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ARGININOSUCCINATE LYASE DEFICIENCY: LONGTERM OUTCOME OF 13 PATIENTS DETECTED BY NEWBORN SCREENING

C Ficicioglu^{a,a}, R Mandell^a, and VE Shih^a

^aMassachusetts General Hospital, Amino Acid Disorders Laboratory, 55 Fruit Street, GRJ239, Boston MA 02114 USA

Abstract

Argininosuccinate lyase deficiency is a urea cycle disorder which can present in the neonatal period with hyperammonemic encephalopathy, or later in childhood with episodic vomiting, growth and developmental delay. Abnormal hair, hepatomegaly, and hepatic fibrosis are unique features of this disorder. Twelve patients with argininosuccinate lyase deficiency were ascertained between 4 and 6 weeks of age by urine amino acid screening. One infant in a previously identified family was diagnosed shortly after birth. Diagnosis was confirmed by enzyme assay in red blood cells and/or skin fibroblasts. At the time of last follow-up, patients had been followed for 13–33 years. All patients were asymptomatic at detection, 7 had slightly increased blood ammonia, and all were initially treated with low-protein diet. Utilization of ¹⁴C-citrulline by intact skin fibroblasts measured by ¹⁴C incorporation into macromolecules was 74–135% of the control mean for 7 of the 8 patients studied. Nine patients had normal development, 4 had learning disability, 6 had EEG abnormalities, 3 had seizure disorder. None had any episodes of hyperammonemic coma. None had hepatomegaly. Patients detected by screening had higher enzyme activity measured by the ¹⁴C-citrulline incorporation assay than comparison groups of patients with neonatal onset and with late onset detected by clinical disease. The ability to utilize ¹⁴C-citrulline by intact fibroblasts seems to correlate with clinical outcome and may have prognostic value. It is likely that early diagnosis and treatment contributed to the relatively mild clinical course of the study group.

Keywords

argininosuccinate lyase deficiency; argininosuccinic aciduria; newborn screening; urea cycle disorder; outcome; hyperammonemia

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Corresponding Author: Vivian E. Shih MD, Massachusetts General Hospital, Amino Acid Disorders Laboratory, 55 Fruit Street, GRJ239, Boston MA 02114 USA, vshih@partners.org.

¹Present Address: The Children's Hospital of Philadelphia, Division of Metabolism, 34th and Civic Center Blvd, Main building 2152, Philadelphia, PA 19104 USA

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Introduction

Argininosuccinate lyase deficiency is a rare, autosomal recessive disorder in the urea cycle. Argininosuccinate (ASA), an intermediate in the pathway of urea synthesis from ammonia, is split into arginine and fumarate in a reaction catalyzed by argininosuccinate lyase (AL; EC 4.3.2.1). As a result of this enzyme defect patients have increased ammonia, both ASA and citrulline accumulate in the blood, and ASA is excreted in the urine. There is considerable phenotypic variability. The most severe cases present in the neonatal period with lethargy, vomiting, hypothermia, hyperventilation, hepatomegaly and progressive encephalopathy. Mortality is high and neurological damage is frequent in survivors. The disease may also be manifested later in childhood with an acute encephalopathy that often is precipitated by infection or high protein intake. Patients may also display chronic symptoms such as episodic vomiting, mental status changes, lethargy, and behavioral abnormalities associated with hyperammonemia. [1] Mild to moderate developmental delay and seizures can be seen even in patients without documented history of hyperammonemia.

We report here a 13–33 year follow-up of 13 patients who were detected through newborn screening. Twelve had mild disease. In addition to outcome, we examine the predictive value of enzyme studies in patients with AL deficiency.

Methods

Amino acids were measured on a Beckman 6300 analyzer with high performance lithium column using the two hour, three buffer method. ASA was measured in boiled samples as the total of the anhydrides. ASA is not detectable in body fluids of normal individuals. Initial plasma and urine ASA values and blood ammonia levels at the time of diagnosis are listed in Table 1.

Patients

Between 1969 and 1978, urine amino acid screening using one dimensional paper chromatography [2] was performed on a total of 633,331 Massachusetts infants between 4 and 6 weeks of age. Twelve infants, including one set of twins, were found to have increased argininosuccinic acid. These infants were referred to the Massachusetts General Hospital Metabolic Disorders Unit for confirmation of diagnosis and treatment. One additional infant was diagnosed shortly after birth due to an older sibling previously identified by screening.

Each patient was the healthy product of a term pregnancy, with normal anthropometric measurements. Patients were 4–6 weeks old at diagnosis and all were asymptomatic. Seven of 13 patients initially had slightly increased blood ammonia. Initial plasma ASA was 6–101 $\mu\text{mol/l}$ and urine ASA was 2667–81900 $\mu\text{mol/g}$ creatinine (Table 1). CSF ASA was increased in the three patients in whom it was measured and was more than twice as high as the corresponding level in plasma. All patients had plasma arginine levels within the normal range. Case 1 was the most severely affected patient with initial ASA levels higher than those of the others.

Following diagnosis the majority of patients were treated with a low protein diet of approximately 1.5 g protein/kg/day, consisting of a limited amount of infant formula with added lipid and carbohydrate, plus cereal, fruit, and vegetables. The patients were monitored at 1 to 3 month intervals during the first 2 years of life and less frequently thereafter. Blood ammonia, amino acids, liver function tests, BUN, urine ASA and orotic acid were measured at every follow up visit and dietary intake was adjusted.

Nine of the 13 patients were given arginine, 25–50 mg/kg/day po. Two solely breast-fed infants never received arginine. Arginine was given to one patient when weight gain began to level off at 18 months of age. In another patient, arginine supplement was discontinued at age 9 years; after one month the plasma level had dropped 19% but still remained in the normal range. One patient received a trial of sodium benzoate (1.8 g/day po) at age 11 years, which was discontinued due to increased urination.

Some families found compliance to the special low-protein diet difficult, particularly since the children appeared to have normal growth and development, and discontinued the diet when the patients were 2–5 years old. Otherwise, the low protein diet was continued throughout the follow-up period. For older children and teenagers the prescribed protein intake was in the range of 1.0 g/kg/day. Some older patients now take an essential amino acid supplement.

Serial EEGs were done routinely. IQ measurements were performed at different ages in five patients using one of the following scales: the Stanford-Binet intelligence scale, the Cattell Infant intelligence scale, WISC-III, and the Merrill-Palmer scale of mental tests. Patients who did well in school without learning difficulties were considered to have normal development. Seven patients are college students or college graduates.

None of the patients in our study group had hepatomegaly on physical exam or liver dysfunction monitored by liver function tests (ALT, AST, alkaline phosphatase, bilirubin). All had normal tyrosine and methionine levels by plasma amino acid analysis. Nor did we note brittle hair, sometimes described in AL deficiency [3]. All patients had normal growth parameters. All our patients displayed elevations of ASA in both plasma and urine. Blood ammonia levels remained in the normal range.

None of our cases manifested hyperammonemic coma during the follow-up period; none required hospitalization for acute encephalopathy. Case 7 had two supervised uneventful pregnancies and Case 10 had two normal pregnancies. All four offspring had normal development at 5–8 years of age.

Table 1 summarizes the outcome of our study group of 13 individuals. Four had a learning disability with verbal IQ range 71–95 and performance IQ range 78–132. One patient was lost to followup for over ten years. This patient went off the low protein diet at around 5–6 years of age, was in special education classes in school, and had low normal development. Six patients showed EEG abnormalities, including abnormal sharp irregular background activity, frequent bilateral paroxysms, and increased slow wave activity. In one patient, abnormal EEG was documented on 4 occasions during a six-year period when the diet was uncontrolled; over the next six years the protein intake was more strictly controlled and EEG was normal on 3 occasions. Three cases showed seizures of various types: staring spells; episodes of night disturbance, vomiting, and stiffened back; and generalized tonic clonic seizure. In one case heavy alcohol consumption and noncompliance to the protein-restricted diet may have precipitated hyperammonemia and subsequent seizures. One additional patient had two febrile seizures with subsequent normal EEG. Patients with clinical seizures were treated with either carbamazepin or levetiracetam.

For comparison, we looked at two other groups of AL deficient patients: those with neonatal-onset and those with late-onset disease. Patients with neonatal onset presented with severe hyperammonemia, lethargy and vomiting in the first 10 days of life. Long-term clinical outcome was not examined in the neonatal onset group. Patients with late-onset were detected when they became symptomatic and were diagnosed between 1.5 and 15 years of age. When not acutely ill, plasma ASA in late onset patients was in the range of 107–152 $\mu\text{mol/L}$ and 30384–60840 $\mu\text{mol/g}$ creatinine in urine; blood ammonia determinations were 75–133 (N: 12–

54). All late-onset patients developed mental retardation (IQ: 40-60), generalized seizures, and intermittent ataxia.

Enzyme Activity

Diagnosis was confirmed by direct measurement of argininosuccinic acid lyase (AL) activity in red blood cell hemolysates and/or fibroblast extracts by the method of Shih et al [4]. In 8 patients AL activity was also assessed indirectly in intact fibroblasts by measuring incorporation of ^{14}C from L-[ureido- ^{14}C]citrulline and ^3H from L-[3,4,5- $^3\text{H}(\text{N})$]-leucine into acid-precipitable material using the incorporation assay described by Jacoby et al [5] with minor modifications.

Enzyme activity in the patients detected by screening is presented in Table 1. Erythrocyte (RBC) AL activity was almost undetectable in 3 individuals. Some residual enzyme activity (0–13% of control mean) was found in the others. Decreased AL activity in fibroblast extracts (0–23% of control mean) was noted for 5 patients. Less symptomatic patients had higher residual activity.

Utilization of ^{14}C -citrulline by intact skin fibroblasts was 74–135% of the control mean for 7 of the 8 patients detected by screening who were studied. The most severely affected patient (case 1) had the lowest incorporation (3%).

Table 2 shows direct enzyme activity and utilization of ^{14}C -citrulline in fibroblasts from patients with different forms of AL deficiency: the newborn screening patients, the group of patients with neonatal-onset and the group of patients with late-onset disease. We found that both the neonatal-onset and late onset patients had low enzyme activity (0–9% of control) in extracts of RBC and fibroblasts. Incorporation of ^{14}C -citrulline into fibroblast protein in both the neonatal-onset and late onset patients was lower than patients detected by screening.

Discussion

Few reports describe long-term outcome of patients with AL deficiency who were detected by newborn screening. Shih [6] described the first youngster so diagnosed, detected at 4 weeks of age. Widhalm et al [7] reported long-term follow-up of 12 patients found by newborn screening. They were treated with protein restriction and L-arginine supplements. All had normal intellectual and psychomotor development. Margalith and et al [8] reported a patient detected through newborn screening and followed over 7 years. Protein restriction and arginine supplements were started at age 8 months and continued intermittently. She had borderline intelligence and mild cerebellar ataxia. Tuchman et al reported that patients with AL deficiency have higher frequency of developmental disabilities, seizure disorders, and abnormal neurologic findings such as abnormal movement, cerebellar dysfunction, tone and reflex abnormalities compared to patients with OTC deficiency [9]. They suggested possible toxic effect of argininosuccinic acid on the brain [9].

Our data suggest the incidence of AL deficiency in Massachusetts at around 1:49,000 live births. All our patients were asymptomatic at diagnosis. They received treatment with a low protein diet, initiated between 3 and 26 weeks of age, designed to prevent potentially neurotoxic hyperammonemia and brain damage. Compliance to the diet varied. Overall growth was normal in each individual. Eight of the 13 had normal intellectual and psychomotor development. Five patients had learning disabilities and borderline IQ; three of these youngsters also had psychosocial problems. Of the 5 patients with learning disability or delay, 4 discontinued the diet early, suggesting a benefit to dietotherapy even with relatively mild disease. Treatment did not prevent the development of EEG abnormalities in 6 of the 13 patients, however poor compliance to the low-protein diet seems to have had an adverse effect. Comparison of siblings

(cases 5 and 6) shows better outcome in the one who remained on diet. In addition, EEG abnormalities resolved in two patients following implementation of a low-protein diet. Initial ASA levels in CSF were more than twice as high as plasma levels in 3 of our patients. Higher ASA in CSF has also been reported in patients with late-onset disease [10]. These findings suggest that ASA or a metabolite of ASA might be neurotoxic in itself.

Hepatomegaly and hepatic fibrosis often is observed in children with AL deficiency, particularly those with neonatal-onset disease. Thus, Zimmerman et al [11] reported an 18 month old boy with neonatal onset AL deficiency who developed severe hepatic fibrosis. The etiology of the hepatopathy is not clear, although it is reasonable to conclude that ASA or a metabolite of ASA is responsible. We did not observe liver dysfunction in our patient cohort.

In this study we examined long-term outcome and the predictive value of enzyme studies in 13 patients detected by newborn screening. We compared the enzyme activity in this cohort to patients with neonatal-onset disease and late-onset disease. Although all three groups of patients had marked argininosuccinate lyase deficiency when measured in RBCs or fibroblast extracts, the newborn screening group had greater ^{14}C -citrulline incorporation into protein compared with the other two groups. Of the patients detected by newborn screening, the most severely affected patient was case 1, who had high ASA excretion, seizures, a learning disability, and an IQ of 71. Without newborn screening, it is possible this patient may have come to attention later in childhood, similar to patients considered to have late-onset disease. This patient also manifested the lowest ^{14}C -citrulline incorporation of the group of patients detected by screening. In this case, the ^{14}C -citrulline incorporation assay may be of more prognostic value than direct assay of AL activity.

Some patients detected through newborn screening had decreased AL enzyme activity but normal utilization of ^{14}C -citrulline values. These patients had notably better outcomes than patients with low levels of both AL enzyme and utilization of ^{14}C -citrulline. This suggests the prognostic value of performing the utilization of ^{14}C -citrulline test along with the other enzyme testing. Patient 4 is an exception. This case had near normal utilization of ^{14}C -citrulline and low enzyme activity; however, unlike other patients with these biochemical findings, developed a mild learning disability. How can we explain this? The finding of learning disability in this case may be a function of genetic family inheritance and thus entirely unrelated to AL deficiency. This suggests the need for further studies that utilize a larger cohort to yield more statistically significant data, and that provide longitudinal assessment of development of all patients, not only those with learning disabilities, as well as assessing development of patient siblings and parents.

The data also raises another question requiring further study: does arginine have any impact on outcomes for asymptomatic patients with AL deficiency detected by newborn screening? Deficient argininosuccinate lyase activity prevents synthesis of arginine, however, all patients detected by screening had plasma arginine levels in the normal range. No patient had significant hyperammonemia or hair abnormality, both of which can respond to arginine. Nor is there any discernible difference in outcomes between those who received and those who did not receive arginine. This suggests that some patients with AL deficiency detected by newborn screening may never need arginine.

In 1972, the short term outcome of Patient 1 was reported as developmentally normal at 2 years of age [6]. Long term follow up of this patient (33 years) showed that she had learning disability, seizures and low IQ. The data illustrates the unreliability of predicting long term outcome from short term follow up and underscores the importance of long term follow up studies.

In conclusion, the large variation in metabolites and enzyme data indicate the heterogeneity among these patients. The most severely affected patients had lower ^{14}C -Citrulline

incorporation test values. The ability to utilize ^{14}C -Citrulline by intact fibroblasts seems to correlate with clinical outcome and therefore may have some prognostic value. Our data suggest that performing the ^{14}C -citrulline utilization test along with the direct enzyme assay can help assess the severity of the disease. The more favorable outcome in patients detected by newborn screening underlines the importance of early detection and treatment.

Acknowledgments

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Table 1

Initial biochemical findings at the time of diagnosis, argininosuccinate lyase activity, treatment, neuro-development and outcome in 13 patients with AL deficiency detected by newborn screening.

Patient	Ammonia [N: 80-110]	ASA Urine, μmol/g [N: 0]	ASA plasma, μmol/L [N: 0]	RBC AL, μmol/h/mg Hgb [N: 0.126± 1.93]	fibroblast AL, μmol/h/mg prot [N: 0.045]	¹⁴ C/ ³ H incorp [N: 1.025 ±.334]	age diet [N: 3 yr]	Neurological	Outcome	Follow- up, yrs
1 [6]	147	101 CSF 282	81900	0.05	<0.001	0.033	Y	Normal EEG → abnormal EEG; seizures	Learning disability, mild developmental delay, VIQ:71/PIQ:78	33
2	137	44	10650	<0.002	ND	ND	Y	normal EEG	Learning disability, mild developmental delay, VIQ:80/PIQ:87	18
3	142	20	16375	<0.002	ND	ND	Y	normal EEG	Learning disability, mild developmental delay, VIQ:72/PIQ:98	18
4	68	48	15975	<0.002	<0.001	0.922	N	normal EEG	Learning disability, VIQ:95/PIQ:132 community college student	19
5	95	41	11300	0.76	ND	ND	Y	abnormal EEG; Seizures	Special classes, low normal development, vocational school graduate	20
6	100	51	15200	0.73	ND	ND	Y	Normal EEG → abnormal EEG	Normal development, college graduate	25
7	137	68 CSF 163	25588	0.002	ND	ND	Y	Normal EEG → abnormal EEG; seizures	Normal development, IQ 125, college student	30
8	94	7	5467	0.77	0.024	1.384	Y	abnormal EEG → normal EEG	Normal development, community college student	24
9	150	36 CSF 176	17360	0.72	ND	1.344	Y	abnormal EEG → normal EEG	Normal development, college student	27

Patient	Ammonia [N: 80-110]	ASA plasma, [N: 0] 34 [N: 0] 34	ASA Urine, [N: 0] 7656 [N: 0] 7656	RBC AL, [N: 0] 10.47± [N: 0] 1.93	Fibroblast AL, [N: 0] 0.126± [N: 0] 0.045	$^{14}\text{C}/^3\text{H}$ incorp [N: 1.025 ±.334]	Age diet [N: 1.153 Y]	Neurological	Outcome	Follow- up, yrs
10	223			0.66	0.014	1.153	Y	normal EEG	Normal development, college graduate	31
11	112	27	5328	0.012	0.011	0.930	N	normal EEG, febrile seizures	Normal development, college student	20
12	107	<6	2667	1.03	ND	1.079	N	normal EEG	Normal development, average student	13
13	95	9	3640	1.39	0.029	0.762	N	no EEG performed	Normal development, "A" student jr. high school	13

ND = not done

Patients 2 & 3 are fraternal twins; Patients 5 & 6 are siblings

Table 2

Argininosuccinate lyase activity and ^{14}C -citrulline utilization in patients with different forms of argininosuccinate lyase deficiency

	RBC AL $\mu\text{m/h/mg Hgb}$	fibroblast AL $\mu\text{m/h/mg protein}$	$^{14}\text{C}/3\text{H}$ incorporation
NB Screening	<0.002–1.39 N=13	<0.001–0.029 N=8	0.033 N=1 0.762–1.384 N=7
Neonatal-onset	ND	<0.001–0.010 N=4	0.010–0.265 N=3
Late-onset	<0.002–0.81 N=4	<0.001–0.014 N=12	0.008–0.214 N=6
Control (Mean $\pm$ SD)	10.47 $\pm$ 1.93 N=13	0.126 $\pm$ 0.045 N=30	1.025 $\pm$ 0.334 N=34

ND = not done