% on the preterm growth curve) and Apgar scores were 8 (1 min) and 9 (5 min). The pregnancy was also complicated by gestational diabetes, maternal systemic lupus erythematosus, and chronic hypertension. The parents are White and Hispanic, non-consanguineous. The infant’s post-delivery history was significant for respiratory distress requiring oxygen supplementation for nine days, intermittent bradycardia, episodes of supraventricular tachycardia (SVT) and patent foramen ovale with left to right shunt. Sepsis was ruled out postdelivery and at day of life (DOL) 17. The first newborn screen (NBS, DOL 3) showed borderline elevated TSH, which normalized on the second NBS (DOL 9). His diet consisted of breast milk supplemented with enriched preemie formula (Neosure), ad libitum.

On DOL 24 the infant developed lethargy and hypothermia and was found to have bradycardia and blood pressure abnormalities, with periods of both hyper- and hypotension. Sepsis evaluation was again negative. He was on total parenteral nutrition (5.5% amino acids with glucose) for two days and then re-started the diet of breast milk and enriched preemie formula. Total protein, arginine and citrulline intake are shown on Fig. 1D and E. Neurologic evaluation at DOL 26 showed nonspecific, diffuse white matter hypointensity on brain MRI interpreted as delayed white matter maturation, and an abnormal EEG with background slowing and scattered multifocal sharp waves without presence of epileptiform discharges. Ammonia was 20 μmol/L on DOL 26. However, plasma amino acid analysis on the following day (DOL 27) showed an elevated ornithine (494 μmol/L; normal range 30–180 μmol/L for patients <1 month old), with normal arginine (55 μmol/L; normal range 30–149 μmol/L for patients <1 month old) and citrulline (21 μmol/L; normal range 7–40 μmol/L, Fig. 1A–C), suggesting a disorder of ornithine metabolism.

Subsequently, the patient experienced two episodes of acute hyperammonemia that required treatment with ammonia scavenger, low protein diet and citrulline supplementation. The first episode started on DOL 30 with vomiting and hypothermia that progressed to agitation and poor feeding, as plasma ammonia gradually increased from 67 μmol/L on DOL 31 to 396 μmol/L on DOL 35 (normal <50 μmol/L) (Fig. 1). These symptoms were accompanied by low plasma ornithine (12 μmol/L; normal range 30–140 μmol/L for patients >1 month old), arginine (21 μmol/L; normal range 35–140 μmol/L) and citrulline (2 μmol/L; normal range 7–40 μmol/L) and elevated glutamine (1551 μmol/L; normal range 400–850 μmol/L) on DOL 32 (Fig. 1). Urine amino acid analysis showed prominent excretion of glutamine, proline, lysine and homocitrulline; urinary orotic acid was also elevated during this episode.

Fig. 1. Biochemical markers and nutritional intake during the course of disease. Blood ammonia (A), plasma glutamine and ornithine (B), plasma arginine and citrulline (C), protein intake (D), and arginine and citrulline intake (E). Shaded areas indicate periods with citrulline supplementation, the arrow indicates institution of ammonia scavenger treatment, and asterisks indicate treatment with an intravenous arginine bolus. Normal ranges, age appropriate for patients <1 month, and >1 month old, respectively, are as follows: glutamine 295–900 μmol/L, and 400–850 μmol/L; arginine 16–140 μmol/L, and 30–149 μmol/L; ornithine 30–180 μmol/L, and 30–140 μmol/L; and citrulline 7–40 μmol/L for all ages.
molecular ammonia level.

Following this acute episode, the patient remained asymptomatic while receiving low protein formula (initially 1.3 g protein/kg body weight/day, gradually increased to 1.7 g, 53% as essential amino acids) supplemented with citrulline (155 mg/kg body weight/day) in addition to ammonia scavenger therapy with glycerol phenylbutyrate (Ravicti, 0.5 mL three times daily). During this time, plasma amino acids analysis showed elevated ornithine with low glutamine, normal arginine and normal to mildly increased citrulline (DOL 37–42, Fig. 1). Despite glycerol phenylbutyrate treatment, discontinuation of citrulline supplementation on DOL 43 triggered a second episode of hyperammonemia (DOL 48, 173 μmol/L) with the patient becoming irritable and refusing feeding. The biochemical features were consistent with the pattern in the first hyperammonemia event: low plasma citrulline, arginine and ornithine, and high glutamine (Fig. 1). The episode resolved with a bolus injection of sodium phenylacetate/sodium benzoate plus arginine and restarting citrulline supplementation. Low protein diet (1.7 g protein/kg body weight/day) supplemented with citrulline (135–104 mg/kg body weight/day), and glycerol phenylbutyrate treatment (0.5 mL three times daily) were continued after discharge with minor adjustments. At 8 months of age glyceral phenylbutyrate was successfully discontinued, while sustaining citrulline supplementation. The infant continued to feed well, maintaining normal growth, and reaching age-appropriate developmental milestones at this time. His follow-up ophthalmic examination at 8 months of age was normal.

Exome sequencing revealed a homozygous nonsense variant of the conserved arginine codon NM_000274.4:c.991C>T, p.Arg331* in exon 8 of the 11-exon OAT gene (MIM *613349). This pathogenic variant is predicted to result in nonsense mediated mRNA decay and a C-terminal truncated OAT peptide [13]. The general population frequency of this allele is 0.000024, with the highest allele frequency in the Latino/Admixed American population (0.00014, GnomAD, rs386833623). This variant has been reported in the homozygous or compound heterozygous form with another pathogenic variant in two individuals affected with GACR [14]. No causative variants in other genes were detected, including in SLC25A15 encoding ORC1, the mitochondrial ornithine transporter which is deficient in the Hyperornithinemia-Hyperammonemia-Homocitrullinuria syndrome (HHH, OMIM 238970). Thus, molecular analysis results confirmed the diagnosis of GACR.

3. Discussion

OAT catalyzes the reversible conversion of ornithine to pyrroline-5-carboxylate (P5C), which can be further metabolized to proline and glutamate (Fig. 2) [15]. Depending on the tissue and the pattern of expression of OAT metabolically-related enzymes, the net flux of the OAT catalyzed reaction may favor either ornithine (and arginine) degradation or synthesis [2,16,17]. For example, the enterocytes of most mammals, including humans, express all enzymes necessary to convert either glutamine or proline to ornithine and on to arginine and, thus, are able to perform de novo arginine synthesis [16–18]. Since the milk of most mammals is relatively deficient in arginine [19], especially to meet the growth demands of rapidly growing infants, the anaplerotic function of this enterocyte pathway, including the OAT catalyzed reaction, is particularly important for neonates. In other cells and tissues, the pattern of expression of OAT-related enzymes is not compatible with ornithine synthesis and, hence, functions to catabolize excess ornithine and arginine [16–18].

The dual function of the OAT catalyzed reaction (ornithine catabolism, ornithine synthesis) and its role in different cell types has been suggested as the main reason for the seemingly paradoxical discrepancy in the presentation of OAT deficiency in children and adults as compared to affected neonates [8,12]. Post-infancy, as the growth rate slows, deficiency of OAT impairs degradation of ornithine derived from dietary arginine with resultant ornithine accumulation (Fig. 2A). By contrast, deficiency of OAT activity in affected neonates results in inability to synthesize ornithine and arginine and, depending on dietary arginine intake and the rate of growth, may result in depletion of arginine and other urea cycle intermediates, with reduced ammonia detoxifying capacity. In rare cases, this results in symptomatic hyperammonemia with encephalopathy, failure to thrive, vomiting and lethargy [8–11].

Nutritional arginine requirements are not clearly defined, primarily

![Mitochondrial matrix and Cytosol](image)

proline dehydrogenase; 2, P5C synthase, short form; 3, glutaminase; 4, ornithine aminotransferase; 5, NAG synthase; 5, ornithine transcarbamylase; 6, CAP synthase; 7, ornithine carrier 1; 8, ASA synthase; 9, ASA lyase. Dashed lines represent positive (→ - →) or negative (−→) small regulation of enzymatic activity [26].
because, unlike essential amino acids, arginine can be synthesized endogenously. In neonatal pigs, endogenous arginine synthesis provides about 60% of total required arginine [20]. The amount of arginine our patient was receiving in formula was comparable to the amount of arginine typically consumed by a healthy neonate on breast milk. However, there is a clear correlation of the periods with lower arginine/citrulline intake with hyperammonemia episodes and low plasma arginine and citrulline levels (Fig. 1). Accordingly, symptoms resolved promptly following the supplementation with arginine and citrulline. This pattern can be explained by insufficient endogenous arginine/citrulline synthesis secondary to OAT deficiency. Our patient first developed lethargy and hypothermia at 24 days of age. We cannot exclude hyperammonemia during this episode, because plasma ammonia was not measured until DOL 26, when it was in the normal range. Nutrition on DOL 25 and 26 was provided parenterally. The arginine content of IV amino acid solutions is approximately four-fold higher than in the enteral formula feeds (Fig. 1E), and therefore the administration of total parenteral nutrition likely led to resolution of symptoms through repletion of urea cycle intermediates and normalization of ammonia levels. Two previous neonatal OAT deficiency cases described clinically asymptomatic infants with mildly elevated blood ammonia, elevated plasma glutamine and low or normal arginine and ornithine suggesting restricted capacity of the urea cycle [9,12]. One of these patients later developed acute symptoms of hyperammonemia [9] while the other maintained normal ammonia on arginine supplementation [12].

The two episodes of documented hyperammonemia in our patient resolved promptly following treatment with ammonia scavengers and replenishing urea cycle intermediates with arginine and citrulline supplementation. The peak ammonia level in our patient (396 μmol/L) was comparable with blood ammonia levels reported in other neonates with symptomatic OAT deficiency (110–812 μmol/L) [8–11]. Elevated ornithine has not been observed before or during the crises in any of the previously published cases [8–11]. The rapid swings of ornithine levels in our patient likely reflect the dynamic balance of growth, nutrition and demand on OAT flux in newborns. In the absence of OAT activity, the normal homeostatic function provided by this reaction is lost, leaving the infant at the mercy of an imperfect balance between nutrition and growth. The fact that this infant was a borderline LGA (95%) premature birth to a diabetic mother may also have contributed to the initial hyperornithinemia.

The low plasma citrulline and arginine levels and elevated urine ornitic acid observed in neonatal hyperammonemic GACR patients can lead to the suspicion of OTC deficiency, an X-linked disorder of the urea cycle. The observation of hyperornithinemia in our patient prior to the first documented hyperammonemical episode is not consistent with this diagnosis. Additionally, the increase of homocitrulline is not compatible with OTC deficiency, as it results from OTC activity on lysine rather than ornithine [21].

The range of plasma ornithine levels in GACR overlaps with that of another disorder of the urea cycle, HHH syndrome: 216–1915 μmol/L in HHH vs. 526–982 μmol/L in GACR [14,22]. HHH syndrome is an autosomal recessive disorder caused by deficiency of the mitochondrial ornithine transporter, ORC1 [23,24]. The key difference between the two disorders is that in HHH, hyperammonemia occurs in the setting of hyperornithinemia, while in GACR, hyperammonemia rarely occurs and only in the setting of subnormal plasma concentrations of ornithine and arginine. Rare elevations of homocitrulline and ornithic acid are prominent in HHH but can be observed in either disorders [1,22]. Indeed, elevated ammonia levels in our patient were associated with low plasma ornithine (Fig. 1) as in previous reports [8–11]. Ultimately, the diagnosis should be confirmed with molecular methods; several clinically available molecular diagnostic hyperammonemia panels include OAT.

Suggested treatment for GACR is based on an arginine-restricted diet with the goal of reducing ornithine accumulation to prevent retinal damage [25]. In cases presenting with hyperammonemia in early infancy, arginine restriction should be avoided to prevent hyperammonemic encephalopathy. In fact, supplementation with arginine or citrulline is necessary in some of these infants to prevent hyperammonemia by replenishing the intermediates of the urea cycle. In our patient, for instance, discontinuation of citrulline supplementation induced the second acute hyperammonemical episode. Based on the previous observations, it would be plausible to assume that dietary restriction of arginine could be initiated in early childhood, under careful monitoring of dietary protein intake and plasma amino acids levels. The optimal timing of this transition has not yet been defined [8,9].

The neonatal presentation of GACR with hyperammonemia does not follow a particular genotype-phenotype correlation, although all neonatal patients described so far carry truncating variants, suggesting the complete lack of enzyme function [14]. The p.Arg331* homozygous variant identified in our patient has also been previously found in the homozygous state in a child of Turkish descent with onset of ocular symptoms at 5 years of age and plasma ornithine level of 650 μmol/L [14]. This patient did not display symptoms of hyperammonemic encephalopathy in early infancy. While it is not clear why GACR patients with the same genotype may or may not have neonatal hyperammonemia, the genetic background could certainly play a role, as well as environmental factors such as nutrition or co-morbidities. Prematurity could be a contributing factor in the case of our patient, as one other OAT deficiency case with a severe neonatal presentation described in the literature was also a premature infant [11].

GACR with OAT deficiency should be considered in neonates/infants presenting with symptomatic hyperammonemia accompanied by a plasma amino acid pattern of high glutamine, low ornithine, citrulline, and arginine, reminiscent of proximal urea cycle defects. Our patient demonstrates that in neonates, ornithine levels may fluctuate between high to low levels with hyperammonemia associated with the latter. Molecular testing of OAT, SLC25A15 and related urea cycle genes confirms the diagnosis.

Contributions of individual authors

Aneta Kaczmarczyk: Conceptualization, Formal Analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualisation. Mark Baker: Resources, Investigation, Writing – Review & Editing. Julianna Diddle: Resources, Investigation, Writing – Review & Editing. Tatiana Yuzuyk: Resources, Conceptualization, Writing – Original Draft, Writing – Review & Editing, Supervision. David Valle: Conceptualization, Writing – Review & Editing, Visualisation. Kristin Lindstrom: Conceptualization, Resources, Writing – Review & Editing, Supervision.

Declaration of Competing Interest

All authors declare no competing financial or non-financial conflicts of interest.

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