Argininosuccinic Acid Lyase Deficiency Missed by Newborn Screen

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Abstract Argininosuccinic acid lyase (ASL) deficiency, caused by mutations in the *ASL* gene (OMIM: 608310) is a urea cycle disorder that has pleiotropic presentations. On the mild end, ASL deficiency can manifest as nonspecific neurocognitive abnormalities without readily identifiable signs to differentiate it from other causes of intellectual disability or learning disabilities. Dietary management and arginine supplementation, if initiated early, may ameliorate symptoms.

Because of the nonspecific nature of the symptoms and the possibility for therapeutic management, ASL deficiency is part of the recommended uniform screening panel for newborn screening in the USA. We report here a case of ASL deficiency that was missed on newborn screening in the USA.

The case reported here has two known pathogenic mutations – one with no residual activity and one with reported 10% residual activity. Review of this newborn screening results showed subtle elevation of citrulline, overlapping the normal range. These findings suggest that newborn screening may be missing other patients with ASL deficiency with at least one hypomorphic allele. This case was diagnosed incidentally, but in retrospect had

symptoms best attributed in full or in part to his ASA deficiency, including protein aversion, developmental delay, and seizures. This case highlights the importance of considering ASL deficiency in patients with nonspecific abnormal neurocognitive signs, such as epilepsy and developmental delay, even when newborn screening was normal.

Introduction

Argininosuccinic acid lyase (ASL) deficiency caused by mutations in the *ASL* gene is a potentially severe, but treatable inborn error of metabolism. There are two forms of ASL deficiency: the newborn form, which can present indistinguishably from proximal urea cycle disorders with neonatal hyperammonemia, and the late-onset form, which has less specific features (Tuchman et al. 2008; Erez et al. 2011; Kölker et al. 2015). The late-onset form of ASL deficiency presents with nonspecific intellectual disability or learning disability (Erez et al. 2011). In some patients, hepatitis and cirrhosis also occur, but these may not be present on initial presentation. Hyperammonemia may be intermittent or completely absent (Erez et al. 2011). This absence of specific features means that a high index of suspicion is required to make the diagnosis. Trichorhexis nodosa is a pathognomic finding of ASA Lyase deficiency but it requires a priori suspicion to detect and is only present in 50% of patients (Fichtel et al. (2007); Ficicioglu et al. 2009). Prior to the newborn screening era, the diagnosis of late-onset ASL deficiency was delayed by more than a year in some cases (Kölker et al. 2015).

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Making the diagnosis of ASL deficiency is critical, because long-term dietary protein restriction and arginine supplementation may improve cognitive outcome (Ficiciglu et al. 2009), although some cases that have been properly identified and treated still have complications, which is thought to be due to depletion of the synthetic pool used for NO and creatine generation. Therefore, because of the benefit in many cases from early initiation of therapy and the absence of specific features, ASL deficiency is on the Recommend Uniform Screening Panel for newborn screening in the USA (American College of Medical Genetics' Newborn Screening Expert Group, 2006).

Here we report a case of ASL deficiency that was missed by properly performed newborn screening in the USA; a pair of siblings with ASL deficiency have been reported to have been missed on newborn screening in Australia (Estrella et al. 2014). ASL deficiency was picked up incidentally on whole exome sequencing (WES) performed for this patient's comorbid dysmorphia. In retrospect, the reported patient had symptoms at least partially explained by his ASL deficiency, including protein aversion and seizures. This case raises the possibility that there may exist several other missed symptomatic cases, highlighting the importance of considering ASL deficiency in patients with nonsyndromic developmental delay, with normal newborn screening.

Clinical Case Report

We present a 26-month-old patient with a reportedly normal New Jersey newborn screen. He first came to attention of clinical genetics at age 26 months, when he was admitted for medically nonresponsive focal seizures. Review of the past medical history was notable for reflux, poor feeding, and lifelong short stature. The patient had an overall disinterest in food, but on open-ended questioning about the dietary history following diagnosis, parents volunteered that he seemed particularly averse to eating meat, and was noted to vomit after being fed meat on several occasions. Developmental history was notable for global developmental delay, with severe expressive language delay out of proportion to his other development. He had hyperactivity. Family history was unremarkable; he was the first child born to nonconsanguineous European and Armenian parents. His mother was pregnant at the time of consultation.

On examination, the patient was small: weight, height, and head circumference were less than the fifth percentile (weight 50th percentile for 10 months; height 50th percentile for 13 months; and head circumference 50th percentile for 11 months). He had dysmorphic features including an upswept frontal hairline, deep-set eyes, large

ears, a bulbous nasal tip, and mild retrognathia. These features led the dysmorphologist to suspect Floating Harbor Syndrome (FHS) (Nikkel et al. 2013); however, the seizures were worse than previously reported in FHS. Therefore, to expedite the workup because of ongoing maternal pregnancy, rapid WES was selected as the best diagnostic modality. WES confirmed the clinically suspected diagnosis of FHS with a *de novo* pathogenic nonsense *SRCAP* mutation (c.7330C > T; p.R2444X); however, additionally there were biallelic known pathogenic mutations in *ASL* (c.765dupG; p.M256DfsX79 and c.1135C > T; p.R379C), with confirmed biparental inheritance. The p.M256DfsX79 variant has been previously reported as a pathogenic variant in a patient with infantile-onset ASL deficiency (Balmer et al. 2014); the p.R379C has been previously reported as a pathogenic variant in a patient with late-onset ASL deficiency, and has been shown to have 10% residual enzyme activity (Engel et al. 2012; Hu et al. 2015).

Because of these genetic results, the patient's previous metabolic laboratory results were evaluated. The newborn screen had shown a very subtle elevation of citrulline, below both the presumptive and borderline cutoff labels established by the state. The New Jersey state newborn screening does not use ASA as a primary target. Based on the R4S post-analytical tool calculated using only citrulline (no – ASA tool), the case score (cumulative) was 2. His subsequent diagnostic plasma amino acids at ages 12 months (obtained for failure to thrive) and 26 months (obtained for epilepsy) as well as cerebrospinal fluid amino acids at age 26 months all showed subtle citrullinemia, whose diagnostic significance was not appreciated at the time due to the low level of the elevation and absence of measured ASA (Table 1). Plasma ammonia level and urine organic acids obtained at 26 months (for epilepsy) were normal. His plasma citrulline level increased dramatically following initiation of tube feeding through his gastrostomy tube at age 14 months (Table 1). The absence of detected ASA is explained because in the reference laboratory, co-elution of ethanolamine, and ASA impairs detection of mild elevations of ASA. Plasma amino acids sent following molecular diagnosis, including dedicated testing for ASA, was performed and showed striking elevation of ASA, confirming the diagnosis (Table 1). Following diagnosis, the patient was started on a low protein diet. Arginine supplementation was unable to be started because it was denied by insurance.

The patient continued to have refractory seizures, with hypsarrhythmia on EEG. He was nonresponsive to trials of multiple antiepileptic agents, including levetiracetam, topiramate, and oxcarbazepine. He had good clinical response to clobazam; however, he developed a severe allergic reaction, and it needed to be discontinued. An interdisciplinary team convened to discuss the risks and benefits of

Table 1 Patient's biochemical results

	Pre-diagnostic testing			Routine care			During steroid wean			
	12 months	26 month	26 months ^a	26 months	26 months	27 months	28 months ^b	29 months	29 months	29 months
Gln (303-837)	808.1	607	1029.2	671.9	620.1	706.4	593	843.8	895.9	678.1
Cit (7-47)	55.0	83.7	10	72.9	110.6	75.6	112	132.3	92.7	73.7
ASA (0)	ND ^c	ND ^c	ND ^c	ND ^c	60.5	ND ^c	57	56.3	51.0	45.2
Arg (15-120)	34.6	37.0	24.3	67.4	29.2	18.5	44	35.7	45.9	45.1
Orn (13-151)	36.9	32.3	2.9	39.9	19.6	17.5	19	28.1	39.2	17.6
Ammonia (9-33)		27	<9	<9	<9	18	13	24	11	26

Units in nmol/mL for all amino acids; units in μ mol/L for ammonia

ND not detected

^a Sample is from CSF. Norms are Gln 228-1008; cit 0-3

^b Sample was performed in alternate lab with reference ranges: gln 329-976; cit 11-45; ASA <2; Arg 31-132; Orn 22-97

^c In this reference lab, ASA coelutes with ethanolamine, so lack of detection is not sensitive

corticosteroid therapy to treat refractory hypsarrhythmia. The perceived risks were that as corticosteroids encourage a catabolic state, proteolysis might be induced, which could result in hyperammonemia; however, because the patient had refractory seizures, limited therapeutic options and had previously clinically responded to corticosteroids, it was determined that the risks outweighed the benefits and high-dose prednisone was initiated. Weekly laboratory studies, including plasma amino acids, were performed during steroid therapy. As expected, corticosteroids provoked increased citrullinemia, hyperglutaminemia, as well as an increase in ASA; however hyperammonemia did not develop (Table 1). Although corticosteroids resulted in fewer clinical seizures and normalization of his EEG, he had a regression in his expressive sign language during steroid therapy. Additionally, clinical seizures and hypsarrhythmia returned following his steroid course. He subsequently has failed to respond to many additional antiepileptic drugs (lacosamide, vigabatrin, and clorazepate). The possibility of ketogenic diet is currently being considered.

The patient did make developmental progress following transition to a low protein diet; however, several medical changes such as changes in his antiepileptic medication were attempted at the same time, so the direct benefits of treatment for ASL deficiency are unclear.

Methods

For all standard runs, amino acids were analyzed using ultra-performance liquid chromatography, as described previously. A standard column temperature of 43° C was used, at which temperature ASA can only be resolved from

ethanolamine when present in high quantities. For the dedicated urea cycle run performed at 28 months of age, liquid chromatography and tandem mass spectrometry were used.

Discussion

Here we present a case of a patient with molecularly diagnosed and biochemically confirmed ASL deficiency who was diagnosed incidentally by WES, sent to diagnose his suspected FHS. Although this patient had biochemical testing prior to diagnosis, his amino acid elevations were too subtle to provoke metabolic referral. This case is the first report of ASL deficiency missed on newborn screen in the USA and this is the second reported family to have ASL deficiency missed on newborn screening (Estrella et al. 2014). In this case, it is difficult to determine the specific contribution of ASL deficiency to the phenotype because of his comorbid FHS; however we speculate that his seizures may be related because they are highly unusual for FHS, both in their semiology (infantile spasms) and in their severity (Table 2). His regression while on corticosteroid therapy is also likely attributable to his ASL deficiency, which has important implications for the considerations of corticosteroid therapy for idiopathic infancy. Similarly, his vomiting may be related because it seemed to improve with transition to a low protein diet and the family voluntarily reported protein aversion. It is possible that his seizures and development would further improve if arginine therapy could be initiated; however, abnormal EEG and neurocognitive features such as intellectual disability have been reported even in patients diagnosed and treated appropriately from birth (Ficicioglu et al. 2009; Mercimek-Mahmu-

Table 2 Patient's phenotypic features

Sign	Patient	ASL deficiency ^a	FHS ^b
Seizures	Medically intractable infantile spasms	32%; no infantile spasms; highly responsive to medical therapy	11% (Nikkel et al. 2013)
Intellectual disability	Moderate intellectual disability, with severe expressive language delay	70%; usually mild-mod; verbal IQ more affected	Common mild-mod. Predominant speech and language involvement
Short stature	Height 50th %ile for 13 months at age 26 months	Not reported	95% below the 5th %ile for height
Reflux	Severe, requires gastrostomy tube	Not reported	25%; severe, may require gastrostomy tube
Protein aversion	Vomiting provoked by proteins	Associated, but not quantified	Not reported
Dysmorphic facies	Deep-set eyes, large ears, bulbous nasal tip, and retrognathia	Not reported	Universal deep-set eyes, large, low-set ears, and broad nasal tip

^a Tuchman et al. (2008), Ficicioglu et al. (2009)

^b Nikkel et al. (2013)

toglu et al. 2010). This is thought to be due to the duality of arginine pools in the cell; arginine used for ureagenesis seems to be distinct from arginine for NO donation and creatine synthesis. Critically, arginine supplementation does not seem to replete the biosynthetic arginine pool (Erez et al. 2011).

This case may represent an unappreciated population of ASL that is missed on newborn screen, since the clinical presentation of ASL deficiency may be quite mild or late onset (Ficicioglu et al. 2009). Although it is difficult to say conclusively how clinically significant his ASL deficiency is because of his comorbid FHS, we can speculate based on his genotype. Our patient had one mutation, p.R379C, associated with the late-onset form of ASL deficiency and known to have 10% residual enzyme activity, and when homozygous results in a phenotype of tremor and mild intellectual disability (Kleijer et al. 2002); his second mutation was a null mutation, p. M256DfsX79, which in the homozygous state causes “severe” disease (Balmer et al. 2014). Together, along with the aspects of the patient's phenotype not explained by FHS that were explicable by ASL deficiency (Table 2), these findings suggest that this patient is clinically affected by ASL deficiency. It is important to note that even patients with biallelic mutations associated with late-onset disease, such as p.R379C, are at risk for clinical consequences from ASL deficiency and may be even less likely than the reported patient to be detected on newborn screening.

For all newborn screening conditions, the possibility of false negative newborn screen exists, due to the nature of newborn screening as a large-scale screening test. Therefore, consideration must be given to both optimization of the newborn screen and to the clinical consideration of disorders of intermediary metabolism in patients with a negative newborn screen. In previous discussion of false negative newborn screening, it has been proposed that ratios be used

to help improve the detection (Hall et al. 2014). In this case, retrospective use of the R4S CLIR system did show an increased index of suspicion that the newborn screening values were abnormal, and the case score was 2 that indicates possible ASA lyase deficiency. This case highlights the importance of clinicians eliciting a dietary history and other clinical features that may suggest an inborn error of metabolism, and having a low threshold to pursue diagnostic laboratory testing, especially for treatable metabolic disease.

In conclusion, newborn screening using citrulline as a primary marker may not be able to detect all cases of ASL deficiency. Including ASA as a primary target for newborn screening may increase the sensitivity of newborn screening for this condition. Although we cannot clearly establish the contribution of ASL deficiency to this patient's phenotype, this case speaks more generally to the importance of considering ASL deficiency in patients presenting with intellectual disability, epilepsy, liver dysfunction, and/or citrullinemia even in the face of normal newborn screening. ASL deficiency is a potentially treatable cause of non-syndromic intellectual disability, epilepsy, and late-onset liver disease, therefore this case highlights how critical it is for clinicians to be aware that a normal newborn screen does not rule out ASL deficiency.

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Details of Ethics Approval

Ethics approval was not required for this study.

Conflicts of Interest

None of the authors have any conflicts of interest to disclose.

Synopsis

Argininosuccinic acid lyase deficiency is an important consideration in a patient with nonsyndromic intellectual disability and can be missed by newborn screening.

Details of the Contributions of Individual Authors

RDG performed clinical and biochemical evaluation of the patient, conceived, and wrote the manuscript. EB and MAD performed clinical evaluation of the patient and were responsible for genetic testing. CF conceived the manuscript, provided oversight, and served as the guarantor for the article.

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