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Ornithine aminotransferase deficiency: Diagnostic difficulties in neonatal presentation

M. A. Cleary[1,7], L. Dorland[2], T. J. de Koning[2], B. T. Poll-The[3], M. Duran[4], R. Mandell[5], V. E. Shih[5], R. Berger[2], S. E. Olpin[6] and G. T. N. Besley[1,*]

[1]*Willink Biochemical Genetics Unit, Royal Manchester Childrens Hospital, Pendlebury, Manchester, UK;* [2]*UMC Utrecht, Department of Metabolic and Endocrine Diseases, Utrecht;* [3]*AMC Amsterdam, Department of Child Neurology, Amsterdam;* [4]*AMC Amsterdam, Laboratory of Genetic Metabolic Diseases, Amsterdam, The Netherlands;* [5]*Amino Acid Disorders Laboratory, Massachusetts General Hospital, Boston, Massachusetts, USA;* [6]*Department of Neonatal Screening, Sheffield Children's Hospital, Sheffield, UK;* [7]*Current address: Great Ormond Street Hospital, London WC1N 3JH, UK*

[*]*Correspondence: Willink Biochemical Genetics Unit, Royal Manchester Children's Hospital, Manchester M27 4HA, UK. E-mail: guy.besley@cmmc.nhs.uk*

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Summary: We describe two unrelated cases of ornithine aminotransferase (OAT) deficiency with rare neonatal presentation of hyperammonaemia. The diagnosis in the neonatal presentation of OAT deficiency is hampered as hyperornithinaemia is absent. Enzyme and mutation studies confirmed the diagnosis. OAT deficiency should be included in differential diagnosis of neonatal hyperammonaemia.

Ornithine aminotransferase (OAT) deficiency characteristically presents with visual disturbance as the progressive destruction of the choroid and retina leads to night blindness and myopia (Shih et al 2000). Patients develop posterior subcapsular cataracts by their late teens and are ultimately usually blind by age 60 years. The biochemical hallmark in this typical presentation is a marked elevation of plasma ornithine (Shih et al 2000). There is only one brief record in humans where low plasma ornithine was measured at age 2 months in an infant who had been diagnosed prenatally (Wang et al 1995). We report two unrelated patients with confirmed OAT deficiency and rare presentation of symptomatic neonatal hyperammonaemia and discuss the difficulties in making this diagnosis in the newborn period. Part of this work has been presented in abstract form (Cleary et al 1999; Dorland et al 1999).

CASE REPORTS

Case 1: A male infant, born to consanguineous Asian parents, developed vomiting, diarrhoea and mild transient hyperammonaemia (180–220 μmol/L) at 13 days of age. He failed

Table 1 Biochemical findings in cases 1 and 2

Age (weeks)	*Plasma ammonia* (μmol/L)	*Plasma ornithine* (μmol/L)	*Plasma arginine* (μmol/L)	*Plasma citrulline* (μmol/L)	*Plasma glutamine* (μmol/L)	*Urine orotic acid* (μmol/mmol creatinine)
Normal range	<80	40–60	55–70	25–50	560–670	0–5
Case 1						
2.5	175					17
3	220	39	40	ND	1108	
4	62	ND[a]	11	ND	1545	
4	42					40
6	812	16	12	ND	2765	34
7	35	680	77	10	217	
8.5		668	71	ND	291	1560
10		413	100	44	687	
13		713	84	25	248	
15		703	225	23	212	
22		1208	203	15	422	
Case 2						
8	320					
10		15	18	<1	1351	39
12		13	14	<1	2016	10
12.5		97	41	12	421	3
14		394	93	35	703	
16	40	481	58	13	481	8
24		388	114	28	556	
48		528	90	22	566	
74		755	130	24	498	
88		693	102	32	491	
311		661	92	24	491	

[a]ND, not detected

to thrive on a combination of breast milk and standard infant formula and required blood transfusions for a severe anaemia. Haemoglobin was 8.8 g/dl; a blood film showed spherocytes, fragmented cells and polychromasia. Plasma glutamine was elevated; ornithine and citrulline were low, but urine orotic acid was slightly raised at 17 μmol/mmol creatinine (see Table 1). However**,** a repeat blood ammonia 4 h after starting intravenous dextrose was normal and remained normal over the next 4 days while standard infant formula feeds were reintroduced.

The main clinical problem was persistent anaemia necessitating packed cell blood transfusions over the ensuing 4 weeks. Bone marrow examination showed an excess of binucleate erythroblasts and occasional trinucleate forms suggestive of a congenital dyserythroblastic anaemia. During this period the infant continued to vomit intermittently and weight gain remained poor.

At age 6 weeks he became encephalopathic within a few hours of completing a blood transfusion. His blood ammonia was 812 μmol/L; he was urgently treated with intravenous sodium benzoate and arginine and protein feeds were discontinued. Blood ammonia fell to normal within 24 h of treatment, but the patient remained irritable with abnormal cycling movements for 3 days. Subsequently he was maintained on a low-protein diet (approximately 1.5 g/kg per 24 h) with oral arginine supplements and sodium benzoate medication. Plasma amino acids at age 6 weeks showed a low ornithine of 16 μmol/L with no detectable citrulline but high glutamine. By 7 weeks there was a marked elevation of plasma ornithine and reduced glutamine. Urine orotic acid was also markedly increased (see Table 1) and homocitrulline was detected in urine; however, this can be a normal constituent for infants on milk formula feeds.

Investigation of [^{14}C]ornithine incorporation into fibroblast cell protein was markedly deficient (see Table 2), consistent with both the ornithine transporter defect (hyperornithinaemia–hyperammonaemia–homocitrullinuria syndrome, HHH syndrome) and ornithine aminotransferase (OAT) deficiency. Subsequent studies showed a severe deficiency of OAT activity, indicating this to be the underlying defect. Mutation analysis of the OAT gene on RNA prepared from cultured skin fibroblasts revealed that this patient is homozygous for the 1192C>T (R398ter) mutation in exon 11. In addition he had the common polymorphism 1134C>T (N378N).

Once the diagnosis of OAT deficiency was established (32 weeks), arginine supplements were discontinued and he was maintained on a low-protein, low-arginine diet (0.2 g protein/kg per/day supplemented with essential amino acids). He has had no further episodes of hyperammonemia. At 1 year he was healthy, had normal growth (weight 10–50th centile, height 3rd centile) and had achieved developmental milestones appropriate for his age. Ophthalmic examination was normal. He was no longer transfusion dependent and the bone marrow now had a normal appearance. The patient is now 6 years old and has remained in good health, although he continues to be anaemic with haemoglobin values of 8.2–10.4 g/dl. His plasma ornithine levels have been maintained below 200 μmol/L for the last 3 years.

Case 2: A female infant born at term to consanguineous Turkish parents developed feeding problems and irritability at 1 week of age. By 8 weeks of age she had failure to thrive and had

Table 2 Fibroblast [^{14}C]ornithine incorporation studies and ornithine aminotransferase (OAT) activity

	[^{14}C]Ornithine incorporation into protein	*OAT activity* (μmol pyrroline-5-carboxylic acid formed per h per mg protein)
Patient 1	0.04[a] [control range 6–10]	None detected[c] [control range 0.47–0.63]
Patient 2	0.008[b] [controls 0.445 ± 0.116]	0.002, 0.001[d] [controls 0.235, 0.223]

Studies on patient 1 were done in Manchester and Sheffield; those for patient 2 were from Boston
[a]nmol/18 h per mg protein
[b]Expressed as ratio to ^{3}H-leucine; method of assay, Shih et al (1982)
[c]Method of assay, Shih and Schulman (1970)
[d]Method of assay, Ohura et al (1983)

frequent vomiting and poor visual fixation. She was not anaemic, had normal motor development, and was not myopathic. Initial metabolic investigations showed hyperammonaemia (320 μmol/L) and orotic aciduria (39 μmol/mmol creatinine). Plasma glutamine and alanine were raised, but arginine and ornithine were low and citrulline was undetectable (Table 1). Homocitrullinuria was initially present. At 12 weeks the infant was treated with sodium benzoate and citrulline and a protein-restricted diet. After treatment with citrulline, the ornithine concentration in blood initially increased (to 481 μmol/L). However, homocitrulline was no longer present in the urine. Ornithine carbamoyltransferase (OCT) activity in the liver was normal and no known mutations were detected in the OCT gene. The diagnosis was unclear and HHH syndrome, perhaps in a variant form, was considered likely.

As in case 1, [^{14}C]ornithine incorporation into fibroblast cell protein was deficient (see Table 2) suggesting HHH syndrome or OAT deficiency. Subsequent studies confirmed a deficiency of OAT activity (see Table 2) as the underlying defect. Mutation studies revealed that this girl was homozygous for the 1276C>T (R426ter) mutation in exon 11. No other mutations were found. Hyperammonaemia and orotic aciduria were observed only in the newborn period and anaemia was never documented. At present the girl is 6 years old and shows a normal growth and psychomotor development (height at 50th centile, weight at 70th centile) with normal findings at recent ophthalmologicalal examination. The compliance with the arginine-resticted diet is very poor, resulting in plasma ornithine concentrations between 700 and 1100 μmol/L.

Neither case was treated or investigated for reduced creatine synthesis secondary to hyperornithinaemia (Heinanen et al 1999), nor was there any evidence of a myopathy.

DISCUSSION

We describe two infants who presented with neonatal hyperammonaemia and orotic aciduria and who responded to treatment for hyperammonaemic encephalopathy. These findings, combined with a subsequent rise of plasma ornithine, led to further metabolic investigations and confirmation of OAT deficiency by enzymatic and molecular analysis. This neonatal presentation is very unusual for OAT deficiency, for which the main clinical feature has been the gradual development of night blindness, myopia, and loss of peripheral vision with progressive gyrate atrophy of the choroid and retina but without increased blood ammonia. On the other hand, the symptoms and findings of patients with hyperornithinaemia–hyperammonaemia–homocitrullinuria (HHH) syndrome are similar to those in the urea cycle disorders, namely, hyperammonic crisis and mental retardation without any retinopathy.

Laboratory tests to confirm these disorders include measurement of OAT activity and the [^{14}C]ornithine incorporation assay in cultured fibroblasts. For gyrate atrophy due to OAT deficiency both tests are abnormal, whereas for the HHH syndrome, a defect of the mitochondrial ornithine transporter, only the incorporation assay is abnormal. These two disorders can be further characterized by mutation analysis.

The combination of clinical findings of hyperammonaemia and the enzyme deficiency of OAT in these two newborns is unusual. The diagnosis in the neonatal presentation of OAT deficiency is more difficult as hyperornithinaemia is absent.

Why does OAT deficiency present a different clinical and biochemical pattern in infancy? Studies on the OAT-deficient mouse model help explain these differences. In 1995,

Wang and colleagues produced a mouse model of OAT deficiency by gene targeting (Wang et al 1995). To their surprise, these mice developed severely low concentrations of ornithine, arginine and citrulline and high ammonia in plasma. These mice could be rescued by arginine supplementation, and after weaning they became hyperornithinaemic and developed retinal degeneration over several months. The authors concluded that the OAT reaction is the only known pathway of ornithine synthesis and that the net flux in the OAT reaction in neonatal mice is in the direction of ornithine synthesis rather than ornithine degradation. However, the OAT reaction is reversible. In physiological states other than the neonatal period, the flux is for ornithine degradation.

This flux of OAT to ornithine synthesis in humans is supported by findings of hypoornithinaemia in a newborn with OAT deficiency. Wang and colleagues had the opportunity to follow an OAT-deficient infant who was diagnosed prenatally since there was a previously affected sibling (Wang et al 1995). Plasma ornithine and arginine levels were subnormal on several occasions between the ages of 2 and 3 months while the patient was on breast milk supplemented with formula, although plasma glutamine and ammonia were elevated. Arginine supplementation corrected the low arginine and at the same time the plasma ornithine became elevated. This plasma amino acid pattern remained after arginine withdrawal.

The biochemical findings in the cases we report also support reverse reaction of OAT in the newborn period in humans. Since there are no reports of unexplained neonatal deaths in families with gyrate atrophy, it seems that the human requirement for endogenous ornithine in the newborn period may be less than that of the mouse.

In the cases we described, the diagnosis was difficult to establish. There is a wide differential diagnosis to be considered in neonatal hyperammonaemia and some confounding factors were present in both cases. In case 1 the presence of congenital dyserythroblastic anaemia led to investigations for a haematological problem. The relationship of anaemia to the eventual diagnosis remains unclear. The initial period of hyperammonaemia was easily managed by stopping protein feeds and giving dextrose. Symptomatic hyperammonaemia did not appear again until the infant was aged 6 weeks. In that intervening period, however, he did not thrive and may have had mild hyperammonaemia. The finding of high plasma ammonia, increased urine orotic acid, and a nondiagnostic plasma amino acid pattern led to ornithine carbamoyltransferase deficiency (OCT) being considered as a possible diagnosis. In a male newborn with OCT deficiency, however, one would have expected a more severe clinical picture with a marked orotic aciduria and difficult-to-control hyperammonaemia. Even after development of hyperornithinaemia, the diagnosis was unclear in our patients, as HHH syndrome seemed a possibility given the presence of homocitrullinuria. Homocitrullinuria, however, is a relatively common finding in infants on formula feeds. Defective ornithine incorporation into protein would be found in both HHH syndrome and OAT deficiency and therefore the final diagnosis was dependent on demonstrating deficient OAT activity in fibroblasts.

There are two other abstract reports of proven OAT deficiency with neonatal presentation (Champion et al 2002; Webster et al 1999). The early laboratory findings in these cases also showed high plasma ammonia with orotic aciduria. In one case there was high plasma glutamine with low ornithine, arginine and citrulline. With treatment, this patient has global developmental delay at 2.5 years, but no evidence of gyrate atrophy (Webster et al 1999).

The other case was a 3-month-old infant presenting with hyperammonaemic encephalopathy (Champion et al 2002).

The exon 11 mutations 1276C>T (R426ter) and 1192 C>T (R398ter) found in our two cases in the homozygous state have been reported previously in patients with gyrate atrophy. A patient of Japanese origin was homozygous for R426ter (Mashima et al 1992) and a pyridoxine-responsive patient with R398ter was a compound heterozygote with A226V in the other allele (Michaud et al 1995). No neonatal illnesses were mentioned in these reports; however, that does not preclude the possibility that a neonatal illness was not recognized as being related to the OAT deficiency.

The two cases described here highlight some important points. First, OAT deficiency must be added to the differential diagnosis of hyperammonaemia in the newborn period. Second, specific OAT enzyme assay is necessary to differentiate OAT deficiency from HHH syndrome. Third, these findings suggest that in humans, as in the mouse model, the OAT reaction in the newborn period acts in the reverse direction, favouring ornithine production.

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