Supplemental Materials for Arginase 1 Deficiency (ARG1-D) Petition

- Letter from Petitioner
- Newborn Screening (NBS) Dr. Angela Sun, Criteria Questions and Answers
    - Responses from Dr. Angela Sun, Biochemical Geneticist at Seattle Children's Hospital, regarding ARG1-D and the Washington State Board of Health's (WSBOH) newborn screening criteria
- ARG-1D Gene Review, Dr. Angela Sun
    - National Institutes of Health (NIH), National Library of Medicine, National Center for Biotechnology Information (NCBI) Bookshelf Gene Review. Sun A, Crombez EA, Wong D. Arginase Deficiency. 2004 Oct 21 [Updated 2020 May 28]. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1159/
- ARG1-D Publications
    - Crombez, E. A., & Cederbaum, S. D. (2005). Hyperargininemia due to liver arginase deficiency. Molecular genetics and metabolism, 84(3), 243-251.
    - Diaz, G. A., Bechter, M., & Cederbaum, S. D. (2023). The role and control of arginine levels in arginase 1 deficiency. Journal of Inherited Metabolic Disease, 46(1), 3-14.
    - Huang, Y., Sharma, R., Feigenbaum, A., Lee, C., Sahai, I., Russo, R. S., ... & Salazar, D. (2021). Arginine to ornithine ratio as a diagnostic marker in patients with positive newborn screening for hyperargininemia. Molecular genetics and metabolism reports, 27, 100735.
    - Therrell, B. L., Currier, R., Lapidus, D., Grimm, M., & Cederbaum, S. D. (2017). Newborn screening for hyperargininemia due to arginase 1 deficiency. Molecular Genetics and Metabolism, 121(4), 308-313.
- Family Testimonies
    - Tanja and Willow's Story
    - Jackson's Story
    - Brandy and Landon's Story
    - Alex and Josh's Story
    - Angela and Isaiah's Story
    - Vanessa and Brie's Story
    - Lincoln's Story

March 31, 2023

Letter from Christine Zahn in support of testing for Arginase 1 Deficiency at newborn screening in Washington State.

My name is Christine Zahn. I am the Director of the Arginase 1 Deficiency Foundation. I am also the grandmother to Willow, an 11-year-old girl diagnosed at the age of 4 years, 11 months with the very rare genetic disorder of Argininemia also known as Arginase 1 Deficiency (ARG1D). It was not until Willow was 3 years of age that she started showing symptoms of spasticity, seizures, and failure to thrive. My perfectly beautiful granddaughter was no longer growing or gaining weight. She could not walk without stumbling and falling. She started having numerous seizures, often 30-50 in a day.

Had Willow, through the very simple heel prick blood test at newborn screening (NBS) shown elevated arginine we would have been alerted that there was potentially something wrong. Instead, Willow's arginine levels rose steadily through her early years of life crossing the brain barrier and injuring her brain. This lack of diagnosis at an early age has left Willow and her care-taking family with lifelong medical issues, medical bills and special needs at a very high cost both emotionally and financially. It's hard to not think about the difference an early diagnosis would have made in Willow's life.

While this may be a very rare disorder, it's a bit surprising how many families we have met that share a similar story with a late diagnosis. Many of the families we know that were diagnosed as toddlers or even later in life will have lifelong medical issues. Sadly, some of the families with late diagnosis are very medically complex needing around the clock care, ports for feeding tubes and medication and many have lost their ability to walk. I am sure there are probably even more children that are misdiagnosed or not diagnosed at all.

Included with our Petition and support from Dr. Sun and Dr. Cedarbaum are stories from many of our families. Each of these families felt it was important to share what living with an ultrarare metabolic disorder is like. As a group of people and a foundation we know the importance of newborn screening and the impact it has made on our loved ones. We ask that Washington State, and all States require testing for Arginase 1 Deficiency for all newborns.

A few facts about Arginase 1 Deficiency and newborn screening:

- Recommended Uniform Screening Panel (RUSP) already lists Arginase 1 Deficiency/Argininemia as an approved secondary NBS.
- Only 17 States including Washington State do not test for Arginase 1 Deficiency - 33 States test for Arginase 1 Deficiency.
- Interestingly Washington State runs the NBS for Idaho which includes testing for Arginase 1 Deficiency.
- Arginase 1 Deficiency rarely has an acute onset so that there is more than sufficient time to institute therapy before any symptoms appear.

The following comments regarding WSBOH criteria for testing are provided by Angela Sun, MD. (Biochemical Genetics, Genetics, Non-Malignant Transplant Program)
On staff at Seattle Children's since August 2012.

Criteria for Testing

Available Screening Technology.
The technology is tandem mass spectrometry, which measures amino acids in newborn dried blood spots. Tandem MS is already being used for NBS conditions such as PKU and MSUD (maple syrup urine disease), so this is easy. Adding arginase deficiency would not require developing, validating, and implementing a new lab test.

Diagnostic Testing and Treatment available.
Biochemical and genetic testing are widely available to confirm the diagnosis. Treatment includes a protein restricted diet, supplementation of essential amino acids with medical formulas, and in some cases, ammonia scavenging medications. In the future, it is possible that enzyme replacement therapy will be available.

Prevention Potential and Medical Rationale - The newborn identification of the condition allows early diagnosis and intervention:

Important considerations:
There is sufficient time between birth and onset of irreversible harm to allow for diagnosis and intervention.

- The symptoms of arginase deficiency appear gradually in the first few years of life. Unlike other urea cycle disorders, infants with arginase deficiency do not present in the first few days of life with catastrophic hyperammonemia, though they can have episodes of hyperammonemia later in life. Therefore, there is ample time between birth and the onset of symptoms to allow for diagnosis and initiation of treatment.

The benefits of detecting and treating early onset forms of the condition (within 1 year of life) balance the impact of detecting late onset forms of the condition.

- There is a spectrum of clinical severity in arginase deficiency. Regardless of severity, most affected individuals have onset of symptoms in the first few years of life. Unlike other conditions on the NBS such as X-linked adrenoleukodystrophy and Pompe disease where patients may not have symptoms until the 4th or 5th decade of life, arginase deficiency does not have this long, asymptomatic period. Furthermore, all patients are started on treatment immediately after diagnosis, whereas those with Pompe disease may not require treatment for decades.

Newborn screening is not appropriate for a condition that only presents in adulthood.

- See above. This is a moot point. That being said, whenever we start NBS for a disease, we end up finding the really mild patients that in the past never came to clinical presentation. We could look into the experience of other states that are already screening for arginase deficiency.

Public Health Rationale: Nature of the condition justifies population based screening rather than risk-based screening of other approaches.

- Arginase deficiency is underdiagnosed as the symptoms can be nonspecific (toe walking, seizures, developmental delay). It is often misdiagnosed as cerebral palsy. Because it is a rare disease, some patients may carry this misdiagnosis their entire life. Only those with access to specialists receive further work-up. Importantly, patient outcomes are improved when treatment is started earlier in life, but the treatment cannot be initiated if the correct diagnosis is not made. Therefore, population-based screening with a cost effective, sensitive and specific laboratory assay is a better diagnostic approach for this disorder.

Cost-benefit/Cost-effectiveness: The outcome outweighs the costs of screening. All outcomes, both positive and negative, need to be considered in the analysis. Important considerations to be included in economic analyses include:

- Cost benefit analysis is complicated. I don't think this would add much cost to the NBS since we are already doing tandem MS. Dr. Cederbaum's paper has some more info here.

Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2023.

Arginase Deficiency

Synonyms: ARG1 Deficiency, Arginase-1 Deficiency, Hyperargininemia

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Summary

Clinical characteristics. Arginase deficiency in untreated individuals is characterized by episodic hyperammonemia of variable degree that is infrequently severe enough to be life threatening or to cause death. Most commonly, birth and early childhood are normal. Untreated individuals have slowing of linear growth at age one to three years, followed by development of spasticity, plateauing of cognitive development, and subsequent loss of developmental milestones. If untreated, arginase deficiency usually progresses to severe spasticity, loss of ambulation, complete loss of bowel and bladder control, and severe intellectual disability. Seizures are common and are usually controlled easily. Individuals treated from birth, either as a result of newborn screening or having an affected older sib, appear to have minimal symptoms.

Diagnosis/testing. The diagnosis of arginase deficiency is established in a proband with suggestive clinical and/or biochemical findings and confirmed by identification of biallelic pathogenic variants in *ARG1* or, in limited instances, by failure to detect arginase enzyme activity (usually <1% of normal) in red blood cell extracts.

Management. *Treatment of manifestations:* Management should closely mirror that for urea cycle disorders, except that individuals with arginase deficiency are not as likely to have episodes of hyperammonemia; if present, such episodes respond to conservative management (e.g., intravenous fluid administration). Treatment should involve a team coordinated by a metabolic specialist. Routine outpatient management includes restriction of dietary protein and consideration of oral nitrogen-scavenging drugs (in those who have chronic or recurrent hyperammonemia). Treatment of an acutely ill (comatose and encephalopathic) individual requires: rapid reduction of plasma ammonia concentration; use of pharmacologic agents (sodium benzoate and/or sodium phenylbutyrate/phenylacetate) to promote excretion of excess nitrogen through alternative pathways; and introduction of calories supplied by carbohydrates and fat to reduc catabolism and the amount of excess nitrogen in the diet while avoiding overhydration and resulting cerebral edema. Standard treatment for seizures, spasticity, developmental delay / intellectual disability, and joint contractures. In thos with persistent hepatic synthetic function abnormalities, fresh-frozen plasma should be considered prior to surgical procedures. In the rare instance of progression to hepatic fibrosis and cirrhosis, liver transplantation can be considered.

Prevention of primary manifestations: Maintenance of plasma arginine concentration as near normal as possible through restriction of dietary protein and use of oral nitrogen-scavenging drugs as necessary to treat hyperammonemia. Liver transplantation eliminates hyperargininemia and presumably the risk for hyperammonemia but is rarely necessary in arginase deficiency.

Surveillance: Regular follow up at intervals determined by age and degree of metabolic stability. Assessment of metabolic control (plasma ammonia, amino acid profile, and nutritional labs) at least monthly for the first year of life and as determined by a metabolic specialist after the first year of life; guanidinoacetate and liver function tests every six to 12 months; monitoring of growth and developmental progress at each visit.

Agents/circumstances to avoid: Valproic acid (exacerbates hyperammonemia).

Evaluation of relatives at risk: Plasma quantitative amino acid analysis, molecular genetic testing (if the family-specific pathogenic variants are known), or enzymatic testing in all sibs (especially younger ones) of a proband to allow early diagnosis and treatment of those found to be affected.

Pregnancy management: In general, affected pregnant women should continue dietary protein restriction and ammonia-scavenging medications (after an appropriate benefit/risk calculation) based on their clinical course before pregnancy.

Other: Immunizations on the usual schedule; appropriate use of antipyretics as indicated (ibuprofen preferred over acetaminophen).

Genetic counseling. Arginase deficiency is inherited in an autosomal recessive manner. At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier. Heterozygotes (carriers) are asymptomatic. Carrier testing for at-risk relatives and prenatal testing for pregnancies at increased risk are possible if the *ARG1* pathogenic variants in the family are known.

Diagnosis

Suggestive Findings

Scenario 1. Abnormal newborn screening (NBS) result

- NBS for arginase deficiency is primarily based on quantification of the analyte arginine on dried blood spots.
- Arginine values above the cutoff reported by the screening laboratory are considered positive and require follow-up biochemical testing (see **Preliminary laboratory findings** below).
- If these studies support the diagnosis of arginase deficiency, additional testing is required to establish the diagnosis (see Establishing the Diagnosis).

Note: (1) Some infants with arginase deficiency may have follow-up arginine levels in the normal range, and thus infants who continue to have elevated arginine-to-ornithine ratios and arginine toward the upper limit of normal should undergo additional diagnostic testing (see Establishing the Diagnosis) [Author, personal observation]. (2) Arginase deficiency is currently a secondary condition on the Recommended Uniform Screening Panel. Thus, not all states will screen for and detect newborns with arginase deficiency.

Scenario 2. Symptomatic individual with either atypical findings or untreated arginase deficiency resulting from any of the following:

- NBS not performed

- False negative NBS result
- Caregivers not compliant with recommended treatment following a positive NBS result

Supportive but nonspecific clinical findings and preliminary laboratory findings can include the following.

Clinical findings

- Slowing of linear growth at age one to three years
- Development of spasticity in the lower extremities
- Plateauing of cognitive development
- Loss of developmental milestones
- Seizures

Preliminary laboratory findings

- **Plasma quantitative amino acid analysis.** Elevation of plasma arginine concentration three- to fourfold the upper limit of normal is highly suggestive of the diagnosis. Plasma arginine elevation is the primary means of ascertainment.

  Note: Up to twofold the upper limit of normal may be seen in infants who do not have arginase deficiency and who are otherwise normal.
- **Plasma ammonia concentration.** Elevation of plasma ammonia concentration may be intermittent. Acute hyperammonemia (plasma ammonia concentration >150 µmol/L) is uncommon.
- **Urinary orotic acid concentration.** Although urinary orotic acid concentration is often elevated, it is not a primary screen for this disorder.

  Note: Because elevations of these metabolites individually are not entirely specific to arginase deficiency, follow-up testing is required to establish or rule out the diagnosis of arginase deficiency (see Establishing the Diagnosis).

Establishing the Diagnosis

The diagnosis of arginase deficiency **is established** in a proband with suggestive clinical and/or biochemical findings and confirmed by identification of biallelic pathogenic variants in *ARG1* (see Table 1) or, in limited instances, by failure to detect arginase enzyme activity (usually <1% of normal) in red blood cell extracts. Because of its relatively high sensitivity, *ARG1* molecular genetic testing is the preferred confirmatory test for arginase deficiency.

Note: Enzyme assay can be helpful if two pathogenic variants are not found on molecular genetic testing.

Molecular Genetic Testing Approaches

Scenario 1. Abnormal newborn screening (NBS) result. When NBS results and other laboratory findings suggest the diagnosis of arginase deficiency, molecular genetic testing approaches can include **single-gene testing** or use of a **multigene panel**:

- **Single-gene testing.** Sequence analysis of *ARG1* detects small intragenic deletions/insertions and missense, nonsense, and splice site variants; depending on the method used, exon or whole-gene deletions/duplications may not be detected. Perform sequence analysis first. If only one or no pathogenic variant is found, perform gene-targeted deletion/duplication analysis to detect intragenic deletions or duplications.

  Note: In individuals of French Canadian ancestry, the c.57+1G>A founder variant may be tested for first.

- **A multigene panel** that includes *ARG1* and other genes of interest (see Differential Diagnosis) is most likely to identify the genetic cause of the condition while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype. Note: (1) The genes included in the panel and the diagnostic sensitivity of the testing used for each gene vary by laboratory and are likely to change over time. (2) Some multigene panels may include genes not associated with the condition discussed in this *GeneReview*. (3) In some laboratories, panel options may include a custom laboratory-designed panel and/or custom phenotype-focused exome analysis that includes genes specified by the clinician. (4) Methods used in a panel may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests.

  For an introduction to multigene panels click here. More detailed information for clinicians ordering genetic tests can be found here.

Scenario 2. Symptomatic individual with atypical findings or untreated arginase deficiency (resulting from NBS not performed or false negative NBS result):

- If arginase deficiency is suspected, single-gene testing or a multigene panel may be performed (see Scenario 1).
- When the diagnosis of arginase deficiency has not been considered, **comprehensive genomic testing** (which does not require the clinician to determine which gene[s] are likely involved) is an option. **Exome sequencing** is most commonly used; **genome sequencing** is also possible.

  For an introduction to comprehensive genomic testing click here. More detailed information for clinicians ordering genomic testing can be found here.

Table 1.

Molecular Genetic Testing Used in Arginase Deficiency

Gene [1]	Method	Proportion of Pathogenic Variants [2] Detectable by Method
ARG1	Sequence analysis [3]	>98% [4]
	Gene-targeted deletion/duplication analysis [5]	<2% [4, 6]

1. See Table A. Genes and Databases for chromosome locus and protein.
2. See Molecular Genetics for information on allelic variants detected in this gene.
3. Sequence analysis detects variants that are benign, likely benign, of uncertain significance, likely pathogenic, or pathogenic. Variants may include small intragenic deletions/insertions and missense, nonsense, and splice site variants; typically, exon or whole-gene deletions/duplications are not detected. For issues to consider in interpretation of sequence analysis results, click here.
4. Diez-Fernandez et al [2018]; data derived from Human Gene Mutation Database [Stenson et al 2020]
5. Gene-targeted deletion/duplication analysis detects intragenic deletions or duplications. Methods used may include a range of techniques such as quantitative PCR, long-range PCR, multiplex ligation-dependent probe amplification (MLPA), and a gene-targeted microarray designed to detect single-exon deletions or duplications.
6. Three single or multiexon deletions have been reported [Korman et al 2004, Wang et al 2012, Diez-Fernandez et al 2018].

Measurement of Red Blood Cell Arginase Enzyme Activity

Most affected individuals have no detectable arginase enzyme activity (usually <1% of normal) in red blood cell extracts.

Note: (1) Although arginase is stable, a control sample should be obtained and treated identically if the cells are to be shipped to a distant site. (2) Liver and red blood cell arginase activity correlate well; therefore, it is not necessary to perform a liver biopsy when enzyme activity can be measured from a blood sample.

Clinical Characteristics

Clinical Description

To date, more than 260 individuals with arginase deficiency have been identified [Uchino et al 1995; De Deyn et al 1997; Crombez & Cederbaum 2005; Schlune et al 2015; Huemer et al 2016; Therrell et al 2017; Diez-Fernandez et al 2018; Chandra et al 2019; Author, personal observation]. The following description of the phenotypic features associated with this condition is based primarily on individuals with severe disease. It should be noted that a phenotypic spectrum exists, and mildly affected individuals exhibit less severe features. Individuals treated from birth (as a result either of newborn screening or of having an affected older sib) appear to have minimal symptoms [Cederbaum et al 2004].

Growth and feeding. Most commonly, growth at birth and through early childhood is normal.

- At age one to three years, linear growth slows and eventually the majority of affected children demonstrate growth deficiency, which persists if arginase deficiency goes untreated.
- Microcephaly is common and is congenital in some cases.
- Feeding issues may develop, leading to inadequate nutrition. Some require a supplemental feeding tube.

Cognitive development. Initially, cognitive development in infancy and early childhood is normal.

- Starting at age one to three years, previously normal cognitive development slows or stops and the child begins to lose developmental milestones.
- If untreated, arginase deficiency usually progresses to severe intellectual disability with accompanying neurologic findings (see **Neurologic features** below).
- Full scale IQ in adults is in the 70s, and about half are able to live independently, though they experience significant memory and fine motor deficits [Waisbren et al 2016]. Mildly affected individuals and those treated early in life may be able to hold a job.
- Some children are more severely affected cognitively, whereas others have more severe spasticity and secondary joint contractures.

Neurologic features. In untreated individuals, progressive neurologic signs typically include the development of severe spasticity with loss of ambulation and complete loss of bowel and bladder control.

- **Spasticity.** Between 80% and 90% of affected individuals develop spasticity of the lower extremities [Huemer et al 2016, Chandra et al 2019].
  - Spastic diplegia typically appears between ages two and four years and is often misdiagnosed as cerebral palsy.
  - Severe spasticity can lead to joint contractures and lordosis.
- **Seizures** occur in 60%-75% of affected individuals and are usually controlled easily by anti-seizure medication [Huemer et al 2016, Chandra et al 2019]. Generalized tonic-clonic seizures are the most common seizure type.
- **Brain imaging** often reveals cortical atrophy. Other parts of the nervous system including basal ganglia, cerebellum, medulla, and spinal cord are largely spared [De Deyn et al 1997].

Hyperammonemia. Unlike the other eight primary urea cycle disorders (see Urea Cycle Disorders Overview), arginase deficiency rarely results in elevated plasma ammonia concentration in the newborn period.

- Episodic hyperammonemia of variable degree may occur during illness but is rarely severe enough to be life threatening, although death has been reported.
- Hyperammonemia presents with vomiting, lethargy, and altered mental status but in some cases is asymptomatic and only recognized if blood ammonia is obtained during an acute illness.
- Older individuals may present with postoperative encephalopathy.

Liver disease. Hepatic dysfunction, if present, is usually mild, manifesting as transaminitis, prolonged coagulation time, and in some cases hepatomegaly. Affected individuals typically do not have bleeding problems from prolonged coagulation time. Rarely, neonatal cholestatic jaundice has been reported [Braga et al 1997, Gomes Martins et al 2010], and cirrhosis can occur. Some adults have developed hepatocellular carcinoma.

Other. Some affected females experience symptomatic hyperammonemia during menstrual cycles. These individuals may require abortive therapy (see Management, Prevention of Primary Manifestations).

Prognosis. While data are not available, the vast majority of affected individuals appear to survive and live long (albeit handicapped) lives.

Genotype-Phenotype Correlations

Genotype-phenotype correlations indicate that the amount of residual enzyme activity modulates the phenotype [Diez-Fernandez et al 2018]. Severe disease is associated with:

- Homozygosity or compound heterozygosity for predicted loss-of-function variants such as c.466-2A>G, c.77delA, c.263_266delAGAA, and c.647_648ins32;
- Missense changes such as p.Ile8Lys or p.Gly106Arg when homozygous or in combination with another severe allele.

Prevalence

Arginase deficiency is one of the rarest urea cycle defects. Its incidence has been estimated at between 1:350,000 and 1:1,000,000; the true incidence in nonrelated populations is unknown.

Arginase deficiency is pan ethnic but may be more common among French Canadians due to a pathogenic founder variant [Uchino et al 1995] (see Table 9).

Genetically Related (Allelic) Disorders

No phenotypes other than those discussed in this *GeneReview* are known to be associated with germline pathogenic variants in *ARG1*.

Differential Diagnosis

Hyperammonemia. Arginase is the sixth and final enzyme of the eight known steps in the urea cycle. See Urea Cycle Disorders Overview for approaches to distinguish:

- Other causes of hyperammonemia from a urea cycle disorder; and
- The differences between the urea cycle disorders themselves.

Spasticity. Arginase deficiency may be misdiagnosed as static spastic diplegia (cerebral palsy). See Hereditary Spastic Paraplegia Overview. It should be noted that arginase deficiency is one of the few treatable causes of spastic diplegia [Prasad et al 1997].

***ARG2*.** A second arginase gene is known (*ARG2*), but no human deficiency state has been identified and it is not clear that elevated plasma arginine would be a part of such a deficiency.

CAT-2. A new metabolic disorder in the human cationic amino acid transporter-2 has been proposed. The biochemical profile includes high levels of arginine, ornithine, and lysine in both blood and urine. The one described affected individual presented with an abnormal newborn screen for arginase deficiency [Yahyaoui et al 2019].

Management

No consensus clinical management guidelines for arginase deficiency have been published. However, general guidelines for the management of urea cycle disorders are available [Häberle et al 2019].

Evaluations Following Initial Diagnosis

To establish the extent of disease and needs in an individual diagnosed with arginase deficiency, the following evaluations summarized in Table 2 (if not performed as part of the evaluation that led to the diagnosis) are recommended.

Table 2.

Recommended Evaluations Following Initial Diagnosis in Individuals with Arginase Deficiency

Evaluation	Comment
Obtain plasma ammonia, amino acid profile, guanidinoacetate, & liver function tests. [1]	Consultation w/metabolic physician / biochemical geneticist
Gastroenterology / nutrition / feeding team eval	• To incl eval of aspiration risk & nutritional status • Consultation w/metabolic dietitian • Consider eval for gastric tube placement in those unable to meet nutritional needs orally.
Developmental assessment	Consider referral to developmental pediatrician.
Neurologic eval	Consider referral to neurologist if spasticity is present or seizures are suspected.
Musculoskeletal eval	To assess for secondary joint contractures & lordosis. Consider referral to rehabilitation medicine.

1. Albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, prothrombin time (PT), and partial thromboplastin time (PTT).

Treatment of Manifestations

The management of individuals with arginase deficiency should closely mirror that described in the Urea Cycle Disorders Overview, with one caveat: individuals with arginase deficiency are less prone to episodes of

hyperammonemia and when present, hyperammonemia is more likely to respond to conservative management such as intravenous fluid administration. However, the individual who is comatose and encephalopathic is at high risk for severe brain damage and should be treated accordingly. Arginine supplementation is obviously contraindicated.

Table 3.

Routine Outpatient Management in Individuals with Arginase Deficiency

Principle	Treatment	Consideration/Other
Restriction of dietary protein [1]	• At least half of dietary protein from natural (complete) sources • Supplementation w/arginine-free essential amino acid formula	• Protein requirement varies by age. Ideally, affected person should be on the minimum protein intake needed to maintain protein biosynthetic function, growth, & normal plasma amino acid concentrations. • Dietary modification does not lead to normalization of plasma arginine concentration but does cause improvement of some clinical symptoms.
Administration of oral nitrogen-scavenging drugs	Sodium benzoate • 250 mg/kg/day Sodium phenylbutyrate • ≤250 mg/kg/day if <20 kg • 5 g/m^2/day if >20 kg	• Medications to be taken in = amts w/each meal or feeding (i.e., 3-4x/day) [2] • Not all affected individuals require nitrogen scavengers. Use only for chronic or recurrent hyperammonemia.

1. The goal should be maintenance of plasma arginine concentration as near normal as possible.
2. Häberle et al [2019], Urea Cycle Disorders Consortium

Table 4.

Acute Outpatient Management in Individuals with Arginase Deficiency

Manifestation/Concern	Treatment	Consideration/Other
Mildly ↑ catabolism [1]	• Carbohydrate supplementation orally or by feeding tube • ↓ natural protein intake [2]	Trial of outpatient treatment at home for 12-48 hrs w/assessments for clinical changes [3]
Fever	Administration of antipyretics (acetaminophen, ibuprofen) if temperatures rises >38.5°C	
Occasional vomiting	Antiemetics	

1. Fever; vomiting, diarrhea, dehydration
2.

Some centers advocate reducing natural protein intake to zero or to 50% of the normal prescribed regimen for short periods (24-48 hours) in the outpatient setting during intercurrent illness.

3. Alterations in mentation/alertness, fever, and enteral feeding tolerance with any new or evolving clinical features should be discussed with the designated center of expertise for inherited metabolic diseases.

Table 5.

Acute Inpatient Management in Individuals with Arginase Deficiency

Manifestation/Concern	Treatment	Consideration/Other
Hyperammonemia (mild to moderate)	Increase caloric intake: • IV fluids w/≥10% dextrose at 1-1.5x maintenance rate [1] • Protein-free oral formula, e.g., Mead Johnson PFD or Ross Formula ProPhree®	Complete restriction of protein should not exceed 24-48 hrs, as depletion of essential amino acids may result in endogenous protein catabolism & nitrogen release. Transition patients from parenteral to enteral feeds as soon as possible.
Hyperammonemia (severe)	Same as above, plus nitrogen scavengers: • Enteral: Sodium benzoate, sodium phenylbutyrate, or glycerol phenylbutyrate • IV: Sodium phenylacetate & sodium benzoate (Ammonul®)	
	Consider intralipids for additional calories or TPN if affected person is unable to tolerate enteral feeds for > few days.	If affected person is unable to hydrate orally, consider placement of NG tube. Avoid overhydration, which can result in cerebral edema. [2]
	Dialysis [3]	It is rare for persons w/arginase deficiency to require dialysis. The ammonia level & clinical status determine need for dialysis.

TPN = total parenteral nutrition

1. High parenteral glucose plus insulin can be used acutely to diminish catabolism.
2. The duration of cerebral edema correlates with poor neurologic outcome.
3. Treatment of choice to most rapidly decrease serum ammonia concentration. The method employed depends on the affected person's circumstances.

Table 6.

Management of Other Complications in Individuals with Arginase Deficiency

Manifestation/Concern	Treatment	Consideration/Other
Seizures	Standard ASM depending on seizure type [1]	Referral to neurologist
Spasticity	Consider a trial of Botox®.	
	Orthotics, walkers, wheelchairs, & other durable medical equipment	Referral to rehabilitation medicine
Persistent hepatic synthetic function abnormalities [2]	• In most cases, only clinical monitoring is necessary. • W/more severe coagulopathy, FFP is administered prior to surgical procedures.	Referral to hematologist for severe cases
Hepatic fibrosis & cirrhosis	Liver transplantation	This is a rare complication.
Joint contractures	• Physical therapy • Tendon release procedures	Referral to orthopedist if severe

ASM = anti-seizure medication; FFP = fresh-frozen plasma

1. Valproic acid should be avoided (see Agents/Circumstances to Avoid).
2. Particularly elevated prothrombin time

The following information represents typical management recommendations for individuals with developmental delay / intellectual disability in the United States; standard recommendations may vary from country to country.

Developmental Disability / Intellectual Disability Management Issues

Ages 0-3 years. Referral to an early intervention program is recommended for access to occupational, physical, speech, and feeding therapy. In the United States, early intervention is a federally funded program available in all states.

Ages 3-5 years. In the United States, developmental preschool through the local public school district is recommended. Before placement, an evaluation is made to determine needed services and therapies and an individualized education plan (IEP) is developed.

Ages 5-21 years

- In the US, an IEP based on the individual's level of function should be developed by the local public school district. Affected children are permitted to remain in the public school district until age 21.
- Discussion about transition plans including financial, vocation/employment, and medical arrangements should begin at age 12 years. Developmental pediatricians can provide assistance with transition to adulthood.

All ages. Consultation with a developmental pediatrician is recommended to ensure the involvement of appropriate community, state, and educational agencies (US) and to support parents in maximizing quality of life. Some issues to consider:

- Individualized education plan (IEP) services:
  - An IEP provides specially designed instruction and related services to children who qualify.
  - IEP services will be reviewed annually to determine if any changes are needed.

- Special education law requires that children participating in an IEP be in the least restrictive environment feasible at school and included in general education as much as possible, when and where appropriate.
- PT, OT, and speech services will be provided in the IEP to the extent that the need affects the child's access to academic material. Beyond that, private supportive therapies based on the affected individual's needs may be considered. Specific recommendations regarding type of therapy can be made by a developmental pediatrician.
- As a child enters teen years, a transition plan should be discussed and incorporated in the IEP. For those receiving IEP services, the public school district is required to provide services until age 21.

- A 504 plan (Section 504: a US federal statute that prohibits discrimination based on disability) can be considered for those who require accommodations or modifications such as front-of-class seating, assistive technology devices, classroom scribes, extra time between classes, modified assignments, and enlarged text.
- Developmental Disabilities Administration (DDA) enrollment is recommended. DDA is a US public agency that provides services and support to qualified individuals. Eligibility differs by state but is typically determined by diagnosis and/or associated cognitive/adaptive disabilities.
- Families with limited income and resources may also qualify for supplemental security income (SSI) for their child with a disability.

Prevention of Primary Manifestations

The treatment goal is maintenance of plasma arginine concentration as near normal as possible through restriction of dietary protein intake, supplementation with arginine-free essential amino acid formula, and use of nitrogen-scavenging drugs as needed to treat hyperammonemia. Liver transplantation eliminates hyperargininemia and presumably the risk for hyperammonemia but (in contrast to other urea cycle disorders) is rarely necessary in arginase deficiency; see also Table 3.

Prevention of Secondary Complications

Table 7.

Prevention of Secondary Manifestations in Individuals with Arginase Deficiency

Manifestation/ Situation	Prevention	Considerations/Other
Hyperammonemic episodes	Ongoing education of affected persons & caregivers re natural history, maintenance & emergency treatment, prognosis, & risks of acute encephalopathic crises	Written protocols for maintenance & emergency treatment should be provided to parents, primary care providers/pediatricians, & teachers & school staff. [1, 2]
	Treatment protocols & provision of emergency letters or cards to incl guidance for care in the event of illness	Emergency letters/cards should be provided summarizing key information & principles of emergency treatment for arginase deficiency & containing contact information for the primary treating metabolic center.
	MedicAlert® bracelets/pendants, or car seat stickers	

Manifestation/ Situation	Prevention	Considerations/Other
	Adequate supplies of specialized dietary products (protein-free formulas; medication required for maintenance & emergency treatment) should always be maintained at home.	For any planned travel or vacations, consider contacting a center of expertise near the destination prior to travel dates.

1. Essential information including written treatment protocols should be provided *before* inpatient emergency treatment may be needed.
2. Parents or local hospitals should immediately inform the designated metabolic center if: (1) temperature is >38.5°C; (2) vomiting/diarrhea or other symptoms of intercurrent illness develop; or (3) new neurologic symptoms occur.

Surveillance

Regular follow up at intervals determined by age and degree of metabolic stability is recommended (see Table 8).

Table 8.

Recommended Surveillance for Individuals with Arginase Deficiency

Manifestation/Monitoring	Evaluation	Frequency
Assessment of metabolic control	Plasma ammonia, amino acid profile, & nutritional monitoring labs	At least 1x/mo for 1st yr of life; thereafter per metabolic specialist
	Guanidinoacetate	Every 6-12 mos
Poor growth	Monitor growth	At each visit
Developmental delay	Monitor developmental milestones	At each visit in those age <18 yrs
	Neuropsychological testing using age-appropriate standardized assessment batteries	As needed
Neurologic deterioration [1]	Neurologic eval	At each visit [2]
Persistent hepatic synthetic function abnormalities	Liver function tests [3]	Every 6-12 mos
Quality of life	Standardized quality of life assessment tools for affected persons & parents/caregivers	As needed

1. Developmental stagnation and/or regression; seizures; spasticity; development of joint contractures
2. Referral to neurologist, orthopedist, and/or physical therapist as indicated
3. Albumin, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, prothrombin time (PT), and partial thromboplastin time (PTT).

Agents/Circumstances to Avoid

Valproic acid should be avoided as it exacerbates hyperammonemia in urea cycle defects and other inborn errors of metabolism [Scaglia & Lee 2006].

Evaluation of Relatives at Risk

Because the age of onset of arginase deficiency is delayed beyond the newborn period and the manifestations can vary, the genetic status of all sibs of a proband (especially the younger ones) should be clarified so that morbidity can be reduced by early diagnosis and treatment in those who are affected. Testing methods can include any one of the following:

- Plasma quantitative amino acid analysis
- Molecular genetic testing (if the family-specific *ARG1* pathogenic variants are known)
- Analysis of enzymatic activity in red blood cells

See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes.

Pregnancy Management

The authors are not aware of any instance in which pregnancy has been reported in a woman with arginase deficiency.

Prior to and During Pregancy

To achieve metabolic control that will enable normal fetal growth and development, affected pregnant women should generally continue dietary protein restriction and ammonia-scavenging medications (after an appropriate benefit/risk calculation) based on their clinical course before pregnancy.

- Protein restriction during pregnancy is challenging given the complications that commonly arise during pregnancy (i.e., nausea, vomiting, anorexia).
- Due to increased protein and energy requirements in pregnancy and, oftentimes, difficulty with compliance, weekly to every two-week monitoring of plasma amino acids and ammonia is recommended, especially in the first and third trimester, and close monitoring immediately after delivery.
- Plasma amino acid levels can help guide quick adjustments to diet in order to achieve normal plasma amino acid profiles that prevent catabolism and hyperammonemia while allowing for normal fetal growth and development.

Fetal Outcomes

There are no well-controlled epidemiologic studies of the fetal effects of sodium benzoate, phenylacetate, or phenylbutyrate during human pregnancy, although there are several case reports.

Redonnet-Vernhet et al [2000] reported a woman with symptomatic ornithine transcarbamylase (OTC) deficiency who was treated with sodium benzoate during the first 11 weeks of gestation and was subsequently transitioned to sodium phenylbutyrate for the remainder of pregnancy. She delivered a healthy female, who at age two years continued to do well.

Lamb et al [2013] reported another woman with symptomatic OTC who was treated throughout pregnancy with sodium benzoate (4 g/4x/day), sodium phenylbutyrate (2 g/4x/day) and arginine (300 mg/4x/day) who delivered a healthy, unaffected male who was doing well at age six weeks.

Ho et al [2019] are the first to document the use of sodium phenylbutyrate throughout two sequential pregnancies in a woman with HHH syndrome:

- In the first pregnancy sodium phenylbutyrate (5.5 g/4x/day) was used as maintenance therapy. This resulted in the delivery of a healthy female who was noted to have typical growth and development at age five years.

- In the second pregnancy, emergency treatment with Ammonul® (sodium phenylacetate/sodium benzoate) to manage hyperammonemic crisis (ammonia 295 µmol/L) was used in addition to maintenance therapy of sodium phenylbuterate (5 g/4x/day).

  Although the mother responded well to emergency treatment, the baby experienced intrauterine growth restriction and remained in the NICU due to prematurity and low birth weight. At age two years, the child exhibited speech delay and autism.

  How severe metabolic decompensation, elevated plasma ornithine, and/or side effects of sodium phenylbutyrate, phenylacetate, and/or benzoate may have contributed to the speech delay and/or autism is not known.

- Ho et al [2019] prefer and recommend the use of sodium benzoate if deemed medically necessary during pregnancy, but did not advise switching maintenance medications during pregnancy

Theoretic Concerns

Sodium benzoate has been reported to lead to malformations and neurotoxicity/nephrotoxicity in zebrafish larvae [Tsay et al 2007]. As a known differentiating agent, sodium phenylbutyrate also functions as a histone deacetylase inhibitor with potential teratogenicity, given its ability to alter gene expression in fetal mice [Di Renzo et al 2007]. Theoretically, the use of benzoate/phenylacetate and in particular sodium phenylbutyrate should be avoided during pregnancy, especially during the first trimester. The use of these medications should be carefully evaluated for each individual (benefit/risk ratio) in consultation with a metabolic genetics specialist.

See MotherToBaby for further information on medication use during pregnancy.

Therapies Under Investigation

A clinical trial for enzyme replacement therapy using pegylated synthetic human arginase I is currently under way (Clinical Trials Identifier NCT03921541).

A variety of genomic therapies are under investigation including mRNA therapy [Asrani et al 2018, Truong et al 2019], *ARG1* gene editing [Lee et al 2016, Sin et al 2017], and viral-mediated gene therapy [Cantero et al 2016].

Search ClinicalTrials.gov in the US and EU Clinical Trials Register in Europe for information on clinical studies for a wide range of diseases and conditions.

Other

Immunizations can be provided on the usual schedule.

Appropriate use of antipyretics is indicated. Ibuprofen is preferred over acetaminophen.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, mode(s) of inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members; it is not meant to address all personal, cultural, or ethical issues that may arise or to substitute for consultation with a genetics professional. —ED.

Mode of Inheritance

Arginase deficiency is inherited in an autosomal recessive manner.

Risk to Family Members

Parents of a proband

- The parents of an affected child are obligate heterozygotes (i.e., carriers of one *ARG1* pathogenic variant).
- Heterozygotes (carriers) are asymptomatic and are not at risk of developing the disorder.

Sibs of a proband

- At conception, each sib of an affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier.
- Heterozygotes (carriers) are asymptomatic and are not at risk of developing the disorder.

Offspring of a proband

- Although most severely affected individuals have not reproduced, those who are successfully treated are likely to be fertile.
- Unless an individual with arginase deficiency has children with an affected individual or a carrier, his/her offspring will be obligate heterozygotes (carriers) for a pathogenic variant in *ARG1*. Note: The rarity of the condition makes it unlikely that an unrelated reproductive partner of the proband whose ancestors do not come from a confined geographic area will be a carrier.

Other family members. Each sib of the proband's parents is at a 50% risk of being a carrier of an *ARG1* pathogenic variant.

Carrier Detection

Molecular genetic testing. Carrier testing for at-risk relatives requires prior identification of the *ARG1* pathogenic variants in the family.

Biochemical genetic testing. The normal mean red blood cell arginase enzyme activity is 100 times the lower limit of detection. Thus, most obligate carriers have been easily distinguished from normal. However, in at least one instance, a mother who was an obligate carrier tested in the mid- to normal range.

Related Genetic Counseling Issues

See Management, Evaluation of Relatives at Risk for information on evaluating at-risk relatives for the purpose of early diagnosis and treatment.

Family planning

- The optimal time for determination of genetic risk, clarification of carrier status, and discussion of the availability of prenatal/preimplantation genetic testing is before pregnancy.
- It is appropriate to offer genetic counseling (including discussion of potential risks to offspring and reproductive options) to young adults who are under treatment for arginase deficiency, are carriers, or are at risk of being carriers.

Prenatal Testing and Preimplantation Genetic Testing

Molecular genetic testing. If both *ARG1* pathogenic variants have been identified in an affected family member, prenatal testing for a pregnancy at increased risk and preimplantation genetic testing are possible.

Biochemical genetic testing. If molecular genetic testing is not possible, prenatal testing for pregnancies at 25% risk may be possible by measuring arginase enzyme activity in fetal red blood cells obtained by percutaneous umbilical blood sampling after 18 weeks' gestation [Hewson et al 2003, Korman et al 2004].

Neither amniocytes nor chorionic villous cells have arginase enzyme activity and thus are unsuitable for prenatal diagnosis using biochemical testing.

Note: Gestational age is expressed as menstrual weeks calculated either from the first day of the last normal menstrual period or by ultrasound measurements.

Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing. While most centers would consider use of prenatal testing to be a personal decision, discussion of these issues may be helpful.

Resources

GeneReviews staff has selected the following disease-specific and/or umbrella support organizations and/or registries for the benefit of individuals with this disorder and their families. GeneReviews is not responsible for the information provided by other organizations. For information on selection criteria, click here.

- **British Inherited Metabolic Disease Group (BIMDG)**
  TEMPLE (Tools Enabling Metabolic Parents LEarning)
  United Kingdom
  Arginase deficiency
- **Medical Home Portal**
  Arginase Deficiency
- **MedlinePlus**
  Arginase deficiency
- **National Urea Cycle Disorders Foundation**
  Phone: 626-578-0833
  Email: info@nucdf.org
  www.nucdf.org
- **Metabolic Support UK**
  United Kingdom
  Phone: 0845 241 2173
  www.metabolicsupportuk.org
- **Newborn Screening in Your State**
  Health Resources & Services Administration
  www.newbornscreening.hrsa.gov/your-state
- **European Registry and Network for Intoxication Type Metabolic Diseases (E-IMD)**
  www.e-imd.org/en/index.phtml
- **Urea Cycle Disorder International Patient Registry**
  Phone: 626-578-0833
  Fax: 626-578-0823
  Email: coordinator@ucdparegistry.org

www.ucdregistry.org

- **Urea Cycle Disorders Consortium Registry**
  Children's National Medical Center
  Phone: 202-306-6489
  Email: jseminar@childrensnational.org
  www1.rarediseasesnetwork.org/cms/ucdc

Molecular Genetics

Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. —ED.

Table A.

Arginase Deficiency: Genes and Databases

Gene	Chromosome Locus	Protein	Locus-Specific Databases	HGMD	ClinVar
ARG1	6q23.2	Arginase-1	ARG1 @ LOVD	ARG1	ARG1

Data are compiled from the following standard references: gene from HGNC; chromosome locus from OMIM; protein from UniProt. For a description of databases (Locus Specific, HGMD, ClinVar) to which links are provided, click here.

Table B.

OMIM Entries for Arginase Deficiency (View All in OMIM)

207800	ARGININEMIA
608313	ARGINASE 1; ARG1

Molecular Pathogenesis

ARG1 encodes ARG1, which forms a homotrimer that requires manganese as a cofactor for catalytic activity and stability. Deficiency of arginase leads to accumulation of arginine and related, toxic compounds such as guanidinoacetate. The block in ureagenesis can lead to hyperammonemia although this is not common as arginase is the last (most distal) enzyme in the urea cycle.

Mechanism of disease causation. Loss-of-function variants cause arginase deficiency.

Table 9.

Notable *ARG1* Pathogenic Variants

Reference Sequences	DNA Nucleotide Change	Predicted Protein Change	Comment [Reference]
NM_000045.3	c.57+1G>A		French Canadian founder variant [Uchino et al 1995]

Reference Sequences	DNA Nucleotide Change	Predicted Protein Change	Comment [Reference]
NM_000045.3 NP_000036.2	c.61C>T	p.Arg21Ter	Common variant in Turkey [Diez-Fernandez et al 2018]
	c.401C>T	p.Thr134Ile	Common variant in Brazil [Diez-Fernandez et al 2018]
	c.703G>A	p.Gly235Arg	Common variant in China [Diez-Fernandez et al 2018]
	c.23T>A	p.Ile8Lys	Severe variants [Diez-Fernandez et al 2018]
	c.77delA	p.Gly27AlafsTer5	
	c.263_266delAGAA	p.Lys88ArgfsTer45	
	c.316G>C	p.Gly106Arg	
NM_000045.3	c.466-2A>G		
	c.647_648ins32		

Variants listed in the table have been provided by the authors. *GeneReviews* staff have not independently verified the classification of variants. *GeneReviews* follows the standard naming conventions of the Human Genome Variation Society (varnomen.hgvs.org). See Quick Reference for an explanation of nomenclature.

Chapter Notes

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Revision History

- 28 May 2020 (ma) Comprehensive update posted live
- 28 August 2014 (me) Comprehensive update posted live
- 9 February 2012(me) Comprehensive update posted live
- 5 October 2010 (cd) Revision: deletion/duplication analysis available clinically

- 1 September 2009 (me) Comprehensive update posted live
- 30 June 2008 (cd) Revision: sequence analysis and prenatal testing available for *ARG1* mutations
- 13 February 2007 (me) Comprehensive update posted live
- 21 October 2004 (me) Review posted live
- 2 March 2004 (sc) Original submission

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Minireview

Hyperargininemia due to liver arginase deficiency

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Abstract

The urea cycle is a series of six reactions necessary to rid the body of the nitrogen generated by the metabolism, primarily of amino acids, from the diet or released as the result of endogenous protein catabolism. Arginase is the sixth and final enzyme of this cycle. Arginase catalyzes the conversion of arginine to urea and ornithine, the latter recycled to continue the cycle. Hyperargininemia due to arginase deficiency is inherited in an autosomal recessive manner and gene for arginase, designated AI, has been cloned. Unlike the other urea cycle enzymes, a second gene encoding arginase, with similar structural properties and enzyme characteristics, exists and has been named Arginase II (AII). Comprehensive histories and physical examinations confirm a strikingly uniform clinical picture and one notably different from patients with other urea cycle disorders. This condition rarely presents in the neonatal period and first symptoms typically present in children between 2 and 4 years of age. First symptoms are often neurologically based. If untreated, symptoms are progressive with a gradual loss of developmental milestones. With adherence to a dietary and drug regimen, a favorable outcome can be expected, with cessation of further neurological deterioration and in some instances, of improvement. This article summarizes the clinical course of selected patients who represent the full spectrum of presentations of arginase deficiency. In addition to the clinical characterization of this disorder; the biochemical, enzymatic, and molecular evidence of disease is summarized. Treatment and prenatal diagnosis are also discussed.

Keywords: Arginine; Arginase; Urea cycle; Treatment

Introduction

The urea cycle is a series of six reactions that have been recruited to rid the body of waste nitrogen (Fig. 1). Arginase is the sixth and final enzyme of this cycle and the most recently evolved; the others having been present for arginine biosynthesis in lower organisms [1]. Arginase catalyzes the conversion of arginine to urea and ornithine, the latter recycled to continue the cycle. The first three enzymes, *N*-acetyl-glutamate synthase (NAGS), carbamoyl phosphate synthase I (CPSI), and ornithine transcarbamylase (OTC) function inside of the mitochondria whereas the latter three, argininosuccinic acid synthase, argininosuccinic acid lyase, and arginase, act in the cytosol [1]. At least two transporters, for ornithine and citrulline (ORNTI) [2] and aspartate (citrin) [3] are also critical to the process. The waste nitrogen for this cycle is generated by the metabolism, primarily of amino acids, either ingested in the diet or released as the result of endogenous protein catabolism [1]. The liver is the only organ in the body to contain all of the enzymes needed for the function of the urea cycle.

Defects of all six steps of the urea cycle are known [1]. All may result in defective function of the cycle and in

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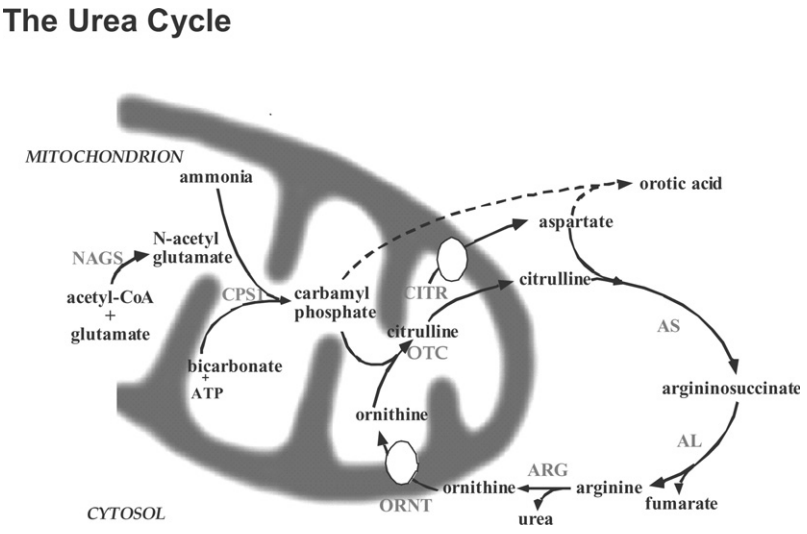

Fig. 1. The urea cycle. The six primary enzymatic steps of the urea cycle are shown in bold capital letters. NAGS, *N*-acetylglutamate synthase; CPSI, carbamylphosphate synthase; OTC, ornithine transcarbamylase; AS, argininosuccinic acid synthase; AL, argininosuccinic acid lyase; and ARG, arginase. ORNT and CITR are respectively, the ornithine and aspartate transporters. Their function is necessary for the movement of substrates in and out of the mitochondrion. Reprinted with permission from Tuchman and Bradshaw [47].

the accumulation of excess ammonia in the body, either continuously or intermittently, with resulting neurological damage, developmental delay, and mental retardation. Defects in any of the first five steps of the cycle have been reported to cause acute neonatal or acute intermittent hyperammonemia.

Hyperammonemia has infrequently been associated with arginase deficiency and presentation in the neonatal period is an uncommon event [4]. Ornithine transcarbamylase deficiency has the highest incidence of the six disorders and arginase and NAGS deficiencies have the lowest incidence. This is ascertained from the number and timing of the case reports, from a fairly comprehensive survey of urea cycle disorders in Japan [5] and from the screening of at least 7000 developmentally handicapped individuals in an institution for the mentally retarded in which no cases of arginase deficiency were ascertained [6]. The true incidence of arginase and NAGS deficiencies is unknown.

The first case of arginase deficiency was probably reported in 1965 by Peralta Serrano [7], but no comprehensive evaluation or enzymatic assay was done. The first family known to have this disorder was reported by Terheggen and associates in 1969 [10–13]. Subsequently, more than 30 cases have been reported in the literature and a larger number are known to our metabolic team and to DeDeyn et al. [8] who published a review in the proceedings of a conference on guanidino compounds, held in Montreal in 1994. The summary material in this article derives from our own extensive experience and the collective experience reflected in that article, encompassing professor DeDeyn's remarkable feat of having visited the majority of known patients in the world prior to that time. The disorder is inherited in an autosomal recessive manner with frequent instances of consanguinity. A pocket of increased frequency may occur amongst the French Canadian due to a well known bottleneck in the founder population of the lake region of northern Quebec province [9].

The gene for liver arginase, designated AI, was cloned in 1986 by Mori and co-workers [10] and ourselves [11,12] and we have subsequently defined a number of natural mutations in the gene [12–14]. Ash and co-workers [15] have crystallized rat liver arginase and have super-imposed the homologous human enzyme on the coordinates derived from the rat. In addition, the perturbation in protein structure and function caused by these mutations has been described [16]. Unlike the other urea cycle enzymes, a second gene encoding arginase, with similar structural properties and enzyme characteristics, exists and has been named Arginase II (AII) [17–20]. Arginase II is most abundantly expressed in kidney and prostate and is located in the mitochondrial matrix. It appears to be induced in AI deficiency and may mitigate the degree of hyperargininemia and hyperammonemia in this disease [21]. The function of AII is not well-defined or proven and is subject of intense study.

Clinical presentations and course

The patients whom we have seen represent the full spectrum of presentations. Thus we have chosen to report them as individuals.

Patient 1, now 13 years, was diagnosed at 4 years of age after presenting with growth failure starting at 2 years, gait abnormalities since 3 years, bilateral lower extremity spasticity, and a seizure disorder. Physical examination on presentation showed growth failure, decreased range of motion, increased tone, and extreme

hyperreflexia of the lower extremities. The deep tendon reflexes in the upper extremities were also increased, but to a lesser extent. There was no history or evidence of developmental delay. The newborn period and infancy was complicated by feeding intolerance, first with breast milk and then with many different formulas, possibly indicating protein intolerance. The patient has been below the 3‰ for height since 2 years of age, weight was initially at the 3‰ and has increased to the 10‰ since starting treatment. The patient was diagnosed with a seizure disorder at 4 years of age with generalized atypical spike and wave over the left frontal temporal region found on EEG. He had one episode of metabolic crisis requiring hospitalization associated with gastroenteritis at 6 years of age at which time he had encephalopathy with increased spasticity and transient hemiparesis and new cranial nerve deficits. Plasma ammonia peaked at 120 μg/dL and all new symptoms resolved within 36 h. Currently, his plasma arginine level, on a protein-limited diet with a limited amount of sodium benzoate, approximately 1/4 the recommended dose, is about three times the upper limit of normal. His muscle tone in the upper extremities is normal, as are his deep tendon reflexes. In the lower extremities he is hypertonic and spastic, the reflexes are excessive, and he has 1–2 beats of clonus at the ankles. The ankles cannot be brought to 90° with the floor and he is a little clumsy. He has never had hepatomegaly. This child has always been intellectually advanced and continues to excel in a traditional classroom. In the course of his care he was diagnosed with, "reactive spasticity" and received several botox treatments with a remarkable response. He has required no treatments for several years. His clinical condition is stabilizing or improved on therapy.

Patient 2, deceased at 22 years of age from cerebral edema with uncal herniation, presented at 2 years of age and was diagnosed at 5 years of age [22]. Irritability, frequent vomiting, and mild developmental delay marked the first 2 years of life. Physical examination on presentation showed increased tone, increased deep tendon reflexes, mild spasticity, hyperreflexia, and clonus in the lower extremities and hyperreflexia in the upper extremities. This patient had progressive deterioration with slow progressive loss of speech and general cognitive function until dietary therapy and sodium benzoate was started. Weight and height had always been much below the 3‰. This patient had moderate to severe mental retardation when treatment was started and management was difficult. This patient showed great behavioral difficulties even with ammonia and arginine levels within the normal range. The patient often refused his medication and would steal food. When diet and medications were carefully monitored the patient was able to maintain a very modest elevation of arginine. During periods of noncompliance ammonia levels were generally three times the upper limit of normal. The patient also presented with and continued with an uncontrolled generalized seizure disorder with approximate frequency of 1 seizure per month.

Patient 3, now 31 years, was diagnosed at 11 years after presenting with hyperammonemia, somnolence, and confusion [23]. Diet therapy and sodium benzoate were started at the time of diagnosis. At presentation he was below the 3‰ for both height and weight, there was some restricted motion at the ankles, increased tone in all extremities, and increased reflexes in the lower extremities. This patient was reported to reach all early developmental milestones on time until 8 years when his intellectual development began to slow. The patient was placed into and graduated from a school for children with learning disabilities. At 5 years, the patient reportedly became clumsy and would fall very easily; the patient was also noted to vomit frequently and began to self-select against high protein containing food. At 6 years this patient developed spastic diplegia and had his first surgery at 8 years for heel cord lengthening and was ambulatory without assistance until his mid-teenage years when he required the use of a walker due to the spasticity in his lower extremities. At 24 years the patient suffered a traumatic right lower extremity fracture, healed well, but never regained the same level of ambulation and is now predominantly wheelchair dependent. At 27 years the patient had surgery which involved bilateral adductor and hamstring releases and bilateral Achilles tendon releases. At present he is stable but noncompliant with both diet and sodium benzoate. The patient's care has also been complicated by depression that began around 15 years. He has never had a seizure. Physical examination is significant for short stature, hyperreflexia in both upper and lower extremities, spasticity of his lower extremities, hyperreflexic with cross adductor responses. He has flexion contractures in bilateral knees, hips, and feet. There is no hepatomegaly. He never had a second episode of hyperammonemia.

Patients 4 and 5 are siblings [24]. The first born is a female, now 42 years, diagnosed at 12 years after presenting with moderate to severe developmental delay and severe lower extremity spasticity. This patient had mild developmental delay first noticed at 4 years. Her development progressed until 6 years; she was bilingual, able to speak in sentences, and able to walk with assistance. She began to deteriorate to the point that at age 12 years she was unable to speak or stand. She also developed a seizure disorder at 8 years that is under reasonable control. At the time of presentation she was below the 3‰ for height, weight, and head circumference, she had episodic irritability, hyperreflexia, spasticity in the all extremities, and contractures of ankles and knees. At the age of 14 years she underwent bilateral adductor myotomies, obturator neurectomies, and Achilles tendon release. She was started on diet of essential amino acids excluding arginine at 16 years after

previously being treated with a protein-restricted diet. Sodium benzoate therapy was started at 18 years. After starting a modified diet she had some improvement in spasticity, toilet training, and language. At present remains in excellent control, is stable neurologically at a baseline maintained since starting a modified diet and has regained limited language skills. Exam is significant for atrophic lower extremities with severe weakness, moderate upper extremity strength, contractures of bilateral knees and ankles, and hyperreflexia in all extremities.

Her younger brother, deceased at 22 years from cerebral edema, was diagnosed at 6 years after an episode of coma with hepatomegaly. This patient had normal development until 3 years when he began to have difficulties walking. His development continued to deteriorate to the point that at 6 years he was unable to walk secondary to severe spasticity, contractures, and weakness. Exam at that time also showed height, weight, and head circumference below the 3‰, mild spasticity of the upper extremities with mild weakness, and hyperreflexia in all extremities. He also developed a seizure disorder at 7 years. He was started on a synthetic diet at 8 years after previous treatment with a low protein diet started at 6 years and was started on sodium benzoate at 11 years. His neurological status stabilized after starting a low protein diet and he showed marked improvement after starting a synthetic diet. Prior to death he was speaking in short phrases in both Spanish and English, was able to perform simple tasks and had limited ambulation. His death was due to hyperammonemia following aspiration pneumonia. He had suffered from bulbar paraparesis, a condition only partially improved by treatment.

Clinical characteristics

Both clinical case reports and the comprehensive examination by us and Dr. DeDeyn confirm a strikingly uniform clinical picture and one remarkably different from patients with other urea cycle disorders [4]. The condition rarely presents in the neonatal period and most patients are described as normal, or at the outer limits of normal, in early life. The first symptoms are often noted between 2 and 4 years of age and consist of clumsiness, tripping, falling, and diminished growth. If untreated, the symptoms are progressive, resulting into frank spasticity and a gradual loss of developmental milestones. Patient 4 was documented to be normal in development and bilingual at age 6 years and by age 12 years had progressed to the point of being alingual.

The most prominent physical findings are spastic paraparesis or paraplegia with lesser effects on the upper extremities, increased deep tendon reflexes, scissoring and cross adductor responses, toe walking, loss of intellectual milestones, poor growth, and seizures with EEG abnormalities. Many patients require heel cord lengthening and obdurator release, sometimes repeatedly. The growth which is normal for several years falls off and all patients are far below the 3‰ for height. Ataxia has been reported in patients, but if present, this is a minor finding. Brain imaging has revealed cerebral atrophy. Strikingly, the cerebellum appears unaffected and there is no impairment of hearing or vision. In general, peripheral nerve testing has been normal or nearly so.

A minority of patients have either persistent or intermittent episodes of irritability, nausea, poor appetite, and vomiting, sometimes progressing to lethargy. Many recover with symptomatic treatment with or without intervenous ammonia diverting drugs. A minority have presented with acute episodes of hyperammonemia which in general are less severe than those that occur with other urea cycle defects. Two of our own patients have died during episodes of acute hyperammonemia and we are aware of others who have suffered a similar fate.

Hepatomegaly is present during acute hyperammonemia episodes but is generally absent at other times. Liver cirrhosis was reported in at least one patient [25]. We have found persistent abnormalities in clotting studies in two patients [4,5], but neither was severe or symptomatic. The severe neurologic disease has led to a number of secondary skeletal abnormalities. One of our patients had episodes of seizures, coma or both associated with her menstrual periods. This was eliminated with hormonal suppression of ovarian cycling and ultimately by hysterectomy without oophorectomy [26].

Biochemical characteristics

The first patients were ascertained at a time when plasma amino acid determinations were more difficult and the first findings frequently were a urine amino acid pattern reminiscent of cystinuria, due to an overflow of the dibasic amino acids which shared a common kidney transport system [22,24,27–30]. Today, plasma amino acid determination in individuals with developmental delay or neurological difficulties would reveal an elevated level of arginine [24]. If the patients are chronically mildly hyperammonemic, glutamine levels may be elevated as well. Arginine levels were increased 5- to 15-fold and sometimes more [21] and proved to be relatively constant for most patients, barely responding to protein intake variation within a normal and growth sustaining range [24]. Orotic acid in the urine is frequently increased [21]. We and others have observed higher urea levels in these patients than in patients with other urea cycle defects and these levels have risen with increase protein intake (Table 1) [24]. Similarly, urinary urea excretion increases as the protein intake increases and

the plasma arginine remains unchanged [24]. This led us to propose the existence of a second form of arginase and this was subsequently proven.

Plasma ammonia levels are usually normal when arginase deficient patients are well, although glutamine levels may be increased [4]. Some patients have consistently mild elevations of ammonia and often are symptomatic as a result. The majority of patients have experienced one or more episodes of hyperammonemia and ammonia levels as high as 400 μM have been seen. Two patients for whom we cared, who subsequently died, had astoundingly high levels of ammonia (6000 μM) and plasma arginine had risen to 1500 μM or higher [21].

All patients studied had elevated levels of arginine in the cerebralspinal fluid and two we studied had a number of other amino acid elevations as well (Table 2) [24]. The basis for this is not understood, but levels improve with therapy. We and our colleagues recently reported a newborn with AI deficiency that succumbed after 3 days of age after ammonia levels, never higher than 194 μg/dL, were brought under control and normalized. In this patient, however, while the glutamine level in plasma was within normal limits (909 μmol/L; nl 332–1084) the glutamine level in CSF (9587 μmol/L; nl 385–771) was unprecedently high and is inferred to have caused the fatal neurotoxicity [31]. Such a disparity between ammonia levels and those of glutamine have been seen in other patients with urea cycle disorders and may indicate that ammonia may not be the best and only marker for the severity of the pathologic effects of urea cycle defects.

Guanidino compounds derived directly from arginine have been studied in many arginase deficient patients as a result of the painstaking efforts undertaken by Marescau et al. [4,32,33]. There was a general increase in the guanidino compounds synthesized from arginine. Creatinine was normal in all patients and creatine was elevated in some. It is not clear whether or not these perturbations contribute to the pathogenesis of this disorder. One patient has undergone testing for activity of the urea cycle by isotope dilution methods and was found to be deficient [34].

Table 1
Urea cycle and related amino acids in the plasma (mM) of pateints 4 and 5

	Protein intake[a]					Normal values[b]
	Patient 5			Patient 4		
	1.0	2.0	3.0	1.0	2.5	
Arginine	637	591	677	786	913	21–151
Citrulline	53	38	46	40	58	12–55
Ornithine	46	39	40	75	95	30–126
Lysine	127	86	80	87	194	83–237
Glutamine	392	440	451	556	607	415–694
Urea	13.5[c]	14.7	18.4	15.5	20.3	20–30

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[a] Protein intake in g/kg body weight/day. All specimens were collected 3 h after a meal containing 1/3 of the prescribed diet. (One fasting sample was not distinguishable from the postprandial one.)

[b] Dickinson et al. [7].

[c] Expressed as mg/dL.

Table 2
CSF amino acid levels (mg/dL) 2.5–3.0 g protein/kg/day (3 h pp)

Amino acid	Patient 4	Patient 5	Controls	X Normal
Arginine	1.71	1.21	0.35 ± 0.1	4
Citrulline	0.16	0.072	0.035 ± 0.14	
Ornithine	0.62	1.25	0.075 ± 0.024	10–20
Lysine	0.21	0.43	0.27 ± 0.1	
Aspartate	0.40	0.38	0.012 ± 0.007	30
Serine	1.82	1.99	0.40 ± 0.24	5
Glutamine	47.4	16.0	7.4 ± 2.1	2–6
Glycine	0.64	0.78	0.05 ± 0.01	15
Other amino acids				2–5

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Arginase activity in arginase AI deficient patients

Arginase activity is very low or absent in the red blood cells of all patients in whom it was tested. White blood cell arginase, and in one instance stratum corneum, enzyme levels were similarly diagnostic [24]. Arginase activity in the liver has been reported in a smaller number of patients. In each, it was reduced to 10% of normal or less [21,24,35–37]. This accords with independent enzymological and immunological studies suggesting substantial correlation between red blood cell and liver arginase.

Very early in the investigations of patients with arginase deficiency it became clear that there were some striking biochemical differences between arginase deficient patients and those with other urea cycle disorders. The patients infrequently had episodes of hyperammonemia, these episodes were usually self-limited when they occurred, the peak ammonia levels were rarely as high as in the other urea cycle disorders (even in what turned out to be patients with two nonsense mutations), and urea levels, always in the normal range, rose with increased protein intake [24]. The most likely explanation for this combination of findings, the existence of a second arginase activity, turned out to be correct.

Spector et al. [38] first observed augmented levels of arginase activity in kidney when liver arginase activity was greatly reduced. This has subsequently been confirmed by us in four other patients [31,37]. Levels of the second, mitochondrial arginase (AII) were elevated as much as 40-fold under conditions of extreme hyperammonemia and hyperargininemia (Table 3). In 1996, when AII was cloned, sequenced, and compared to AI, it was clear that the two arginases had a common ancestry, with the duplication in the parent gene having occurred at east 300 million years ago at the time of the evolution of amphibians [17–20].

Table 3
Arginase II activity in tissue extracts of hyperargininemic patients

Tissue	Normal	Activity (nmol/mg/30 min)		
		Patient 2	Patient 5	Patient[a]
Kidney	200 (350)	5192	8564	1430
Liver	300 (19,500)	334	532	980
Brain	50	492	N.D.	N.D.
Heart	N.D.	N.D.	N.D.	10
Spinal cord	N.D.	N.D.	N.D.	10

[a] Ref. [35].

From these data we have inferred that the higher or augmented levels of AII in baseline conditions and its further induction under conditions of higher plasma arginine and ammonia is responsible for the higher ureagenesis in AI deficiency. We infer further that the milder course of AI deficiency as compared with other urea cycle disorders is a result of this second isozyme.

Studies in the AI knockout mouse have so far not shed light on this issue [39]. The mouse which succumbs at 2 weeks with hyperammonemia may have a 2-fold induction of AII, levels which would not reach statistical significance. Moreover, the AI/AII double knockout does not increase the vulnerability further. Studies of arginase expression by in situ hybridization suggest that AI and not AII is the gene most prominently transcribed in utero [40]. AII expression in kidney only begins at birth. Thus AII may play a more prominent role in AI deficiency in the model as well when we can bring the knockout animals to weaning and beyond.

Pathology and pathogenesis of neurological disease

Liver biopsy during acute episodes has shown swollen hepatocytes without obvious abnormalities of lipid accumulation, mitochondrial derangement or biliary defects. This is consistent with the tendency of ammonia to cause hydropic and reversible changes in hepatocytes [37]. In a number of patients, chronic fibrosis and cirrhosis were seen. As noted previously, some of patients have had abnormalities of liver function consistent with the ongoing and permanent hepatic damage, whereas the elevated transaminase levels often return to normal between episodes [4]. Complete autopsies done in a number of patients have failed to reveal any consistent abnormalities or abnormalities that might be associated with the primary enzymatic defect [4,24]. Neuropathology has confirmed the cerebral atrophy found on imaging in those patients, but there were no specific abnormalities otherwise.

The pathogenesis of the neurologic disease is less clear. It is apparent that the intermittent or chronic elevation of blood ammonia underlies the episodic irritability, anorexia, and vomiting. With control of the ammonia levels these symptoms disappear. Similarly, we have observed acute psychosis during minor hyperammonemia in an older patient and this too regressed with control of ammonia levels. The extreme and progressive cerebral and motor neuron disease is clearly different from other urea cycles disorders, even those with recurrent acute or chronic hyperammonemia and clearly must relate in one or another way to the elevated arginine level. Whether it is arginine or one of its metabolites, such as guanidino compounds, is unclear [4,32,33]. Of the latter, α-keto-γ-guanidino valeric acid has been shown to cause seizures in rabbits. Similarly, arginine may be a precursor to glutamate or GABA and thus cause damage on an excitotoxic basis. Arginine is also a precursor of nitric oxide and elevated levels of arginine may cause greater synthesis of nitric oxide and oxidative damage. This may represent an important unexplored area of investigation and provide accessory approaches to the care of patients with this disorder. Marescau et al. [4] champion other guanidine compounds as the primary brain toxins.

Molecular studies

Uchino et al. [12] and Vockley et al. [13,14] have carried out mutation analysis in patients with arginase I deficiency. Nonsense mutations and small deletions were found in a large minority of the patients and were scattered randomly throughout the coding sequences. In contrast, the missense mutations were found exclusively in those residues that have been conserved in evolution and imply a critical role for these amino acids in the stability of catalytic function. Uchino et al. concluded that some correlation between mutations and disease severity existed, whereas Vockley et al. were less impressed in their series of patients. Vockley et al. [13] expressed the mutant proteins in an *Escherichia coli* expression system and demonstrated reduced activity in all cases save one. In that instance, the semi-conservative nature of the amino acid change and the evolutionary data predicted no loss of activity.

The human arginase missense mutations were superimposed on the crystal structure of rat liver arginase with which it shared 87% homology at the amino acid level. In each instance, in which a natural or a site directed mutant caused loss in activity, the crystal structure model predicted significant perturbation in the active site. No distortion in structure was seen with the neutral mutation. More recently, Kim et al. have isolated an alternatively spliced AI message which contained an eight amino acid insertion near the N-terminal region of the protein. This in frame 24 base insertion encoded a protein that was 10% as active as the wild type [41]. Again, the crystal structure predicted a lesser degree of active site perturbation than one would expect since the inserted amino acids lie on the external surface of the

protein in an area unlikely to impinge heavily on the active site or on the formation of the trimer. The effect of these changes on multimer formation is being studied.

Studies of red blood cells from AI deficient patients demonstrated that immunologically cross reacting material was absent from all but one patient who had normal arginase band after SDS–PAGE electrophoresis [23]. Thus both most missense and nonsense mutations had a destabilizing effect on the protein, presumably after it had been translated. What if any significance this may have is uncertain.

Treatment

Hyperargininemia is a favorable candidate for standard urea cycle therapy; limitation of natural protein intake, essential amino acid supplementation, and ammonia diversion to salvage pathways (Table 4) [20]. Arginase deficient patients are less prone to acute, uncontrolled hyperammonemia and may have no or only moderate levels of ammonia elevation on a diet containing natural foods. For those patients and families able to comply with the onerous regimen, treatment has been encouraging. At least one patient and possibly others, treated from birth with a protein limited diet and essential amino acids have no apparent defects in his 30's [42]. Another, younger individual, also treated from birth is doing similarly well [43]. Based on our own experience comprising more than 75 patient years, reasonable adherence to the treatment regimen is very likely to halt the disease progress, will relieve acute symptoms of ammonia and "arginine" toxicity, and will permit recovery of some lost functions over time. Unfortunately, only the minority of patients can adhere to a diet rigorous enough to get arginine levels into or near the normal range. In some of these patients, the spasticity of the legs may continue to progress, albeit at a very slow rate. The, "good news" is that successful treatment is unlikely to be undone by severe and brain-damaging episodes of intermittent hyperammonemia.

Prenatal diagnosis and newborn screening

The arginase AI gene is located at 6q23 and arginase deficiency is inherited as an autosomal recessive disorder [44]. The recurrence risk in subsequent births to the same parents is 25%. If the mutation(s) in the patient is known, prenatal diagnosis can be accomplished by mutation analysis in chorionic villous tissue or in amniotic fluid cells. Some years ago we demonstrated that AI is expressed in fetal red cells at 16–20 weeks of gestation and at levels comparable, albeit somewhat lower than postnatal levels [45]. Percutaneous umbilical blood sampling (PUBS) was recently used to predict a normal sibling to an arginase deficient patient [46], as well as an affected patient [42]. The ability to do prenatal diagnosis for a condition whose treatment from birth, although onerous, is successful raises an ethical issue. Some may find it unethical to terminate in such a circumstance, despite this being legal.

The advent of newborn screening by tandem mass spectrometry now allows for measurement of blood levels of arginine. We recently reported the diagnosis of a patient with arginase deficiency from a newborn blood spot [31]. An informal and unscientific poll of participants in a metabolic disease listserv resulted in the report of four more cases diagnosed on newborn screening and one that was missed. From this information it appears likely that many, if not most patients with this condition will be diagnosed prior to the onset of symptoms and the occurrence of permanent neurological injury. This is indeed good news for patients with this disorder, even if it means being sentenced to a life-long ascetic regimen.

Table 4
Treatment results for patient 4

Plasma parameter (mg/100 ml)	Control	Benzoate		Phenyl acetate	Both med	Normal diet both med	Normal values
		5 g/day	10 g/day	10 g/day			
Patient 4							
Arginine	6.49	4.95	2.98	1.95	1.82	5.89	0.37–2.63
Ornithine	0.42	0.48	0.40	0.41	0.39	0.33	0.39–1.67
Citrulline	0.33	0.29	0.15	0.10	0.046	0.13	0.21–0.97
Lysine	3.22	2.11	1.82	1.88	2.27	2.58	1.21–3.47
Glutamine	9.13	8.26	5.10	3.28	3.31	6.94	6.06–10.14
Urea	5.00	4.70	2.18	<1.00	3.50	6.48	20–40
Patient 5							
Arginine	7.81	6.27	3.29	1.83	1.90	4.93	0.37–2.63
Ornithine	0.46	0.39	0.43	0.38	0.35	0.43	0.39–1.67
Citrulline	0.51	0.32	0.16	0.10	0.075	0.23	0.21–0.97
Lysine	3.05	1.78	1.79	1.75	2.15	1.64	1.21–3.47
Glutamine	9.28	7.83	4.15	2.59	3.28	4.44	6.06–10.14
Urea	6.14	4.66	2.82	1.90	1.64	4.92	20–40

Summary

Hyperargininemia due to liver arginase deficiency is a treatable inborn error of the urea cycle. With adherence to the dietary and drug regimen, a favorable outcome can be expected, with cessation of further neurological deterioration and in some instances, of improvement. It appears that this favorable outcome is due, in part, to the augmented expression of a second arginase gene. The existence of this second locus provides a unique approach to treatment, if only expression could be greatly enhanced, especially in liver. Arginase deficiency is also a good candidate fore gene therapy, an approach that may be more distantly in the future.

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REVIEW

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The role and control of arginine levels in arginase 1 deficiency

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Abstract

Arginase 1 Deficiency (ARG1-D) is a rare urea cycle disorder that results in persistent hyperargininemia and a distinct, progressive neurologic phenotype involving developmental delay, intellectual disability, and spasticity, predominantly affecting the lower limbs and leading to mobility impairment. Unlike the typical presentation of other urea cycle disorders, individuals with ARG1-D usually appear healthy at birth and hyperammonemia is comparatively less severe and less common. Clinical manifestations typically begin to develop in early childhood in association with high plasma arginine levels, with hyperargininemia (and not hyperammonemia) considered to be the primary driver of disease sequelae. Nearly five decades of clinical experience with ARG1-D and empirical studies in genetically manipulated models have generated a large body of evidence that, when considered in aggregate, implicates arginine directly in disease pathophysiology. Severe dietary protein restriction to minimize arginine intake and diversion of ammonia from the urea cycle are the mainstay of care. Although this approach does reduce plasma arginine and improve patients' cognitive and motor/mobility manifestations, it is inadequate to achieve and maintain sufficiently low arginine levels and prevent progression in the long term. This review presents a comprehensive discussion of the clinical and scientific literature, the effects and limitations of the current standard of care, and the authors' perspectives regarding the past, current, and future management of ARG1-D.

KEYWORDS

arginase deficiency, guanidino compounds, hyperargininemia, inborn error of metabolism, urea cycle disorder

Synopsis

ARG1-D is a distinct urea cycle disorder with a progressive neurologic phenotype. This review presents a comprehensive discussion of evidence from genetically manipulated mouse models and observations from clinical practice that implicate high arginine levels directly in disease pathophysiology.

1 | INTRODUCTION

Arginase 1 Deficiency (ARG1-D) is a rare, progressive inborn error of metabolism that results in persistent hyperargininemia and debilitating cognitive, neurologic, and mobility impairments.[1,2] These clinical impairments consistently manifest in patients with ARG1-D, although with varying age of onset and rate of progression.[2,3] Although ARG1-D shares some clinical characteristics with other urea cycle disorders (UCDs), there are several distinct biochemical and clinical features of ARG1-D that suggest a unique mechanism driving development and progression of disease manifestations. Neurotoxic effects of elevated levels of arginine and arginine metabolites, as well as a mechanistic role of chronic hyperargininemia in the development and progression of neurologic manifestations have long been proposed,[4–10] and are supported by both empirical studies and clinical evidence discussed here.

1.1 | Arginase 1 deficiency is a distinct urea cycle disorder

The urea cycle comprises six consecutive enzymatic reactions and two transporters in the liver that detoxify ammonia through conversion into urea, which is excreted through the kidneys. Under normal conditions, arginase 1 hydrolyzes arginine into ornithine and urea in the final step of the cycle. Mutations in the *ARG1* gene lead to impaired or absent arginase 1 activity and, as a direct effect of defective metabolism, intracellular hepatic arginine accumulates at levels approximately 50-fold higher than normal (Figure 1).[11] This excess arginine is released to the plasma and subsequently accumulates in other organs (including brain and cerebrospinal fluid [CSF]),[8,12] as a result of arginine being readily transported and maintained in an equilibrium between different tissues and plasma.[13–15] Elevated levels of arginine and arginine-derived guanidino compounds, putative neurotoxins generated through downstream enzymatic pathways external to the urea cycle,[8,11,12,16–18] are well-documented in the plasma/serum and CSF of patients with ARG1-D as well as rodent models of this multisystem disorder.[1,4,8]

Biochemically, markedly elevated plasma arginine is the most readily apparent feature of ARG1-D. Normal plasma arginine levels range from 40 to 115 μmol/L[19] but are typically >300 μmol/L in ARG1-D and often much higher[20]; levels >10-fold normal have been reported. In contrast, arginine levels are low in other UCDs because of upstream metabolic abnormalities that diminish endogenous arginine production—in fact, arginine supplementation is indicated for all UCDs other than ARG1-D.[20] In most UCDs, hyperammonemia is a common and potentially life-threatening complication. Hyperammonemic episodes, often severe, may occur throughout life and can cause encephalopathy, neurocognitive sequelae, or even death. Symptomatic hyperammonemia and hyperammonemic crisis are comparatively less common in ARG1-D, probably because upstream ammonia detoxification processes (through activity of

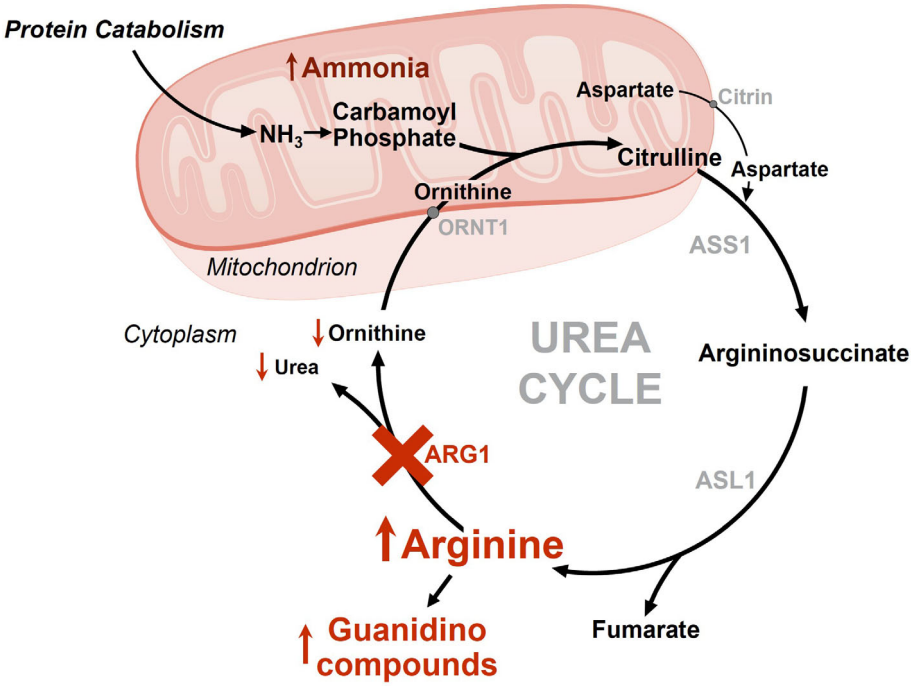

FIGURE 1 Urea Cycle Dysfunction in Arginase 1 Deficiency. Loss of arginase 1 enzymatic activity results in pathologic accumulation of arginine, decreased levels of its urea cycle products (ornithine and urea), and increased levels of guanidino compounds. ARG1, arginase 1; ASL1, argininosuccinate lyase; ASS1, argininosuccinate synthetase 1; ORNT1, ornithine transporter 1. Adapted with permission from Blair NF, Cremer PD, and Tchan MC. *Pract Neurol.* 2015;15:45–48. doi:10.1136/practneurol-2014-000916

enzymes preceding arginase 1 in the urea cycle) remain intact.[20,21]

The importance of hyperammonemia in most other UCDs is reflected in their management and clinical course, wherein early manifestations of the more complete enzyme deficiencies become apparent in the first days or weeks of life and commonly involve signs and symptoms driven by ammonia accumulation (e.g., cerebral edema, lethargy, anorexia, hypothermia, neurologic posturing, seizures, and coma). Cases of severe neonatal/infantile hyperammonemia in ARG1-D have been described but are uncommon.[22–26] Instead, the classic phenotype of ARG1-D involves insidious onset with manifestations developing typically in the first years of life and worsening progressively over time at variable rates; newborns typically appear healthy.[2,17,27–29] The clinical profile of ARG1-D includes seizures, developmental delay, and cognitive impairment as common manifestations. Unlike other UCDs, however, developmental delay and cognitive impairment in ARG1-D are progressive. Furthermore, spastic diplegia is a hallmark clinical feature of ARG1-D that differentiates this disorder from other UCDs, with its pathogenesis distinct from the known toxic effects of ammonia.[20,21,30] Patients with ARG1-D exhibit progressive spasticity that predominantly affects the lower limbs and worsens in severity and impact over time. As a result, these patients may initially stumble and appear clumsy, develop gait abnormalities and mobility impairments, and eventually lose the ability to walk independently.[17,29] Based on this clinical profile, ARG1-D is uniquely recognized among UCDs as a clinical mimic of cerebral palsy and hereditary spastic paraplegia.[31–34]

The pathophysiologic profile of ARG1-D strongly suggests that elevated arginine, rather than hyperammonemia, plays the key role in development and progression of manifestations.[2,4,28] Plasma arginine levels may be within or near the normal range in the immediate postnatal period, when infants appear phenotypically normal.[30] Furthermore, progression of manifestations with increasing duration of disease suggests a cumulative effect of persistently high arginine.[35,36] Clinical recognition of the importance of plasma arginine levels in ARG1-D is reflected in current management guidelines.[20] Treatment of other UCDs is focused on reducing risk of hyperammonemia and addressing acute hyperammonemic episodes,[20] but, as noted previously, hyperammonemia is less of a concern in ARG1-D. Preventing symptomatic hyperammonemia does not prevent progression or improve long-term outcomes in these patients. Instead, current guidelines for ARG1-D management focus on lowering plasma arginine, and specifically recommend maintaining plasma arginine levels at <200 μmol/L or as low as possible (aiming for the upper reference range).[20]

1.2 | Empirical studies implicate arginine in development and progression of ARG1-D manifestations

Multiple mouse models support a key role of arginine accumulation in ARG1-D pathophysiology. These animals demonstrate markedly elevated plasma arginine and guanidino compounds that lead to phenotypic abnormalities consistent with disease manifestations observed in patients with ARG1-D, including failure to thrive, seizures, spasticity, gait abnormalities, and mobility impairment.[37–42]

The most extensively characterized *Arg1* knockout model, first described by Iyer et al., was developed through replacement of *Arg1* exon 4 (the active site) with a neomycin resistance gene by homologous recombination that results in a total lack of liver arginase 1.[37] These mice are phenotypically indistinguishable from wild-type littermates at birth and appear to behave normally for the first 10–12 days of life. Onset of hyperargininemia and hyperammonemia is followed by weight loss, central nervous system dysfunction (which manifests as gait instability, tremor, ataxia, lethargy, decerebrate posture, and seizure-like activity), encephalopathy, liver pathology, metabolic crisis, and death. Plasma arginine levels are approximately four-fold greater in these animals than wild-type comparators and rise to >10-fold normal levels during metabolic crisis. As expected, hyperargininemia is accompanied by marked increases in guanidino compounds in the serum as well as in the brain.[39,41,42] These mice ultimately succumb to severe hyperammonemia at approximately 14–21 days of life. The biochemical and phenotypic abnormalities stemming from arginine accumulation in these mice have been recapitulated in 2 different conditional adult-onset knockout mouse models generated using tamoxifen-inducible Cre-Lox mice with floxing of *Arg1* exons 7 and 8.[40,43] As is characteristic of the exon 4–targeted *Arg1* knockouts, the adult-onset models demonstrate hyperargininemia upon tamoxifen induction and ensuing signs of physical, neuromotor, and behavioral abnormalities such as growth disparity, weight loss, hunched body posture, difficulty standing, gait abnormalities (e.g., instability, staggering, irregular steps, and shortened stride length), and progressive ataxia.[40,43] Also like the exon 4–targeted knockouts, both inducible models follow a rapid course of metabolic disruption, including increased arginine, guanidino compounds, and ammonia, among other perturbations, as well as lethal hyperammonemia within 2–3 weeks.[40,43] A consistent observation across all three models is that hyperargininemia is established by the time that phenotypic abnormalities become apparent, suggesting that the phenotype may be biochemically driven by accumulation of arginine and arginine metabolites resulting from

hepatic *Arg1* disruption.[37,40,43] This hypothesis is further supported by a recent characterization of another genetic mouse model that lacks expression of *Arg1* in neural cells only.[44] Although small decreases were noted in the volume of two brain structures involved in motor activity of these neural-specific knockouts, others were unchanged and their gait was largely unaltered compared with wild-type controls. Assessment of blood amino acids revealed that arginine levels were also unchanged in mice lacking neural expression of *Arg1*. The striking contrast between this genetically manipulated model and the global or liver-specific *Arg1* knockouts led the authors to two important conclusions: (1) that hyperargininemia and neurologic manifestations of ARG1-D are driven by the toxic metabolic environment that results from loss of hepatic arginase 1, and (2) that reducing arginine levels in the blood represents the best chance to avoid neurologic manifestations.[44]

Restoration of *Arg1* in transgenic mouse models of ARG1-D has provided further evidence of a key role of arginine in disease manifestations, as pioneered by Gerald Lipshutz's group at UCLA. In an early proof-of-concept study, the exon 4–targeted *Arg1* knockout mice[37] were treated with *Arg1* gene transfer delivered using an adeno-associated viral (AAV) vector administered on the second day of life.[38] Whereas all untreated knockouts died within 24 days, AAV-treated mice (i.e., those with hepatic arginase 1 restored) demonstrated normal plasma arginine levels, less-severe hyperammonemia, improved weight gain, and prolonged survival with 89% alive through >8 months. Results of a detailed characterization of the biochemical, neuromotor, and neurobehavioral phenotype of knockout mice with AAV-mediated restoration of *Arg1* also supports this hypothesis.[39] Brain development at 4 months was similar between treated knockouts and wild-type littermates, with no abnormalities or lesions evident in key brain structures such as olfactory bulbs, cerebral cortex, basal ganglia, hippocampus, thalamus, cerebellum, or pons. A battery of functional assessments revealed no differences in exploratory activity, cerebellar function, spatial learning, or behavioral responses, or in body posture, tremor, locomotor activity, gait, grip strength, or righting reflexes. Notably, normal brain development and neurologic phenotype were observed in the context of reversal of metabolic abnormalities. By 3 weeks after AAV-mediated gene transfer, brain and serum levels of arginine were normalized or below control levels. Furthermore, serum guanidino compounds, which were markedly elevated in untreated knockout mice, were decreased to near-normal levels in sera and brain tissue of treated knockouts.[39]

The prominent, progressive spasticity observed in patients with ARG1-D and the analogous neuromotor abnormalities observed in *Arg1*-deficient mice prompted further investigations of the motor cortex in untreated knockout mice and knockouts with AAV-mediated *Arg1* hepatic gene therapy.[41,45] Altered circuitry in the motor cortex was observed in untreated knockouts at postnatal day 15 (after development of hyperargininemia) with decreased dendritic arborization, decreased numbers of excitatory and inhibitory synapses, and abnormal synaptic transmission, suggesting a potential arginine-driven neural mechanism of motor dysfunction in ARG1-D.[45] Neuronal structure and cortical circuitry were virtually normal in knockout mice with neonatal *Arg1* restoration.[45] In a second study, microarray expression analysis in untreated *Arg1* knockouts suggested abnormalities in myelinating oligodendrocytes that were supported by evidence of marked subcortical dysmyelination in key motor structures including the corpus callosum and caudate putamen.[41] Compared with wild-type mice, the *Arg1*-deficient mice had fewer myelinated axons in the motor cortex, pyramidal tract, and corticospinal tract, as well as decreased thickness of the myelin sheath where myelination was evident; axonal degeneration and decreased dendritic complexity were also observed. Among the knockouts receiving AAV treatment at postnatal day 2, myelinated axon density, oligodendrocyte wrapping of axons, and axonal integrity were largely normal, indicating prevention of neural abnormalities through restoration of functional arginase 1 and normalization of plasma arginine.[41] The results of these studies implicate arginine levels, rather than residual or induced brain arginase 1 or 2. Wild-type mice express only low levels of arginase 1 and arginase 2 confined to specific brain areas. Furthermore, there is little to no increase in brain arginase 2 in *Arg1* knockout animals, and little to no increase in brain arginase 1 in genetically corrected *Arg1* knockouts. Finally, in a more recent mouse model also spearheaded by the Lipshutz group, a lipid nanoparticle carrying human *Arg1* mRNA was administered intermittently to constitutive *Arg1* knockout mice to restore their hepatic arginase 1 activity.[46] Normalization of plasma arginine and reductions in guanidino compounds were achieved in the mice receiving lipid nanoparticle/*Arg1* mRNA compared with untreated knockouts and wild-type controls; these biochemical changes were associated with a dramatic recovery of myelin density, increased myelin sheath thickness, and normal growth and survival.[46]

This evidence of abnormal neurophysiology and dysmyelination is consistent with the limited available observations reported in children with ARG1-D. In one patient with toe walking and spastic paraplegia, among other signs of pyramidal tract dysfunction affecting his lower limbs, neurophysiologic assessment revealed prolonged latency of motor evoked potentials, indicating

involvement of the corticospinal tract.[47] In another case, a patient exhibited the characteristic ARG1-D clinical profile and trajectory, with an uneventful infancy before insidious onset of progressive neurologic deterioration.[48] At 2.5 years of age, spastic diplegia and permanent loss of speech were evident, followed by loss of locomotion over the course of several months, and ultimately spastic tetraplegia and reliance on a wheelchair at only 3 years and 10 months of age. Plasma ammonia was only slightly elevated above normal levels, whereas arginine levels were nine-fold higher than normal in the plasma and 2.5-fold higher than normal in the CSF. Electroencephalography (EEG) showed multifocal discharges with abnormal background activity and absence of short latency responses in brainstem evoked potentials. Magnetic resonance imaging revealed dysmyelination as well as an undersized cerebellum, enlarged cerebral ventricles, and thinning of the corpus callosum. At 9 months after diagnosis of ARG1-D and initiation of dietary restriction, background activity on EEG was normalized and short latency responses were improved in parallel with lowering of arginine levels. Increased severity of protein restriction produced further reductions in arginine that were accompanied by additional improvement or normalization of brainstem evoked potentials. Importantly, objective improvement of neurologic function was reflected through improvements in alertness and motor activity.[48] Lastly, a patient evaluated in a study conducted with the Urea Cycle Disorders Consortium (UCDC) was also found to have corticospinal tract abnormalities consistent with his neuromotor deficits.[49] This patient exhibited characteristics not uncommon in ARG1-D in the early postnatal period, including poor feeding, vomiting, and poor growth; however, developmental milestones were normal. Before diagnosis at 4 years of age, he demonstrated increasing fall frequency and decreasing motor skills followed by development of significant lower-limb spasticity and seizures. At diagnosis, plasma arginine was predictably elevated and treatment with dietary restriction and ammonia diversion was initiated. At 16 years of age, his cognitive performance with regard to visual memory and language was normal and he was succeeding scholastically, but impairments in complex problem-solving and organization were evident in addition to impaired motor strength. At 17 years, he was ambulatory without orthotics or assistive devices but had increased tone, hyperreflexia, and clonus in the lower extremities consistent with the spasticity that manifested in his early childhood. Diffusion tensor magnetic resonance imaging revealed altered integrity and microstructural damage in the white matter of regions involved in motor function—specifically, the central pons extending into the cerebellum at the level of corticospinal tract crossing as well as adjacent to the corpus callosum. There was also a significant reduction in corticospinal tract fiber count compared with matched control subjects, further indicating neuronal damage to motor circuitry in ARG1-D.[49] Abnormalities in neuromotor circuitry and corticospinal tract damage in particular have not been reported in patients with more proximal UCDs and were not observed in patients with ornithine transcarbamylase deficiency ($n = 23$) in the UCDC diffusion tensor imaging study, which further implicates arginine in the pathology of ARG1-D.[49]

We believe that these neurologic abnormalities reflect the neurotoxic effects of arginine and/or the guanidino compounds that accumulate in conjunction with, and as a result of, arginine elevation.[8,11,12,17] Guanidino compounds, both as a class and individually, have neurotoxic effects on the brain and on brain cells in culture.[16,18] For example, guanidino compounds that are increased in ARG1-D, such as guanidinoacetic acid and guanidinovaleric acid, have been empirically shown to induce epileptiform and convulsive activity in rodents.[18] Likewise, argininic acid and guanidinovaleric acid, at levels comparable to those observed in ARG1-D, alter evoked depolarizing responses in cultured spinal cord neurons.[50] Although this neurotoxicity has been demonstrated independent of the effects of arginine, guanidino compounds (and their effects) in ARG1-D are inextricably linked with elevation of arginine, the proximal substrate.[42] It has also been suggested that ornithine deficiency, which is observed in pyrroline-5-carboxylate synthetase deficiency (P5CSD), or distorted arginine/ornithine imbalance, which occurs in hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome, may play a role in the spasticity sometimes observed in these two disorders.[34,51–53] However, ornithine deficiency is not an established feature of ARG1-D, and both P5CSD and HHH syndrome differ from ARG1-D in that spasticity occurs with variable prevalence and late onset, in contrast with the regular and relentless occurrence of spasticity in ARG1-D. As such, they would not support ornithine deficiency as an important pathogenic contributor in ARG1-D. Lastly, downstream effects of excessive arginine on nitric oxide and promotion of oxidative stress and excitotoxicity have also been hypothesized.[28,49]

1.3 | Clinical evidence implicates arginine in development and progression of ARG1-D manifestations

With an estimated global prevalence of only 1:726000,[54] the rarity of ARG1-D poses a challenge to characterizing the pathophysiology and mechanisms driving progression

in humans. Most clinical evidence to date is largely anecdotal and based on individual cases, familial series, or retrospective case analyses. Nonetheless, the clinical evidence is consistent with empirical evidence from mechanistic studies in mouse models.

The first ARG1-D patients described in the literature were three female siblings, the older two of whom developed seizures, psychomotor delays, and spasticity within the first years of life.[55–57] At presentation and biochemical evaluation for the two older sisters (ages 5 years and 1.5 years), significantly diminished/near-undetectable arginase one enzymatic activity and markedly increased arginine levels in serum and CSF were evident; only mild hyperammonemia was observed. Because of this family history, a third sister was assessed at birth and was found to also have ARG1-D.[57] Despite early initiation of treatment with a low-protein diet at 8 weeks of age, her plasma arginine remained significantly elevated at ~800 μmol/L and onset of motor abnormalities was evident by age 5 months. She experienced a progressive clinical course similar to her siblings, with psychomotor delays and lower-limb spasticity evident by 3 years of age.[57]

Over the ensuing five decades since these first patients were described, a clear clinical and pathophysiologic profile of ARG1-D been borne out with striking consistency through numerous reports: deleterious effects of high arginine and/or dysregulation of arginine metabolites become evident typically in the first years of life and increase in severity and extent throughout the patient journey.[2–4,7,9,28,30,32,34,47,58–65] Elevated plasma arginine, whether as the primary driver or proximal causal component of downstream toxicity, is associated with progressive intellectual disability, global developmental delay, seizures, and uniquely, with progressive spasticity. Of note, seizures in ARG1-D patients are not attributed exclusively to hyperammonemic events.[8,12] The neurotoxic effects of guanidino compounds, which increase in the plasma and CSF as a result of elevated plasma arginine, contribute to seizure susceptibility.[16–18] The morbidity associated with ARG1-D puts these patients at risk for early mortality.[30] The specific factors driving early mortality in ARG1-D are not yet clear; precipitating events reported in the literature are diverse and the end stages of disease remain to be more fully characterized.

A detailed clinical characterization of the relationship between biochemistry and functional outcomes in multiple UCDs, performed by the UCDC, more directly implicates chronic high arginine as the driver of development and progression of ARG1-D manifestations.[36] Patients with ARG1-D were at greater risk than those with other UCDs for low IQ and poor performance in all neuropsychologic domains assessed. Consistent with the known biochemical profiles of ARG1-D and other UCDs, mean lifetime plasma ammonia was lower in the ARG1-D cohort compared with other UCDs (mean, 78.87 μmol/L vs. 127.22–139.59 μmol/L) and hyperammonemic episodes were less common. Elevated arginine (several fold normal in the ARG1-D cohort) was more tightly associated with poorer functioning in global and memory domains than any other biochemical marker evaluated, and higher plasma arginine levels were significantly correlated with poorer motor composite scores. Lastly, increasing cumulative arginine exposure (in terms of longer duration of disease) was an indicator of worse neuropsychiatric outcome among patients with ARG1-D. Individual case reports of patients with long-term follow-up have also documented phenomena strongly suggestive of arginine toxicity in ARG1-D. Periods of worsening hyperargininemia, whether because of poor treatment adherence or other factors such as biologic stressors, were accompanied by worsening of cognitive and mobility impairments that returned to patients' functional baseline upon re-establishment of their typical plasma arginine levels.[59,65–67]

In the first reports of ARG1-D treatment, dietary protein restriction was able to control hyperammonemia but not plasma arginine (which was lowered from pretreatment levels but remained elevated), and achieved only limited clinical improvement.[10,55–57] An important outcome of this early work was the exclusion of ammonia and the implication of arginine as a direct driver of disease pathophysiology; additionally, these reports established the challenge of lowering arginine levels in patients with ARG1-D. In subsequent reports, rigorous management with chemically defined amino acid diets was more effective, lowering plasma arginine and achieving clinical stability or improvement even in patients with advanced/severe disease **(**Table 1**)**.[9,48,59,61] In adolescent/teenage siblings with established neurocognitive and neuromotor manifestations of ARG1-D, lowering of plasma arginine with a chemically defined diet resulted in clinical improvement.[9,61] Specifically, spasticity was lessened and mobility improved, independent feeding and toilet training were regained, and language improved.[61] Initiation of treatment in younger patients has been described to yield more meaningful clinical benefits.[58,63] In a patient with overt cognitive impairment, lower-limb spasticity, and toe walking, treatment was initiated upon diagnosis at age 7 years.[63] Limitation of natural protein lowered her plasma arginine by approximately 50%, from 8-fold to 4-fold normal levels. Further restriction with a second dietary formulation was able to reduce plasma arginine to near the upper limit of

TABLE 1 Clinical Evidence Supporting the Effectiveness of Arginine Reduction* for Improving Outcomes in ARG1-D

	Pre-treatment History of Manifestations	Plasma Arginine[a]	Clinical Outcomes
Case report			
Cederbaum[9] Cederbaum[61]	• 6 years: severe spasticity, hyperreflexia • 15 years: progressive physical and intellectual deterioration (severe psychomotor impairment with no speech or language comprehension, no interaction with environment, severe spasticity with difficulty moving, decreased gag reflex, poorly coordinated swallowing)	• Pretreatment: 7-fold ULN • Initial restricted diet: 5- to 6-fold ULN • Stricter diet: 2-fold ULN	• Regained ability to dress, feed self, brush teeth, use toilet independently • Improved language and regained capacity to respond to simple commands • Diminished spasticity and improved mobility
Cederbaum[9] Cederbaum[61]	• 2.5 years: clumsiness, hyperreflexia, ankle clonus, spasticity (predominantly affecting lower limbs) • 8 years: wheelchair-dependent, severe psychomotor impairment with no speech and minimal language comprehension, no bladder/bowel control, tiptoe gait, reduced gag reflex, poorly coordinated swallowing	• Pretreatment: 5-fold ULN • Initial restricted diet: 4-fold ULN • Stricter diet: near-normal	• Regained ability to speak and construct phrases/sentences • Regained bowel/bladder control • Regained ability to feed self, brush teeth independently • Improved concentration • Improved spasticity and mobility
Brockstedt[48]	• 2.5 years: spastic diplegia, loss of speech, worsening of mobility impairment • 3 years 10 months (diagnosis): intellectual disability, no intelligible speech, spastic tetraplegia (predominantly affecting lower limbs), wheelchair-bound, no reproducible short latency response in brainstem acoustic evoked potentials	• Pretreatment: 907 μmol/L (9-fold ULN) • Initial restricted diet: 4-fold ULN • Stricter diet: 2- to 3-fold ULN	• Improved alertness and motor activity • Improved/normalized brainstem evoked potentials
Lambert[63]	• 5 years: motor and cognitive impairment • 6 years 1 month: lower-limb spasticity, hyperreflexia, tiptoe gait, • 6 years 11 months (diagnosis): spasticity, tiptoe gait, hyperactivity • 7 years 7 months: progressive worsening of motor deficits	• Pretreatment: 895 μmol/L (10.5-fold ULN) • Initial restricted diet: 6-fold ULN • Stricter diet: 1.7-fold ULN	• Progressive improvement of muscle strength, mental skills, and mobility • Patient a community ambulator; able to run, ride bike, climb stairs
Snyderman[58]	• 3 months: vomiting, lethargy, tremor • 5 months: seizures, hyperreflexia, bilateral ankle clonus • 20 months: seizure recurrence, ataxia • 4 years (diagnosis): intellectual disability, tiptoe gait, ataxia, hyperactivity	• Pretreatment: 9.4 mg/dL (6-fold ULN) • Initial restricted diet ineffective • Stricter diet: 2- to 3-fold ULN	• Reduced hyperactivity • Improved ataxia and coordination • Improved mental capacity

(Continues)

TABLE 1 (Continued)

	Pre-treatment History of Manifestations	Plasma Arginine[a]	Clinical Outcomes
Snyderman[5]	• 2.5 years: vomiting, lethargy, hyperreflexia • 3 years 7 months (diagnosis): intellectual disability, developmental delay, hyperactivity, tiptoe gait, lower-limb spasticity	• Pretreatment: 9.95 mg/dL (7-fold ULN) • Initial restricted diet: 4-fold ULN • Stricter diet: 2- to 3-fold ULN	• Reduced hyperactivity • Improved ataxia and coordination • Improved mental capacity
Snyderman[59]	• Patient identified at and treated from birth (pre-symptomatic)	• Until 4 months of age: normal • Beyond 4 months: 2- to 3-fold ULN	• Physiologically, neurologically, and mentally normal • Average developmental assessments, through 2.5 years
Observational study			
Huemer[28]	• Three patients identified at and treated from birth (pre-symptomatic)	• Pretreatment: not applicable • Last follow-up at age 1–3 years: 256–574 μmol/L (ULN not available)	• Last follow-up at age 1–3 years: asymptomatic course in all three patients

Abbreviation: ULN, upper limit of normal.

[a]ULN reflects normal range or control range as defined in each case report.

*Arginine-lowering intervention comprised dietary protein restriction with essential amino acid supplementation for all patients.

normal, which was accompanied by substantial lowering of guanidino compounds. Over 2.5 years of treatment, the patient's cognitive function improved, spasticity was markedly decreased, and muscle strength improved; the patient was ultimately able to run, climb stairs without support, and ride a bicycle, and was a community ambulator.[63] Treatment from birth has actually been shown to delay or reduce progression, with some patients showing no overt manifestations of ARG1-D through 5 years of age (Table 1).[28,59] For example, in a 2016 case series, three patients ascertained through newborn screening and treated from infancy remained clinically asymptomatic through toddlerhood, the age of most recent follow-up.[28]

Additional clinical evidence supporting the relationship between plasma arginine levels and clinical outcomes comes from a 1984 case report of a boy with ARG1-D who lost his ability to stand, sit, or crawl by himself at the age of 3 years and was also experiencing rigidness in his legs and spastic quadriplegia. At the age of 5 years, an experimental therapeutic approach involving transfusion of healthy red blood cells (i.e., with functional arginase) was used to lower plasma arginine.[23,68] This approach, though not without limitations precluding clinical adoption, markedly decreased arginine levels in the serum and, as a result, in the CSF and resulted in clear clinical improvements, such that the boy became able to sit and roll by himself and manifested reduced spasticity.[68]

1.4 | Current management is insufficient to maintain adequately low arginine and does not prevent ARG1-D progression in the long term

Dietary protein restriction to lower plasma arginine levels is the mainstay of management for all UCDs, with particularly extreme protein restriction required in ARG1-D.[20] The flux of arginine into plasma depends on three key sources (dietary arginine intake, de novo arginine synthesis via the intestinal-renal axis, and whole-body intracellular protein turnover), of which the endogenous flux from protein turnover is the major contributor in humans.[15] Dietary restriction in ARG1-D is focused on limiting exogenous supply of arginine, but cannot address the endogenous production[15]; even during periods of good adherence to a restrictive diet, elevated arginine levels can persist.[3] As in all UCDs, ammonia is a product of the catabolism of every amino acid and the flux through the cycle is far greater than the flux for the catabolism of other individual carbon skeletons. Patients with ARG1-D may receive nitrogen scavengers as part of their management[20] to address ammonia levels but also to lower arginine levels based on a potential downstream effect of offloading nitrogen from the urea cycle. The combination of dietary protein restriction and ammonia diversion therapy can stabilize the ammonia levels in all UCDs in non-catabolic situations but is insufficient to lower plasma arginine levels to anywhere near the

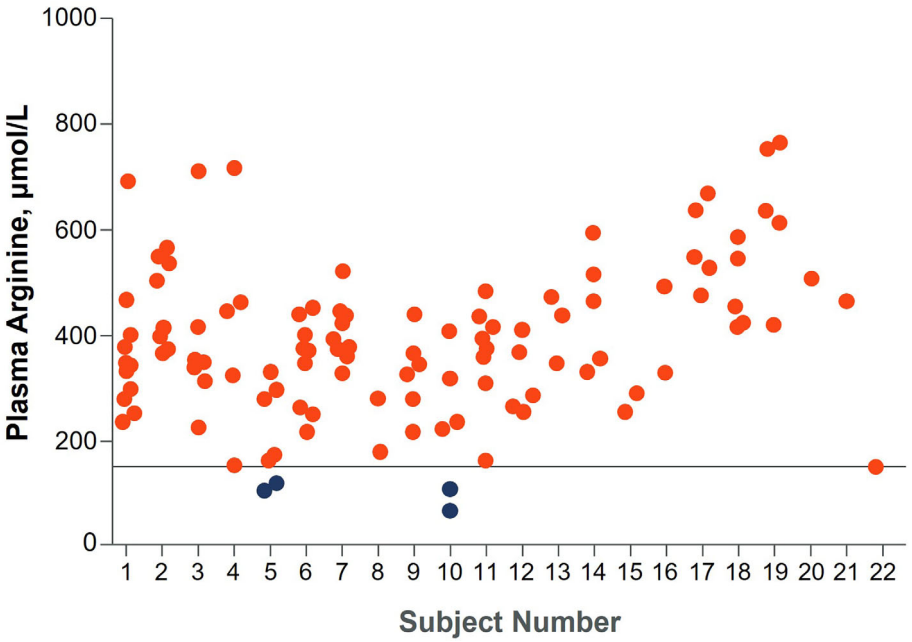

FIGURE 2 Plasma Arginine Levels With Current Standard of Care. Analysis of data from patients ($n = 22$) with Arginase 1 Deficiency in the Urea Cycle Disorder Consortium database. Dashed line indicates upper limit of normal applied to the study's laboratory assessments; current guidelines recommend maintaining plasma arginine <200 µmol/L. Blue dots represent arginine levels below the applied upper limit of normal of 150 µmol/L. Adapted with permission from Burrage LC, Sun Q, Elsea SH, et al. *Hum Mol Genet*. 2015;24 (22):6417–6427. doi:10.1093/hmg/ddv352

normal range in ARG1-D.[60] Nonetheless, as is evident throughout the literature and based on our clinical experience, even suboptimal reduction of plasma arginine can halt or delay progression and even improve patient outcomes, demonstrating the importance of effective arginine-lowering management approaches.

With currently available approaches, achieving and maintaining adequate reduction of plasma arginine in the long term is extremely difficult; thus, the guideline-recommended level of <200 µmol/L[20] is rarely achieved. As a result, most patients deteriorate over time and poor long-term outcomes are the norm.[2,6,28,65] In an analysis of published case reports of patients with ARG1-D, the median plasma arginine level (reported at any time in the patient journey, $n = 112$ patients) was 572 µmol/L; levels under treatment with dietary protein restriction ($n = 33$ patients) were also markedly elevated at a median of 400 µmol/L.[30] Even with a relatively young median age of 11 years in these patients, significant disease progression was evident, with lower-limb spasticity and intellectual disability reported in 84% and 82%, respectively.[30] Similarly, analysis of UCDC plasma arginine data from patients receiving standard of care management (22 patients; 1–13 measurements per patient) revealed that nearly all samples evaluated (97%) were above 150 µmol/L (value used as the upper limit of normal) and very few were <200 µmol/L; no patient had levels in the normal range in all samples/timepoints assessed **(**Figure 2**)**.[60] Median age at the most recent visit was 14.75 years; diagnosis was made at a median age of 3.25 years. Despite standard of care management in this cohort and the relatively young age at diagnosis/treatment initiation and follow-up, 89% of patients had developmental delay or intellectual disability; abnormal reflexes and abnormal tone were evident in 53% and 63% of patients, respectively, and 60% were nonambulatory.[60]

2 | SUMMARY

Collectively, the scientific literature demonstrates a key mechanistic role of elevated arginine as the proximal or direct driver of disease in ARG1-D. The ARG1-D biochemical profile and clinical manifestations are distinct from most other UCDs in which hyperammonemia is the primary concern. Persistent high plasma arginine in ARG1-D is accompanied by consistently and progressively manifesting debilitating neurologic and functional impairments, whereas reducing plasma arginine improves manifestations even in patients with established disease. Since tissue and CSF levels of arginine are in equilibrium with those of plasma, lowering plasma arginine to guideline-recommended levels is an important therapeutic approach, and is supported by improvements in neurologic and functional manifestations occurring with modulation of plasma arginine levels in ARG1-D demonstrated both in animal models and clinically. Furthermore, worsening severity of disease manifestations during acute decompensation events indicate toxic effects of spikes in arginine levels. Even when lowering of arginine is suboptimal, intervention with current management approaches has been shown to delay development of manifestations and to reverse many aspects of established cognitive and mobility impairment at both

the neurophysiologic and functional levels. However, the aggregate data from the UCDC highlight the difficulty in maintaining adequately low arginine levels with the current standard of care, even at highly specialized centers and with rigorous individualized disease management strategies; long-term outcomes remain poor with many patients developing significant disability over time. There is an urgent need for effective treatments that maintain long-term reduction, or even normalization, of plasma arginine levels in patients with ARG1-D to address the underlying mechanism of disease, thereby preventing progression and improving outcomes.

3 | PERSPECTIVE

As we seek better outcomes in ARG1-D, improvements in newborn screening algorithms for ARG1-D will allow diagnosis to be made with high sensitivity and specificity.[35,69] Whereas standard of care can maintain arginine levels in an acceptable therapeutic range for the first months or years of life, we have seen how difficult this can be in the longer term. Enzyme therapy has been shown to lower plasma arginine levels to the therapeutic range and to substantially reduce guanidino compounds; these biochemical changes are accompanied by meaningful improvements in mobility.[70–72] This potential therapy currently awaits FDA approval and represents the first step in advancing treatment of ARG1-D, which has been awaiting a therapeutic breakthrough for 40 years. Both gene and mRNA therapies have been validated in animal models[39,73] and must be demonstrated to be effective and safe in humans before they can be considered part of this more hopeful future of ARG1-D treatment. One of the authors (SDC) has been investigating ARG1-D for nearly 50 years and has been hoping for these promising therapeutic breakthroughs.

AUTHOR CONTRIBUTIONS

George A. Diaz: planning and design, drafting the article, revising the article critically for important intellectual content, and approval of the final version of this work.
Mark Bechter: planning and design, drafting the article, revising the article critically for important intellectual content, and approval of the final version of this work.
Stephen D. Cederbaum: planning and design, drafting the article, revising the article critically for important intellectual content, and approval of the final version of this work.

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ETHICS STATEMENT

All authors were compliant and followed the ethical guidelines, according to the requirements of JIMD.

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CONFLICT OF INTEREST

Dr Diaz has served as an advisor and clinical trial investigator for Aeglea. Dr Cederbaum has served as a consultant and/or advisor for several biopharmaceutical companies, including Aeglea. Dr Bechter is an Aeglea employee (Medical Affairs). No author received compensation for their role in writing this article.

DATA AVAILABILITY STATEMENT

There is no data associated with this manuscript.

INFORMED CONSENT/ANIMAL RIGHTS

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Arginine to ornithine ratio as a diagnostic marker in patients with positive newborn screening for hyperargininemia

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ABSTRACT

Arginase deficiency is a rare inborn error of metabolism that interrupts the final step of the urea cycle. Untreated individuals often present with episodic hyperammonemia, developmental delay, cognitive impairment, and spasticity in early childhood. The newborn screening (NBS) algorithms for arginase deficiency vary between individual states in the US but often include hyperargininemia and elevated arginine to ornithine (Arg/Orn) ratio. Here, we report 14 arginase deficiency cases, including two patients with positive NBS for hyperargininemia in whom the diagnosis of arginase deficiency was delayed owing to normal or near normal plasma arginine levels on follow-up testing. To improve the detection capability for arginase deficiency, we evaluated plasma Arg/Orn ratio as a secondary diagnostic marker in positive NBS cases for hyperargininemia. We found that plasma Arg/Orn ratio combined with plasma arginine was a better marker than plasma arginine alone to differentiate patients with arginase deficiency from unaffected newborns. In fact, elevated plasma arginine in combination with an Arg/Orn ratio of $\geq$1.4 identified all 14 arginase deficiency cases. In addition, we examined the impact of age on plasma arginine and ornithine levels. Plasma arginine increased 0.94 μmol/L/day while ornithine was essentially unchanged in the first 31 days of life, which resulted in a similar increasing trend for the Arg/Orn ratio (0.01/day). This study demonstrated that plasma Arg/Orn ratio as a secondary diagnostic marker improved the detection capability for arginase deficiency in newborns with hyperargininemia, which will allow timely detection of arginase deficiency and hence initiation of treatment before developing symptoms.

Abbreviations: NBS, newborn screening; Arg, arginine; Orn, ornithine; Arg/Orn, arginine to ornithine ratio; DOL, day of life; DBS, dry bloodspot; ROC, receiver operating characteristic.

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1. Introduction

Arginase, sometimes referred to as arginase-1 (E.C·C 207800), is the final enzyme in the urea cycle, a six-enzyme, two-transporter pathway responsible for the detoxification of ammonia in the body and its conversion to urea [1]. The inherited deficiency of arginase has been shown to cause a unique syndrome, the hallmarks of which are high plasma arginine (Arg) levels, low to absent (<1% of normal) arginase activity in red blood cells, progressive spasticity, and slowing and eventual loss of cognitive milestones [2]. Current standard treatment for arginase deficiency includes lowering plasma arginine levels by dietary protein limitation, essential amino acid supplementation, and nitrogen scavengers which may have a favorable outcome both in preventing the progression of symptoms and partial reversal of some symptoms [2,3]. Moreover, enzyme replacement therapy and various DNA and RNA therapies are in clinical or preclinical development [4]. Thus, it is essential to identify patients presymptomatically and treat cases as early as possible. Most importantly, cases should not be missed. The advent of expanded newborn screening (NBS) has enabled the early diagnosis of arginase deficiency in many patients with the condition in countries with this program. Screening has allowed patients to be identified prior to the development of symptoms [5]. In the US, the U.S. Recommended Uniform Screening Panel included hyperargininemia as a secondary target for newborn screening. The primary marker for screening is the arginine level.

Arginine is a conditionally essential amino acid that plays an important quantitative and qualitative role in a number of biological pathways. It is a precursor for nitric oxide, polyamines, creatine and possibly glutamate and proline, especially in the postnatal period. The plasma arginine (Arg) level is influenced by dietary intake, endogenous synthesis both within and independent of the urea cycle, and protein turnover [6]. Given that plasma Arg level could be normal in newborns with arginase deficiency, it is necessary to utilize a secondary marker to improve test sensitivity for arginase deficiency. We have demonstrated that newborn screening can effectively and efficiently identify newborns with arginase deficiency. The use of the ratio of arginine to ornithine (Arg/Orn) or to the product of phenylalanine×leucine as secondary markers will ascertain virtually all affected individuals, with an acceptable false positive rate [5]. The challenge now is to be equally effective with clinical confirmation and treatment.

Here, we report two patients who were positive for arginase deficiency on newborn screening but who received delayed diagnoses because follow-up testing indicated normal or near normal plasma arginine levels. By incorporating the plasma Arg/Orn ratio as a secondary diagnostic marker, these two patients would have received diagnoses earlier, and the symptoms of arginase deficiency in one patient could have been prevented or lessened. An algorithm was developed using the data from 12 other patients who received a diagnosis of arginase deficiency during the newborn period and the unselected newborn population. We also showed that plasma arginine (Arg) and ornithine (Orn) levels, and the Arg/Orn ratio were relatively stable over the first month of life, obviating the need to stratify the control data.

2. Methods

2.1. Study design

In this study, we aimed to evaluate the plasma Arg/Orn ratio as a secondary diagnostic marker in newborns with positive NBS for hyperargininemia. A study request email was sent to Metab-1, an electronic mailing list for professionals in the field of inborn errors of metabolism. Cases with a confirmed diagnosis of arginase deficiency by molecular analysis, arginase activity assay in red blood cells or both were collected from respondents. The plasma Arg, Orn, and Arg/Orn ratio from these cases were analyzed and compared to those in newborns without arginase deficiency in the Quest Diagnostics database. In addition, we evaluated the correlation between age in days and plasma amino acid levels for Arg, Orn, and the Arg/Orn ratio in the unselected newborn population. The institutional review board at UCLA reviewed the study protocol and granted permission for this study.

2.2. Data collection

Patient data and demographics were collected from care providers with all protected health information removed. The results of NBS and plasma Arg, Orn, and Arg/Orn ratio levels from 14 individuals with arginase deficiency were collected for this study. The plasma Arg and Orn levels from individuals without a confirmed diagnosis of arginase deficiency between 0 and 31 days of life were acquired from the Quest Diagnostics (San Juan Capistrano, CA USA) Biochemical Genetics Laboratory database, which included 6587 samples over 6 years spanning January 2013 to December 2019.

2.3. Data analysis

The plasma Arg, Orn, and Arg/Orn levels from the 14 cases with arginase deficiency were compared to the distributions from newborns without arginase deficiency. The 97.5th percentile of this population was determined and McNemar's test was used to compare the number of cases below the 97.5th percentile between plasma arginine and Arg/Orn ratio [7]. The area under the receiver operating characteristic curves (AUC) formed using Arg and Arg/Orn ratio to discriminate between those with and without arginase deficiency were also compared by Delong's test to compare two correlated ROC curves [8]. The relationships between age and plasma Arg, Orn, and Arg/orn ratio were analyzed by linear regression. The p value <0.05 was considered statistically significant. Analysis was performed using R software version 3.4.3 [9].

3. Results

3.1. Delayed diagnosis of arginase deficiency owing to normal plasma arginine level

Patient 1 was a 4-year-old male who was born at full term following an uncomplicated pregnancy. An NBS sample collected at 17 h of age was positive for hyperargininemia (101 μmol/L, cutoff <50 μmol/L) and elevated Arg/Orn ratio (10.1, cutoff <1.4). Confirmatory tests including plasma amino acids, ammonia, and comprehensive metabolic panel were sent on day of life (DOL) 5. The plasma Arg level was within the normal range (134 μmol/L, normal range 14–135 μmol/L). He had a normal history and examination at the follow-up clinic visit. The patient was discharged with a false-positive newborn screening result for hyperargininemia. After a period of normal development, the patient developed bilateral lower extremity spasticity at 18 months of age. Plasma Arg was markedly elevated at 587 μmol/L (normal range 30–147 μmol/L). Results of an RBC arginase enzyme activity assay confirmed arginase deficiency with undetectable enzyme activity. Since the diagnosis, the patient has been on protein restriction with nonessential amino acid-free formula and has shown improvement in development and spasticity.

Patient 2 was a 4-year-old male who was born at 38 weeks following an uncomplicated pregnancy. NBS performed at 25 h of life showed elevated arginine (68 μmol/L, cut-off <50 μmol/L) and Arg/Orn ratio 4.4 (cut-off <1.4). Plasma amino acid performed on DOL5 showed mildly elevated arginine (150 μmol/L, normal range 14–135 μmol/L) with normal ammonia level. Repeated plasma amino acid levels were mildly elevated for arginine. The child continued to have normal growth and development. Subsequently, RBC arginase enzyme activity assay and plasma amino acid were performed simultaneously when the patient was 7-month-old. Plasma arginine level was normal (113 μmol/L, normal range 12–133 μmol/L) while RBC arginase enzyme activity was

undetectable, confirming arginase deficiency. The patient was followed in a clinic monthly. Plasma amino acids were also monitored monthly, and treatment was initiated following an arginine level of 402 μmol/L (normal range 30–147 μmol/L) at 9 months of age. With protein restriction and glycerol phenylbutyrate (Ravicti®) treatment, he continues to have a normal neurologic exam, though is noted for speech delay.

3.2. Arginine to ornithine ratio as a secondary marker for arginase deficiency

Given that plasma Arg levels can be normal in patients with arginase deficiency during the neonatal period, it is clearly necessary to add a secondary marker to improve the diagnostic sensitivity in NBS cases positive for hyperargininemia and arginase deficiency. The Arg/Orn ratio and other ratios have been popular second-tier discriminators used in NBS to reduce the number of false positive cases. Therefore, we collected the plasma Arg, Orn, and Arg/Orn ratio data on their initial NBS follow-up from 14 arginase deficiency patients identified by screening in the newborn period (Table 1). We found that all cases of arginase deficiency had an Arg/Orn ratio equal to or greater than 1.7 (range 1.7–16.0). Interestingly, the two cases with delayed diagnoses have the lowest plasma arginine levels as well as the lowest Arg/Orn ratios of the group. To compare the validity of using the plasma Arg level and the Arg/Orn ratio to discriminate true positives from false positive cases, we evaluated the distribution of Arg and Arg/Orn ratio in neonates with and without arginase deficiency. Data from the unselected newborn population revealed plasma Arg levels ranging from 10 to 191 μmol/L (2.5–97.5%tile), Orn levels from 27 to 312 μmol/L (2.5–97.5% tile), and Arg/Orn ratios from 0.1–1.6 (2.5–97.5%tile). Four of 14 (29%) of patients with arginase deficiency had an initial plasma Arg level (range 134–192 μmol/L) below or slightly above the 97.5th percentile (191 μmol/L) in unselected newborn population, while all 4 patients had an Arg/Orn ratio below the 97.5th percentile (ratio 1.6), which was a statistically significant difference ($p = 0.046$, Fig. 1). In addition, discrimination of newborns with arginase deficiency and unselected newborn population by ROC curve analysis was better with Arg/Orn ratio than with arginine alone (AUC = 0.998 vs. 0.980, respectively; p-value = 0.005).

3.3. Trend of plasma arginine and ornithine levels in neonatal period

A previous study suggests that plasma Arg levels change with age in children [10], which could have an impact on the diagnosis of arginase deficiency. To examine the age-related change in plasma Arg levels, we analyzed plasma Arg levels of 6587 unselected newborn population (0–31 days) obtained from Quest Diagnostics database (Fig. 2). We found that plasma Arg levels increased with age, averaging 0.94 μmol/L per day in the first 31 days of life. Plasma Orn levels, in contrast, were essentially unchanged during the neonatal period (−0.02 μmol/L per day). As a result, the Arg/Orn ratio showed a similar trend of increasing with age (0.01 per day) as the Arg alone.

4. Discussion

Arginase deficiency is a rare urea cycle disorder with an estimated minimal incidence of 1 in 1.1 million newborns in the United States [5]. It is a treatable disorder for which newborn screening and early diagnosis are now recognized as a high priority. In our previous study published in 2017 [5], we demonstrated a NBS algorithm for arginase deficiency, in which Arg in combination with Arg/Orn ratio can identify all affected individuals with a relatively low false-positive rate. To date, hyperargininemia in combination with a secondary discriminator such as Arg/Orn ratio is the most commonly used algorithm that has identified virtually all arginase deficiency newborns in screened patients [5]. However, our data showed the plasma arginine levels in newborns with arginase deficiency have significant overlap with that from the newborns without arginase deficiency. This is an important challenge in the diagnosis of arginase deficiency following a positive NBS and has led to the delayed diagnosis in the two patients reported in this study. The delayed diagnosis resulted in developmental delay, cognitive impairment, and spasticity in one patient, which could have been prevented or lessened by dietary modification if the NBS findings had been confirmed in the neonatal period.

The Arg/Orn ratio has been widely used as a second-tier discriminator in NBS for hyperargininemia and has been very successful in discriminating the affected from the unaffected in NBS. The NBS cutoff for Arg/Orn ratio ranges from 0.45 to 1.5 between the states in the US [5]. Interestingly, the two cases with delayed diagnosis of arginase deficiency in our study have the lowest plasma arginine levels (134 and 150 μmol/L) as well as the lowest plasma Arg/Orn ratios (2.2 and 1.7) on the initial NBS follow-up when compared to other arginase deficiency cases (2.9 to 16.0). At least for the small set of case study, the findings demonstrated that the Arg/Orn ratio could provide additional discriminating power to distinguish mild Arginase deficiency cases from normal newborns.

All 14 cases of arginase deficiency would be identified if an Arg/Orn ratio ≥ 1.7 was used as a secondary diagnostic marker. Since the number of newborns with arginase deficiency included in this study is limited, it is possible to have a case with an Arg/Orn ratio lower than 1.7, although it is unlikely to be much lower based on our experience in NBS. We suggest continued use of the NBS algorithm for arginase deficiency outlined in the paper by Therrell et al. [5]. With this approach, screen-positive patients will have a high probability of being true positive for arginase deficiency. In the follow-up confirmatory test, we recommend

Table 1
Plasma and NBS Arg, Orn, and Arg/Orn ratio in patients with arginase deficiency.

	Plasma					NBS		
Patient	Arginine (μmol/L)	Ornithine (μmol/L)	Arg/Orn (μmol/L)	Range (μmol/L)	DOL	Arginine (μmol/L)	Ornithine (μmol/L)	Arg/Orn (μmol/L)
1[a]	134	60	2.2	14-135	5	101	10	10.1
2[a]	150	86	1.7	14-135	5	68	16	4.3
3	263	91	2.9	14–135	15	188	46	4.1
4	192	60	3.2	14–135	16	138	26	5.3
5	268	67	4	14–135	12	351	N/A	N/A
6	179	43	4.2	6–140	2	100	29	3.4
7	233	51	4.6	N/A	N/A	261	N/A	N/A
8	299	63	4.7	6–140	5	177	22	8
9	204	42	4.9	15–160	6	248	17	15
10	282	51	5.5	N/A	N/A	233	N/A	N/A
11	881	110	8	N/A	3	377	16	22.9
12	259	32	8	N/A	6	137	9	16.1
13	528	56	9.4	20–148	7	242	N/A	N/A
14	930	58	16	14–135	7	218	N/A	N/A

[a] Cases with delayed diagnosis of arginase deficiency.

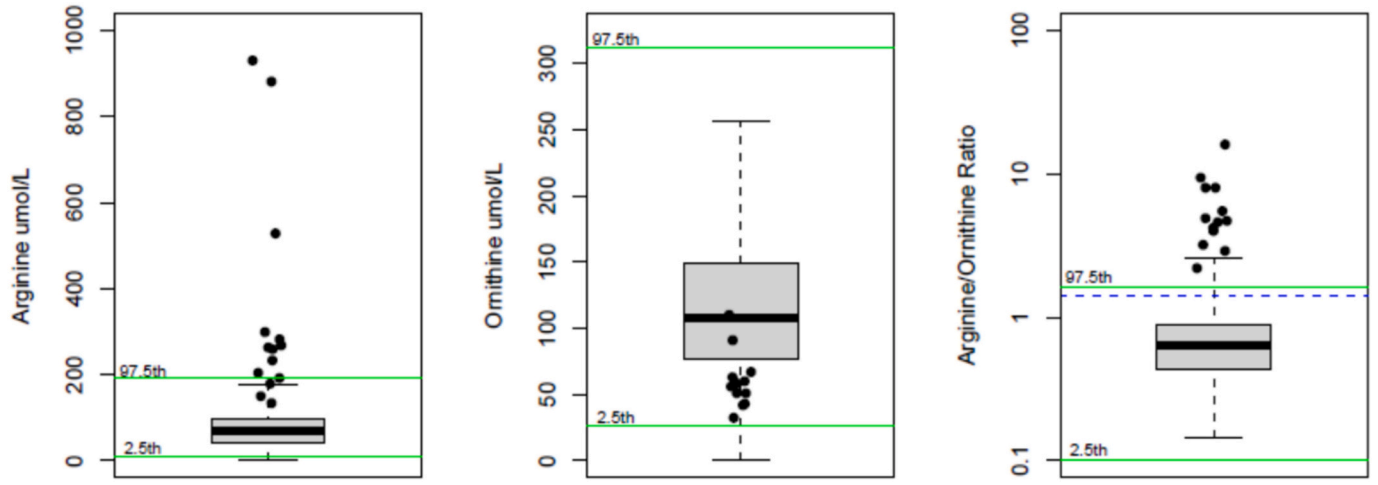

Fig. 1. The distribution of Arg, Orn, and Arg/Orn ratio in newborns.
The blue dotted line represents an Arg/Orn ratio of 1.4. The distribution of unselected newborns is represented by the box and whiskers while the points represent the cases with arginine deficiency.

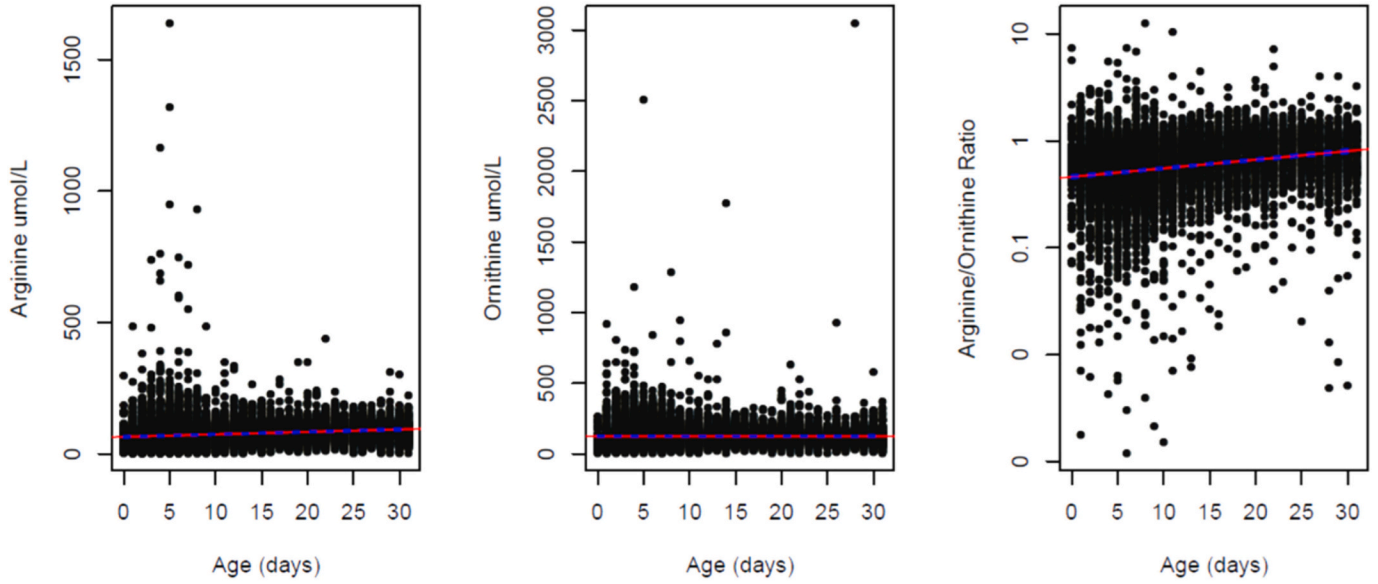

Fig. 2. The distribution of Arg, Orn, and Arg/Orn ratio during the neonatal period.
The colored line represents the trend of respective amino acids over time.

using the plasma Arg/Orn ratio $\geq$ 1.4 as the cutoff to confirm the diagnosis of arginase deficiency, which is approximately the 96th percentile among unselected population (Fig. 3). A ratio of $\geq$1.4 is also the cutoff for the DBS Arg/Orn ratio used in the California NBS program [5]. However, no referred patient should be discharged from care without a normal RBC arginase activity level or absence of *ARG1* gene mutation, because of the potential overlap with normal values and the high prior probability from the NBS algorithm. Conversely, an Arg/Orn ratio above 1.4 alone is not a valid criterion for suspecting arginase deficiency in patients who had a negative NBS or a normal plasma arginine level.

In our efforts to determine the appropriate approach to ensure accurate confirmation of arginase deficiency following a positive screening, we also established the normal values of Arg and Orn in the newborn period in a large dataset of more than 6000 newborns. The analysis demonstrated that the arginine and Arg/Orn values change slightly during the first 31 days of life whereas no significant change of ornithine values occurs, suggesting an age-related adjustment of reference range is not necessary for the neonatal period.

Author contributions

Y.H., R.S., A.F., D.W., S.C., F.L.L., P.T., D·S. were involved in conception, study design, data collection, interpretation, and manuscript preparation. C.L., I.S., R.S., J.N., S.S.B., K.J. were involved in data collection and manuscript review. C.M.R. provided statistical analysis. Y.H. and P.T. take responsibility for the collection of data, the analyses, interpretation, and publication. All authors have given approval for publication of this manuscript.

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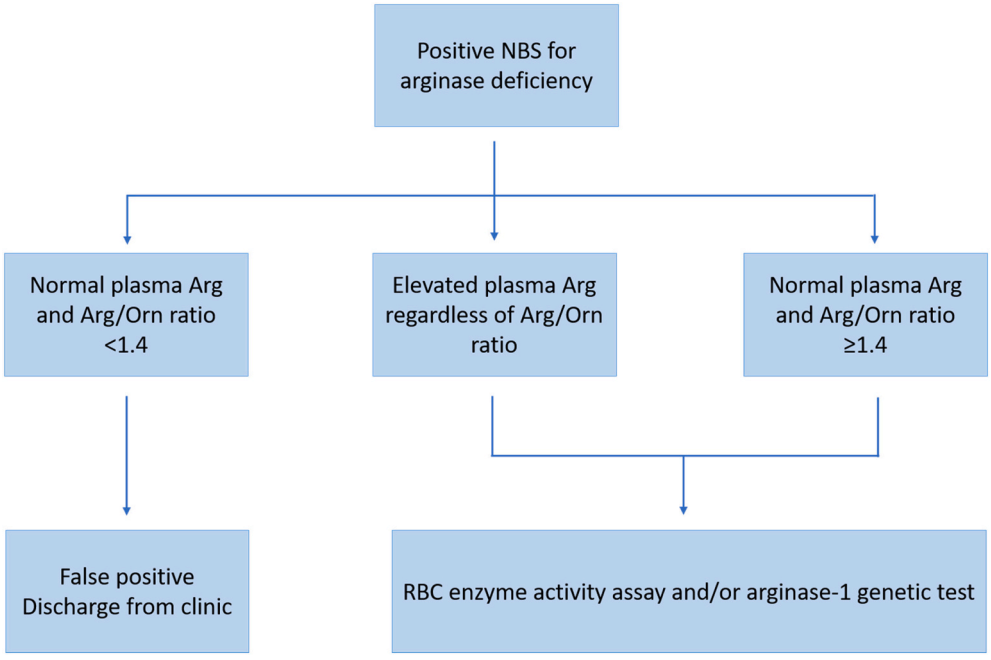

Fig. 3. Proposed algorithm for arginase deficiency workup following a positive NBS.

Ethics statement

This study involved retrospective analysis of existing patient data that were collected without patient identifiers. This study was reviewed by the institutional review board at UCLA with permission to proceed granted.

Declaration of Competing Interest

P.T, F.L.L, D.S, R.S, and C.M.R are employee of Quest Diagnostics. Other authors declare no potential conflicts of interests. None of the authors have financial gain or loss from the results of this study.

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We thank all patients, their parents, and caregivers for sharing clinical information.

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Newborn screening for hyperargininemia due to arginase 1 deficiency

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ABSTRACT

Hyperargininemia caused by Arginase 1 deficiency is a rare disorder of the urea cycle that can be diagnosed by elevation of arginine in newborn screening blood spots when analyzed by tandem mass spectrometry. Hyperargininemia is currently included as a secondary target on the U.S. Recommended Uniform Screening Panel, which directly influences state-based newborn screening. Because of the apparent low disease frequency and lack of case detection and treatment data, detailed attention has not been given to a model newborn screening algorithm including appropriate analytical cutoff values for disease indicators. In this paper we assess the frequency of hyperargininemia in the U.S. identified by newborn screening to date and document the current status and variability of hyperargininemia newborn screening across U.S. newborn screening programs. We also review other data that support improved screening efficacy by utilizing the arginine/ornithine ratio and other amino acid ratios as discriminators in the screening algorithm. Analysis of archived California screening data showed that an arginine cutoff of 50 μM combined with an arginine/ornithine ratio of 1.4 would have resulted in a recall rate of 0.01%. Using an arginine cutoff of 60 μM and an arginine/(phenylalanine x leucine) ratio of 1.4, reportedly used in one screening program, or the R4S Tool Runner, would have resulted in a recall rate of <0.005%. All 9 diagnosed patients would have been found for either protocol. Thus, use of appropriate ratios as part of the screening algorithm has the potential to increase both screening sensitivity and specificity. Improved newborn screening effectiveness should lead to better case detection and more rapid treatment to lower plasma arginine levels hence improving long term outcome of individuals with hyperargininemia.

1. Introduction

Arginase 1 is the 6th and final enzyme and one of 8 proteins that are commonly thought of as comprising the urea cycle (see Fig. 1). Its products are urea and ornithine, the latter recycled into the nitrogen elimination pathway and the former excreted in the urine Deficiency of arginase 1 resulting in hyperargininemia is one of the least frequent disorders of the urea cycle and its more indolent, late-onset presentation usually leads to its diagnosis only after irreversible neurological symptoms have occurred. These symptoms initially include loss of intellectual milestones, spasticity and mild liver dysfunction. Later, more severe liver abnormalities such as liver fibrosis, cirrhosis and even hepatocellular carcinoma may occur [1,2]. A strict dietary and pharmacologic regimen has been shown to reduce the plasma arginine level to normal or near normal levels [3]. Even in the presence of irreversible neurological damage, improvement in neurological function can occur. The few older patients treated from birth were much less severely affected than their symptomatically diagnosed family members despite sub-optimal adherence to the treatment regimen [4].

There is limited information regarding hyperargininemia incidence or prevalence. Reports of incidence vary by an order of magnitude: 0.5 to 5.0 per million [5,6]. A relatively large U.S. study estimated 1.1 cases per million births [7], but it used an indirect methodology that introduces uncertainty about the precision of the result.

The advent of expanded newborn bloodspot screening (NBS) for amino acid disorders using tandem mass spectrometry (MS/MS) includes the possibility to determine arginine levels, thus allowing for the detection of increased risk for hyperargininemia at or near birth. The overlap between normal arginine levels in affected and unaffected newborns is sufficiently great so that determining optimal arginine cutoff levels in NBS is problematic. The goal of laboratory algorithms used in NBS is to minimize or eliminate late diagnosed (missed) cases (false

Abbreviations: ACHDNC, Advisory Committee on Heritable Disorders in Newborns and Children; MS/MS, tandem mass spectrometry; NBS, newborn bloodspot screening; NCHS, National Center for Health Statistics; R4S, Region 4 Stork; SACHDNC, Secretary of Health and Human Services' Advisory Committee on Heritable Disorders in Newborns and Children; RUSP, Recommended Uniform [Newborn] Screening Panel.

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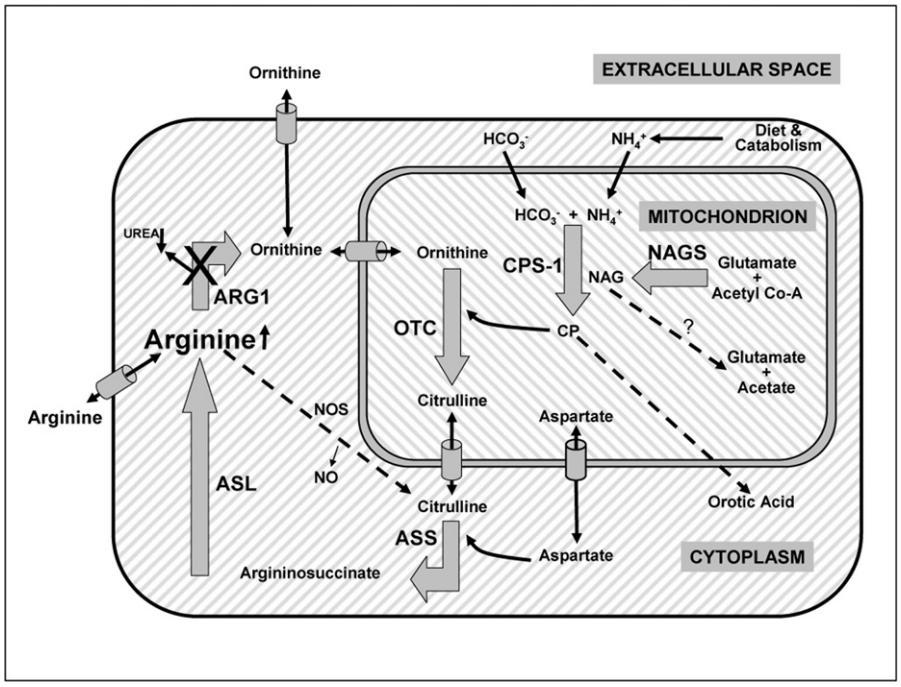

Fig. 1. The Complete Urea Cycle: Focusing on the left side of the figure, the consequences of Arg1 deficiency are illustrated. Arginine accumulates both intra- and extracellularly and urea production is diminished. Ornithine production should be diminished, but neither this nor lowered ornithine in man has been formally demonstrated.

negatives) while reducing unnecessary follow-up (false positives). Because MS/MS simultaneously detects many amino acids, the possibility for assessing various amino acid ratios as a second-tier screening strategy exists. Such ratios have been found useful in improving screening algorithm efficiency for some screened conditions [8,9], including use of the arginine to ornithine ratio (Arg/Orn) for hyperargininemia [10]. The utilization of other individual amino acid ratios [e.g. Arginine to Alanine (Arg/Ala), Arginine to Phenylalanine (Arg/Phe), Citrulline to Arginine (Cit/Arg), etc.] are also possible and provide additional variables for consideration in establishing the most effective screening algorithm.

While NBS is widely acknowledged as a critical public health prevention strategy [11], currently capable of identifying in excess of 50 different congenital inherited disorders including hyperargininemia, a national newborn screening requirement does not exist in the U.S. Instead NBS is state-based with national recommendations provided by the Secretary of Health and Human Services in consultation with an Advisory Committee on Heritable Disorders in Newborns and Children (ACHDNC; previously called the SACHDNC) tasked with providing real time analysis of the national screening situation. In 2005, the SACHDNC accepted a report from the American College of Medical Genetics and Genomics (ACMGG), which included a Recommended Uniform Screening Panel (RUSP) to be considered for implementation by each state screening program [12,13], and recommended its implementation by the Secretary. The RUSP was originally developed using an empirical scoring system and included both 'core' and 'secondary' conditions depending on treatability, screening test availability, family benefits, and other relevant information available at the time [12]. The Secretary accepted the SACHDNC recommendation and the RUSP now strongly influences the conditions included in state screening mandates, particularly the core conditions. A formal nomination and evidence review process has since evolved for nominating and adding conditions to the RUSP [14,15]. Part of this process involves assessment of public health impact and readiness to include the proposed condition.

We report here a basic assessment of public health readiness useful in assessing whether hyperargininemia should be adopted as a core condition on the RUSP. Since hyperargininemia is already included as a RUSP secondary condition, our primary goal was to determine the degree of screening homogeneity across state NBS programs, to approximate a national incidence of the disease from NBS findings, and to consider screening algorithm alternatives for program improvement. Specifically, we surveyed state NBS programs to determine whether they screened for hyperargininemia and if so, whether it was included in their mandated screening panel, what laboratory screening results triggered follow-up actions, whether (and which) second-tier ratio calculations were part of screening algorithm, and the number of cases of hyperargininemia confirmed since their screening program began. Further, we reviewed possible alternative screening laboratory algorithms for possible impact in improving overall NBS effectiveness using archived laboratory data and diagnosed case information from the California NBS program.

2. Methods

In mid-November 2015, a short questionnaire was emailed to state newborn screening laboratory and/or follow-up personnel identified as primary program contact persons (see Acknowledgments). The questions sought to assess the extent to which U.S. newborn screening programs include arginase 1 deficiency in their newborn screening panel and related screening information. Included were questions regarding whether arginase 1 deficiency screening was formally a part of the screening mandate, what and how laboratory data were assessed, follow-up processes, and case detection information. After an initial 2-week response period, a follow-up email was sent to programs that had not responded. Additional email and telephone follow-up resulted in completed surveys for all 51 state programs (50 states and the District of Columbia). All data were reviewed and summarized, and in mid-2016 a table containing the summarized data was circulated to all respondents for approval. Corrections and updates were made as necessary and the case data were updated through the end of 2015.

In addition to reviewing the relevant literature, we assessed the available data from U.S. NBS programs on confirmed cases and screened newborns as part of an ongoing effort to better define U.S. incidence. Since many U.S. NBS programs do not link NBS data with birth records, reliable national data giving unduplicated counts of births screened are not available. Instead, we used national data on births by place of occurrence available from the Centers for Disease Control and Prevention's

National Center for Health Statistics (NCHS). We assumed complete birth coverage by a screening program in order to provide consistency in evaluating the populations for which NBS was available within specific jurisdictions. In cases where hyperargininemia screening began on a date other than the first day of the year, we approximated the number of babies screened by assuming an even distribution of births daily throughout the year and calculated the number of births from the first day a state's hyperargininemia screening effort began.

To evaluate the validity of the various hyperargininemia screening algorithms reported by state NBS programs (i.e. Arg cutoffs and second-tier amino acid ratios), we analyzed newborn screening data from California on newborns screened from July 2005 through December 2015 ($n = 5.4$ M). Preliminary to a broader data analysis we first evaluated archived laboratory data for the 9 confirmed cases of hyperargininemia diagnosed from newborn screening during this time period in order to determine the detection capability of the screening algorithms. Specifically, we used ratio data for six different amino acids (Arg, Cit, Orn, Ala, Phe, Leu) in combination with various Arg cutoff levels. For an additional comparison, we used the analysis tool (Tool Runner) available as part of the international MS/MS database, Region 4 Stork (R4S). Further, we approximated the impact on follow-up that might result if various combinations of Arg cutoffs, Arg cutoffs in combination with various amino acid ratio cutoffs, and the R4S Tool Runner were used by applying each combination to the 2015 California newborn screening data ($n = 486{,}591$).

3. Results

Of the 51 U.S. jurisdictions surveyed (Table 1), 33 reported that hyperargininemia is one of the conditions for which all newborns are required to be screened, with the earliest screening reported in Massachusetts in 1999. Of the 18 other jurisdictions, an additional 5 reported that hyperargininemia would likely be detected by the screening algorithm currently in use for other metabolic conditions, and screen positive cases would be followed up accordingly, even though screening is not required. Thirteen state screening programs reported no screening for hyperargininemia (Alabama, Arizona, Arkansas, Florida, Kansas, Maryland, Montana, Nebraska, South Carolina, Virginia, Washington, West Virginia and Wisconsin). One jurisdiction indicated that arginine levels were not officially reported as part of the program, even though they were observable, since hyperargininemia was not included in the program's current screening mandate.

Between 1999, when screening for hyperargininemia first began, and the end of 2015, slightly over 29 million newborns were born in U.S. jurisdictions that included NBS for hyperargininemia with 26 confirmed cases identified. Assuming that all of eligible newborns (29,107,011) were screened, the prevalence of hyperargininemia across U.S. jurisdictions screening during this period was approximately 1:1,119,500 (95% confidence interval 1:640,392 to 1:1,763,942). Since we know that not all eligible newborns were screened or screened appropriately, the estimate represents the minimum prevalence.

While most survey respondents noted that an elevation of Arg was the trigger leading to further investigation of the possibility of hyperargininemia, the analytical cutoffs requiring additional follow-up (screen positive) varied widely. Multiple cutoffs related to age at time of screening and to birth weights were reported by some programs. Most reported using two Arg cutoff levels leading to two different follow-up pathways, repeat filter paper screening or referral for clinical evaluation. A few programs reported that elevated Arg values above a single cutoff simply resulted in clinical referral (i.e. a repeat filter paper was not part of the general follow-up protocol, although a repeat specimen after clinical referral might occur). The range of Arg values considered actionable (requiring additional follow-up of any type) varied from 20 μmol/L to 130 μmol/L. Some programs chose lower cutoffs for Arg initially and used a second-tier ratio or combination of ratios of other amino acids to ultimately reduce the number of patients recalled. Arg/Orn was the most popular second-tier discriminator with cutoffs for actionable levels varying from 0.45 to 1.5. Other ratios in use included Arg/Ala, Arg/Phe, Cit/Arg and Arg/[Phe × Leu].

Using archived analytical MS/MS data from confirmed cases in California, we constructed a table (Table 2) to illustrate the results that would be obtained using the various reported screening protocols. As a point of reference, 5.4 million specimens were screened during this time period and 138 were originally found to have both Arg ≥ 50 μmol/L and Arg/Orn ≥ 1.40, the criteria for screening positivity during this time period. Clinical evaluation resulted in the 9 cases referenced.

In order to assess the potential impact of some of these protocols on screening follow-up, we developed a table (Table 3) to show the percentage of newborns that would require additional follow-up based a proposed protocol using 2015 California NBS data. We chose consider Arg alone, Arg in combination with Arg/Orn, and Arg in combination with Arg/[Phe × Leu]. We also included the R4S Tool Runner multivariate/metabolic profile analysis [8,9]. While the California hyperargininemia protocol resulted in 0.01% follow-up, two other protocols (utilization Arg and Arg/[Phe × Leu] and R4S Tool Runner appeared to be at least as good.

4. Discussion

Ideally, newborn screening for a particular disorder would be uniform between jurisdictions and have a very low false negative rate and a low recall (false positive) rate. Screening algorithms would be based on sufficient case detection evidence to validate the algorithm with modification as new data accumulates. It is clear from the information presented here that NBS for hyperargininemia in the U.S. is far from ideal with significant variability between state screening programs. Variations appear to be based on anecdotal findings without a solid scientific basis (Table 1). Reliable national NBS incidence data beyond that reported here do not exist.

There are essentially two screening strategies to minimize the number of newborns recalled for additional testing without missing cases for most screening disorders. For hyperargininemia, one strategy uses Arg alone as the indicator of possible disease and the other uses Arg in combination with a ratio (or ratios) of related amino acids. In the first method (Arg alone), in order to eliminate missing cases, relatively large numbers of patients must be recalled to further assess other laboratory and clinical information before confirming the presence of disease (Table 3). Reduced recall can be accomplished by raising the cutoff value thus increasing the possibility for missing cases. The second strategy reduces the numbers recalled through a filtering process. A lower Arg cutoff can be used to initially create a larger pool of potentially abnormal patients (thus lowering the chances of missing a case) whose numbers are then reduced by examining laboratory values for other analytes, assessed as various amino acid ratios. The difficulty comes in determining which ratios are reliable discriminators of disease.

For either of these screening strategies, large numbers of screens are required in order to estimate the sensitivity and specificity of the screening algorithm. Screening algorithms for NBS can be developed using multivariate pattern recognition software and metabolic profile scoring along with large datasets such as the international R4S [16]. Additionally, tools such as the R4S Tool Runner have been created to provide further assistance in assessing various ratios as their value as secondary discriminators for various diseases [8,9].

While most U.S. NBS programs are aware of and participate in contributing to the R4S database, our state survey data indicated that its use in developing state NBS algorithms was minimal and only one state program reported routinely using Tool Runner as a NBS aid. In order to consider the possible effectiveness of the various hyperargininemia NBS protocols reported, we retrospectively compared various algorithms using California NBS data. In addition to the routine Arg/Orn ratio used in California and many other programs, the ratio of Arg/(Leu × Phe) is used in the New England Regional Program.

Table 1
Data from US newborn screening programs screening for hyperargininemia (arginase 1 deficiency).

State	Start Date	Cases	Screenable births	Screening result requiring follow-up screen	Screening result leading to clinical referral
Alaska[a]	10/01/03	0	135,112	110 μmol/L ≤ Arg < 180 μmol/L	Arg ≥ 180 μmol/L
California	07/07/05	9	5,499,189	All abnormal findings referred for clinical evaluation	Arg ≥ 50 μmol/L; Arg/Orn ≥ 1.4
Colorado[a]	07/01/06	0	642,633	Arg ≥ 100 μmol/L	Two successive findings of Arg ≥ 100 μmol/L
Connecticut	01/01/05	1	432,966	Arg ≥ 55 μmol/L (age < 10 days); ratios assessed: Arg/Ala; Arg/Orn; Arg/Phe; Cit/Arg [see footnote [b]]	See footnote [b].
District of Columbia	07/01/00[c]	0	224,217	125 μmol/L ≤ Arg < 270 μmol/L	Arg ≥ 270 μmol/L
Delaware[a]	01/01/03	0	154,811	Arg ≥ 33 μmol/L (age < 7 days)	Arg ≥ 43 μmol/L (Age < 7 days); Arg ≥ 58 μmol/L (age ≥ 7 days)
Georgia	01/01/07[d]	0	1,238,019	Arg ≥ 120 μmol/L (wt < 2500 g); Arg ≥ 105 μmol/L (wt ≥ 2500 g)	Clinical referral decided by follow-up contractor on basis of results
Hawaii	09/01/03	0	231,769	110 μmol/L ≤ Arg < 180 μmol/L	Arg ≥ 180 μmol/L
Idaho[a]	10/01/02	1(Prenatal)	301,083	110 μmol/L ≤ Arg < 180 μmol/L	Arg ≥ 180 μmol/L
Illinois	07/01/02	1	2,255,001	30 μmol/L ≤ Arg < 50 μmol/L (Arg/Orn ≥ 0.45) 40 μmol/L ≤ Arg < 50 μmol/L (Arg/Orn not elevated)	Arg ≥ 50 μmol/L
Indiana	05/01/04	0	1,010,397	100 μmol/L ≤ Arg < 180 μmol/L; Assessing Arg/Orn, Arg/Phe	Arg ≥ 180 μmol/L
Iowa	08/01/03	0	488,156	Any abnormal findings referred for clinical evaluation	Arg > 35 μmol/L; Arg/Orn > 0.6 (previously 1.0)
Kentucky	12/05/05	0	551,556	Any abnormal findings referred for clinical evaluation	Arg > 55 μmol/L (not on TPN)
Louisiana	11/01/04	0	710,746	Any abnormal findings referred for clinical evaluation	Arg > 120 μmol/L and Arg/Orn >1
Maine	07/01/01	0	191,996	60 μmol/L < Arg <132 μmol/L and Arg/[Leu × Phe] > 0.006	See footnote [e].
Massachusetts	02/01/99	1	1,232,171	60 μmol/L < Arg <132 μmol/L and Arg/[Leu × Phe] > 0.006	See footnote [e].
Michigan	04/01/05	1	1,259,984	Arg ≥ 50 μmol/L; Arg/Orn ≥ 0.8 (age < 180 h) Arg ≥ 90 μmol/L; Arg/Orn ≥1 (180 h ≤ age < 1 yr) If on TPN, inconclusive, request repeat	Arg ≥ 90 μmol/L; Arg/Orn ≥ 1.5(age < 180 h) Arg ≥ 110 μmol/L; Arg/Orn ≥ 1 (180 h ≤ age < 1 yr) If on TPN, inconclusive, request repeat
Minnesota	05/21/01	0	1,021,894	125 μmol/L ≤ Arg < 270 μmol/L	Arg ≥ 270 μmol/L
Mississippi	06/01/03	0	512,771	125 μmol/L ≤ Arg < 270 μmol/L	Arg ≥ 270 μmol/L
Missouri	07/01/05	0	827,604	100 μmol/L ≤ Arg < 150 μmol/L	Arg > 150 μmol/L
Nevada[a] (post 7/2015)	07/01/15	0	456,419	100 μmol/L ≤ Arg < 200 μmol/L (age ≤ 7 days) 130 μmol/L ≤ Arg < 200 μmol/L (age > 7 days)	Arg ≥ 200 μmol/L or 2 successive findings requiring a repeat (see preceding column)
Nevada[a] (pre 7/2015)	07/01/03	1		110 μmol/L ≤ Arg < 180 μmol/L	Arg ≥ 180 μmol/L
New Hampshire	03/01/10	0	73,715	60 μmol/L < Arg <132 μmol/L and Arg/[Leu × Phe] > 0.006	See footnote [e].
New Jersey	03/01/09	2	700,946	100 μmol/L ≤ Arg < 200 μmol/L	Arg ≥ 200 μmol/L or 2 successive findings of 100 μmol/L ≤ Arg < 200 μmol/L
New Mexico[a]	01/01/07	0	242,641	110 μmol/L ≤ Arg < 180 μmol/L	Arg ≥ 180 μmol/L
New York	05/02/05	5	2,622,604	52 μmol/L ≤ Arg < 115 μmol/L	Arg ≥ 115 μmol/L or 2 successive findings of 52 μmol/L ≤ Arg < 115 μmol/L
North Carolina	08/31/15	1	41,177	100 μmol/L ≤ Arg < 150 μmol/L	Arg ≥ 150 μmol/L
North Dakota	08/05/04[d,f]	0	124,460	All abnormal findings referred for clinical evaluation	Arg > 35 μmol/L; Arg/Orn > 0.6 (previously 1.0)
Ohio	06/28/02	0	1,959,931	Arg ≥ 110 μmol/L (Follow-up action decided by specialist)	Arg ≥ 110 μmol/L (Follow-up action decided by specialist)
Oklahoma	05/27/08	0	397,331	100 ≤ Arg < 200 μmol/L (low risk)	Arg ≥ 200 μmol/L (high risk) or 2 successive findings of 100 ≤ Arg < 200 μmol/L
Oregon[a]	10/28/02	1	619,068	110 μmol/L ≤ Arg < 180 μmol/L	Arg ≥ 180 μmol/L
Pennsylvania	07/01/09[d]	0	923,002	125 μmol/L ≤ Arg < 270 μmol/L	Arg ≥ 270 μmol/L (URGENT Repeat Request)
Rhode Island	07/01/06	0	114,270	60 μmol/L < Arg <132 μmol/L and Arg/[Leu × Phe] > 0.006	See footnote [e].
South Dakota	06/01/07[d]	0	108,987	All abnormal findings referred for clinical evaluation	Arg > 35 μmol/L; Arg/Orn > 0.6 (previously 1.0)
Tennessee	01/01/06	2	873,371	95 μmol/L ≤ Arg < 125 μmol/L	Arg ≥ 125 μmol/L.
Texas[a]	05/25/15	0	245,806	100 μmol/L ≤ Arg < 140 μmol/L (age ≤ 7 days) 115 μmol/L ≤ Arg < 150 μmol/L (age > 7 days)	Arg ≥ 140 (age ≤ 7 days); Arg ≥ 150 (age > 7 days); 2 successive findings requiring a repeat specimen (see preceding column)
Utah[a]	01/01/06	0	537,200	All abnormal findings referred for clinical evaluation	Arg > 20 μmol/L for first screens (<7 days) Arg > 35 μmol/L for second screens (≥7 days))
Vermont	01/01/03[d]	0	77,051	60 μmol/L < Arg <132 μmol/L and Arg/[Leu × Phe] > 0.006	See footnote [e].
Wyoming[a]	07/01/06	0	66,962	Arg ≥ 100 μmol/L	Two successive findings of Arg ≥ 100 μmol/L
Total		22	29,107,011		

[a] Routinely requires or recommends two screens on all newborns with high compliance – usually > 90%.
[b] Abnormal Arg on initial screen in newborn < 10 days old is repeated in duplicate and assessed using R4S ARG Tool Runner: Arg/Ala > 0.19; Arg/Orn > 0.70; Arg/Phe > 1.00; Cit/Arg < 0.32. If two out of the three analyses are outside of normal limits and the R4S ARG Tool indicates a possible abnormal result, the result is reported as abnormal. Any borderline or questionable results are reported as abnormal.
[c] Exact start date is not known, only the year. We have used 07/01/00 as the start date in order to approximate the number or screenable births.
[d] Not required, but likely to be detected by laboratory methodology currently in use.
[e] Arginine result is assessed versus ratios for Arg/[Leu × Phe] and Arg/Orn; Risks are assessed as follows: Moderate Risk: 60 μmol/L < Arg < 132 μmol/L with Arg/[Leu × Phe] > 0.006 and Arg/Orn > 1.5 or 132 μmol/L < Arg < 200 μmol/L with no ratios elevated; High Risk: 132 μmol/L < Arg < 200 μmol/L with either ratio elevated (high risk); Arg > 200 μmol/L (high risk) regardless of ratios.
[f] Pilot screening MS/MS screening began on 11/15/02 with official implementation on 08/05/04.

Using these two ratios and an initial cutoff value for arginine of 50 μM, all 9 known cases of arginase 1 deficiency detected in California over the 2005–2015 period (Table 1) would have been ascertained with no false screen positive cases reported. While the low frequency of arginase 1 deficiency in the population precludes absolute statements, we can state with certainty that the sensitivity of this screening protocol is

Table 2
Distribution of markers relevant for hyperargininemia screening among California newborns diagnosed with arginase 1 deficiency.

Case	Arg (μmol/L)	Cit (μmol/L)	Orn (μmol/L)	Ala (μmol/L)	Phe (μmol/L)	Leu (μmol/L)	Arg/Orn	Cit/Arg	Arg/Ala	Arg/Phe	Arg/Leu	Arg/[Phe × Leu]	R4S Score[a]
1	68	12	16	227	51.5	75.3	4.25	0.18	0.30	1.32	0.90	0.0175	112
2	101	22	25	318	62.3	80.8	4.04	0.22	0.32	1.62	1.25	0.0201	130
3	138	17	26	431	61.5	99.1	5.31	0.12	0.32	2.24	1.39	0.0226	210
4	142	15	26	237	70.7	97.1	5.46	0.11	0.60	2.01	1.46	0.0207	257
5	182	23	30	365	88.2	103.7	6.07	0.13	0.50	2.06	1.76	0.0199	248
6	209	16	23	366	54.8	142.9	9.09	0.08	0.57	3.81	1.46	0.0267	419
7	209	16	29	200	43.6	61.6	7.21	0.08	1.05	4.79	3.39	0.0778	423
8	233	19	52	314	60.6	151.6	4.48	0.08	0.74	3.84	1.54	0.0254	371
9	422	29	19	377	65.3	160.6	22.21	0.07	1.12	6.46	2.63	0.0402	468

Abbreviations: Arg = arginine; Cit = citrulline; Orn = ornithine; Ala = alanine; Phe = phenylalanine; Leu = leucine.
[a] R4S Results: Hyperargininemia highly likely if R4S Score ≥ 125; Hyperargininemia likely if 40 ≤ R4S Score < 125.

very high and the specificity approaches 100%. Because cases of metabolic disorders missed by newborn screening are reportable in California, it is relatively certain that no unknown cases of the disorder are present in California newborns, although early misdiagnosis is always a possibility. Thus, despite its rarity, screening for arginase 1 deficiency is practical when these ratios are used. Moreover, when each case was examined using the R4S Tool Runner, all were ascertained with a calculated false positive rate of <0.005%.

Determining status and value of newborn screening for hyperargininemia is complicated by a scarcity of published case detection and treatment data. Available case detection data do not support specific ethnic or geographic predilections, although hyperargininemia appears to be higher in Japan, Portugal and among French-Canadians of pioneer origin. While NBS in the U.S. is still not universal, 38 U.S. state programs currently report arginine results, and are therefore likely to observe hyperargininemia cases. While the apparent 1:1.2 M minimum incidence observed through NBS to date is similar in magnitude to that of some of the other metabolic conditions currently included in NBS, continued data collection is needed to establish a more reliable incidence.

The ACMGG ACT sheet for the follow-up of presumptive positive newborn screening results (i.e. elevated arginine) suggests obtaining a repeat arginine level along with a quantitative urinary orotic acid level [17]. While elevated orotic acid in the urine of hyperargininemia patients was reported by one of us, S.C., in 1981 [18] and subsequently found to be elevated in a number of infants with the disorder, we believe that this test has been insufficiently validated to be reliable for diagnosis. On the other hand, red blood cell arginase enzyme levels and mutation analysis of the exons of the arginase gene have been demonstrated to be reliable and together are the follow-up methods of choice [4]. Although the enzyme assay has been validated only in symptomatic patients, extrapolation to those who may be less severely affected seems appropriate. The normal levels in infants appear to be the same as in older children and adults, although this has not been studied extensively.

There is little question that as we become more proficient in the diagnosis of arginase 1 deficiency individuals with intermediary elevations in arginine on newborn screening and partial defects in enzymatic activity will be found. There are no reliable data to determine a safe level of arginine and indeed this may differ from patient to patient and vary with age. With any disorder, time and experience help with these decisions and they may be slow in coming with a disorder so relatively rare. In the meantime, NBS offers a means of early detection and treatment, and programs should consider using available resources such as R4S as a means to harmonizing screening algorithms. Because all relevant metabolites are already captured in routine MS/MS screening, adjustment of the interpretive algorithm is all that is needed to immediately implement one of the suggested approaches. As with any new and rare newborn screening condition, it will be critical to maintain a national (or international) database of relevant screening data (screening algorithm, time of screening, demographics of detected patients, etc.) that can be periodically analyzed in order to refine the screening algorithms being used [16,19].

Table 3
Estimated screen positive rate for arginase 1 deficiency based on alternative screening strategies.
(Data from California, 2015, $N = 486{,}591$).

Method	Arg	Arg/Orn	Arg/[Phe × Leu]	Newborns requiring follow-up
Using specified cutoff concentration for Arg	33			2.18%
	40			1.39%
	50			0.84%
	100			0.11%
	110			0.07%
	125			0.05%
Using specified cutoff for Arg and indicated ratio cutoff(s)	30	0.45		0.26%
	35	0.6		0.07%
	50	0.8		0.02%
	50	1.4		0.01%
	60	1.5	>0.0006	<0.005%
Using R4S Tool Runner	Not applicable			<0.005%

Abbreviations: Arg = arginine; Orn = ornithine; Phe = phenylalanine; Leu = leucine; R4S = Region 4 Stork international MS/MS database.

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My daughter, Willow, was diagnosed with Arginase 1 Deficiency a month before her 5th birthday. Because of the delayed diagnosis, she suffers from many irreversible symptoms of the disease resulting in physical, behavioral, and learning disabilities. I will touch on a few of her symptoms here but keep in mind, this is a sampling of what she lives with, not the entirety.

Willow suffers from spasticity in all of her limbs but predominantly in her legs. This diagnosis is probably her most debilitating physical symptom. The spasticity requires her to wear AFOs (braces that wrap around her foot, ankle, and go to her knees) to walk with proper alignment. Even with these, she often trips over her feet and stumbles on uneven ground. Just a crack in the pavement can cause her to fall. She can't walk for long distances either. A trip to the grocery store will wipe her out. She can't take part in a lot of kid focused activities because they usually require too much physical exertion. She gets fatigued very quickly and has to sit out and watch the other kids play.

Willow has foot and toe malformations where her big toes curl under her other toes and her whole foot rolls inward, collapsing over her arch. Because of this, her nail beds are often infected and painful due to ingrown toenails. She is also missing some toenails due to her dragging her feet. She hyperextends her knees causing knee pain, has lordosis which causes back and hip pain and suffers from overall core weakness. With issues such as these comes many specialists and many appointments that require her to miss a lot of school.

Speaking of school, she is about 4 grade levels behind in math. She only just got caught up to grade level with reading and is still multiple grade levels behind in spelling. Her handwriting is often illegible, even after 6 years of physical therapy focusing on this. Willow has a hard time learning and staying focused but she is socially on point. This means that she is able to now recognize her differences and struggles with being so different from her classmates. She never gets to take part in class activities because she gets pulled out for one-on-one help. She frequently will hear of a fun and exciting assignment or activity but usually doesn't get to participate because of this pull-out. I often hear of how she winds up sitting on the bench at recess alone because she isn't able to play on the swings or play tag. Honestly, this part might be the hardest for me. Knowing that my kid is aware of her differences and is sad and alone.

If Willow had been diagnosed with Newborn Screening, many of these symptoms would have been prevented. I believe this to be true because of the individuals we have met who were diagnosed at birth. Their symptoms are much less pronounced, if present at all. We were just at a meet-up for people living with Arginase 1 Deficiency and the children that were diagnosed with Newborn Screening were up running around playing silly games of tag, catch, and just running and jumping around like a typical kid does. The children that were not diagnosed until later in life were stuck at tables due to their lack of physical capabilities or were completely reliant on their wheelchairs or walkers. Some were not able to feed themselves and were often not verbal. Even the children like Willow, who are able to be independent in some ways, were still not able to keep up with their peers diagnosed at birth. It is heartbreaking to see such a stark difference that could have been completely eliminated if only we all had equal access to newborn screening for Arginase 1 Deficiency.

Meet Jackson, age 30, with parents Jean and Leafy
Southern California
Living with ARG1-D

Jackson, age 30, is a self-described cooking fanatic. It is a joyful family experience, involving his mom Jean and stepmom Leafy, bringing this already close-knit family even closer together. “I love to make Thanksgiving dinner, with all the fixings,” Jackson explains. “But on my plate, there won’t be any turkey of course and I select my side dishes very carefully.”

If Jackson doesn’t choose what he eats carefully, the medical consequences are severe. Jackson lives with Arginase 1 Deficiency (ARG1-D) a debilitating and progressive inherited metabolic disorder affecting children, teens, and adults that can significantly impact a patient’s health over time.

Jackson was born with ARG1-D, but it took the family nearly five years to get a diagnosis. “When Jackson was a baby, he slept a lot and we were delighted to have such a ‘good’ baby,” recalled mom Jean. “But we started having some concerns.” Jackson did not nurse, and he was prone to vomiting when exercising or laughing at cartoons on television. At first, Jackson’s pediatrician was not concerned. But when other physical symptoms emerged – toe walking, falling easily, and more vomiting when he exerted himself - Jackson was finally sent for metabolic testing.

Late on a Friday, Jean and Leafy anxiously waited to learn officially what was happening with Jackson. “I knew something was not right,” said Leafy. “They kept testing his blood and he had an extraordinarily high amount of ammonia in his urine.” Finally, the physician had the diagnosis of ARG1-D.

ARG1-D is characterized by complete or partial lack of the enzyme arginase in the liver and red blood cells. Arginase helps to break down and remove nitrogen from the body. The lack of the arginase enzyme results in excessive accumulation of nitrogen, in the form of ammonia, in the blood and arginine in the blood and cerebrospinal fluid. Children may exhibit seizures, spasticity, short stature and intellectual disability. The reason why Jackson had slept so much as a baby was likely that excessive protein was causing him to go into long stretches of comatose state. “He was not simply a good baby sleeping peacefully,” said Jean.

Jean and Leafy had vastly different reactions to hearing Jackson’s diagnosis. For Leafy, “It was a surreal experience. It was like I was hovering from above and listening to a conversation someone else was having. And then I started to panic.” Jean’s reaction was calm. “I thought, okay protein is making him sick, we have to figure out a way to cope. We can fix this.”

While much still is being learned about ARG1-D, back in the late 1990s when Jackson was diagnosed there was even less guidance for patients and caregivers. “We were told ‘don’t give him protein’ and then sent on our way,” said Leafy.

Jean and Leafy struggled for many months to care for Jackson appropriately, given the limited information available about ARG1-D. While trying to manage the diet, Jackson’s weight dropped precipitously, he continued to vomit regularly, and he suffered from spasticity. In addition, while Jean and Leafy tried to manage the care of their son, they also endured judgement from others. One doctor suggested their own anxieties were possibly making Jackson sick, not ARG1-D. And at a rest stop during

a family vacation, an onlooker became upset when the women were limiting Jackson's food despite his pleas for more and threatened to call police. "It was a long and lonely journey not knowing what to do," said Leafy.

Eventually, Jean and Leafy met another family also caring for a child with ARG1-D. The family became a source of support and strength and introduced them to a physician who would change their lives, Dr. Stephen Cederbaum, a geneticist from UCLA that they are still in touch with 26 years after meeting him. Dr. Cederbaum established a treatment plan for Jackson consisting of nutritional drinks, a limited diet, leg injections to help with spasticity and much more. Until he was in grade 7, Jackson needed to eat his lunch alone in the principal's office so he would not come into contact with peanuts (an allergy diagnosed as an infant) or potentially eat high protein foods. He could not go on school field trips without Jean or Leafy, and playdates and sleepovers were arduous to plan. Jackson also had to go for physical therapy to learn simple tasks like how to tie his shoes and button his shirts.

Today, Jackson still follows a similar diet and routine as he had as a child, though therapies to potentially treat ARG1-D are now in development. When he is not cooking with his family, Jackson works at Amazon and enjoys music, art and playing his guitar. He follows a strict diet and the instructions for managing ARG1-D very carefully. In his 30 years he has learned many lessons. "Do your own research, but don't pretend that you alone are the expert," Jackson advises. "Listen to what you're told to do, and don't give up or become complacent." Jackson hopes to see a treatment approved to treat ARG1-D in his lifetime, as he lives with the many restrictions and side effects of this rare disease. He appreciates the researchers and healthcare providers who have devoted many decades of study into ARG1-D. "We have learned so much over the past 26 years." Jean and Leafy are very proud of Jackson's accomplishments. In 2014, he graduated from California Baptist University with a Bachelor of Art in graphic designs.

For Jean, she offers advice for families who are new to ARG1-D. "We went to so many doctors until we found the ones that worked best for us," she said. "Don't' give up or settle, find the right doctor and create a team of specialists that will work together. And yes, you will need a lot of specialists." She also offers practical advice for parents and other caregivers:

- ✓ Get your child used to the taste of the formula early. They need it and they will become accustomed over time like Jackson did.
- ✓ Always make and keep all doctor appointments.
- ✓ Meet regularly with several specialists: metabolic specialist, orthopedic surgeon, ophthalmologist (not optometrist), dentist, and physical therapist. Also, see a neurologist if your child has seizures.

The family remains eternally grateful for finding and connecting with others living with ARG1-D, as the community has grown over the last two and a half decades. They recommend reaching out to community groups like the Arginase 1 Deficiency Foundation for support, an organization Jean, Leafy and Jackson all give back to today. "We have been through it ourselves and we know what it's like," said Leafy. "We are there for each other 24-7. That's what you do when you are part of a family. It does not end with a conversation. We continue to think about them."

Jackson added, "We want to give hope to those who need it. You were given a bad hand, but you can play your cards right. Listen and make the right decisions, and let people help you along the way."

On the value of newborn screening:
"Our diagnosis came many years late because newborn screening was not an option. And I know there are more families just like ours out there." Leafy, stepmom of son with ARG1-D

Lessons learned:

"We went to so many doctors until we found the ones that worked best for us. Don't give up or settle, find the right doctor and create a team of specialists that will work together. And yes, you will need a lot of specialists." Jean, mom of son with ARG1-D

"We worried tremendously in the beginning about everything for our son. But he is smart and strong, and I know he can do anything." Jean, mom of son with ARG1-D

"You were given a bad hand, but you can play your cards right. Listen and make the right decisions, and let people help you along the way." Jackson, living with ARG1-D

On community:

"We have been through it ourselves and we know what it's like. We are there for each other 24-7. That's what you do when you are part of a family. It doesn't end with a conversation." Leafy, stepmom to son with ARG1-D

Jackson was born in January 1991. The day he was born, according to his physician, he was a perfect baby in every way with no known birth defects or disease. That all changed 4 ½ years later on September 28, 1995. The following story could have been avoided had California had Newborn Screening at the time of his birth. They could have started treatment / medications for Argininemia / Arginase 1 Deficiency.

The day of Jackson's diagnosis they told us Jackson would always be small, would stop walking on his own, would develop "tics" with symptomatic Tourette's syndrome, and would probably die from the flu and not Arginase 1 Deficiency. They also told us to stop giving him food with protein. There was no support or recommendation on how to move forward from the worst day of our lives.

Within days of changing his diet, Jackson started throwing up and falling more often throughout the day. If we walked out the front door, we always had the pan and towels to catch his vomit and clean up the floors or furniture. He vomited 6 to 12 times a day, throwing up what little he could eat and all his formula. Because he was unable to walk, run, or laugh without vomiting, his world was limited to riding in a stroller and no cartoons which he would laugh at. This went on for 110 days before an Orthopedic Surgeon gave him Botox shots in his calves. It helped after three days of treatment.

Our doctor asked for a wheelchair for Jackson and our insurance denied it. It took over a year to get the insurance to pay for a wheelchair. Our nights were spent trying to find information on Arginase 1 Deficiency and writing letters to the insurance companies in order for them to pay for or assist in the payment of care. Battling the health insurance companies was a constant, daily struggle. They did not want to pay for any of the drugs / formulas that were recommended to maintain his current health. The insurance company considered his formulas to be "protein drinks / bodybuilding drink" and refused to pay for them. To this day, every year we spend countless hours jumping through all the hoops we have jumped through for the past 25 years.

Because of his unusual diet, allergies, and propensity for injury, we could never hire or trust anyone to help with his daily care. This caused us to eventually lose our business forcing us into bankruptcy because of the amount of time we had to devote to his care.

We are absolutely confident that had Jackson been diagnosed at birth, we could have avoided many of the symptoms of his disabling disease. Most children now diagnosed at birth avoid spasticity, hospital visits, vomiting, seizures, broken bones, horrible mood swings, glaucoma, awful skin outbreaks, and outburst of mood swings from high ammonia.

Jackson continues to have occasional hyperammonemia, he toe walks, is spastic, has osteoporosis, glaucoma, and has suffered through two broken hand due to falls. Whenever he does get a cold or flu, he spends weeks in bed unable to do anything.

Jackson does not make friends and shies away from social events unless attended by family or the ARG1D community / family. He does not like to go out with groups of people because of his walking characteristics and unusual diet. He spends most of his free time in his room, reading or playing games.

We do our best to have a normal life.

I would like to thank you for allowing us to share our lives with you and hope that you will not only consider but pass the newborn screening request to add Arginase 1 Deficiency to the blood test panel. It

has been an absolute honor working on this project with our Arginase 1 family and have 100% confidence in any way that they represent us.

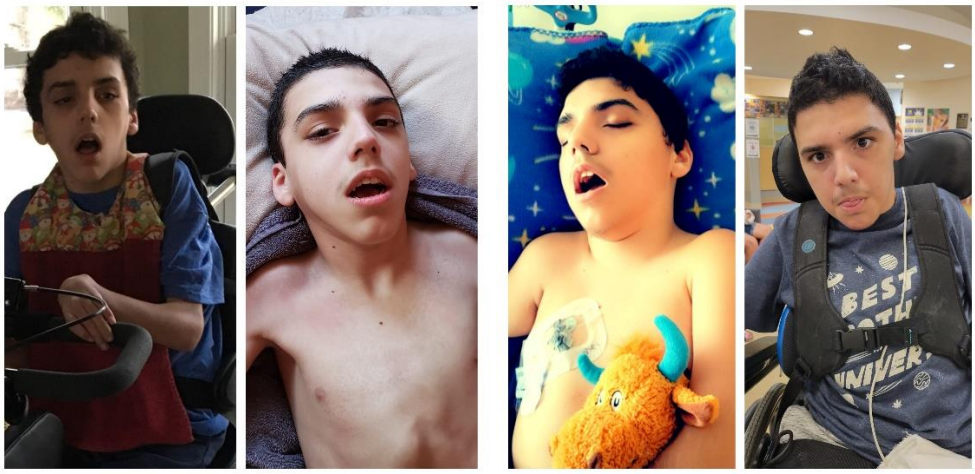
Hi I'm Brandy, Landon's mom and this is Landon's story.

I had a perfectly normal pregnancy. Landon was born via c-section September 3, 2004. Right around 6 months old Landon started having seizures. Immediately, I called my pediatrician, and we began an extremely long journey. He was sent to a neurologist, genetics, and a whole bunch of other specialists. Neurology confirmed the seizures and he started medications to control them. Genetics did testing and found nothing. Mind you this was in 2005 and maybe there wasn't even testing for Arginase 1 Deficiency (ARG1D) at the time. He was diagnosed with Cerebral Palsy and Epilepsy. He had many abilities throughout his life. Crawling, walking, propelling his chair, use of his hands, feeding himself, eating orally and the list goes on of his abilities. Slowly over time, these things stopped one by one with no apparent reason why.

Fast forward to April 2021. Landon and I received our first dose of the COVID vaccine on a Monday. I felt fine and by Friday Landon was eating less, more sleepy, irritable (he was always very irritable but even more so now), low urine output, vomiting and just not himself. Now, also, in this time I was in the process of switching all of our doctors from the past 16 years to new doctors at University of Michigan Motts Children's Hospital. It's one of the top hospitals in the country and I wanted all new eyes on him. I took him in on a Saturday, 6 days after the vaccine and thinking it was just side effects from it. He was lethargic when we got there, just in rough shape. They immediately got to work. Labs, labs and even more labs.

Landon's ammonia came back 1,672 (normal is around 45 and anything over 100 can lead to brain damage). He was immediately placed in PICU, put on a vent and had dialysis to clean his blood to get him to the normal ammonia level range. The doctors were concerned about hyperammonemia. What was he going to be like? Has this caused brain damage? Well 10 days on a vent, blood draws every 2 hours for days, and a feeding tube placed to keep him on a strict formula recipe. He was 62 pounds at 16 years old when I got to the hospital. How did I not notice?

Well, 6,073 days of life and he was FINALLY diagnosed with Arginase 1 Deficiency. It's been a ride. But he needed me as much as I needed him. Almost 2 years later and he's doing AMAZING. He will never get back all the abilities he lost but we are in a good spot right now. There will obviously be bumps in the road but we can handle those. The doctors at Motts not only saved his life that day but saved mine as well, our whole family's actually. Landon is so so loved by everyone. We have a small village. He is his brother's best buddy and his aunts love him to death. Doctors and nurses have become friends and confidantes. Landon is the only person at the genetics clinic at our hospital that has ARG1D, but yet they have taught me everything about it. I'm so happy to have found this group. I wish his diagnosis was found years sooner through newborn screening. I wish he was on the proper formula plan sooner. I wish he was on nitrogen scavenger medications earlier. Maybe he would still be able to do some of the things or all of the things he used to do. But most of all regardless of everything he is STILL HERE and that's truly what matters the most. He is my heart, my existence, my sun, moon, and stars.



Left two photos are prior to diagnosis, right two photos are post diagnosis. 40 pound weight gain due to proper nutrition and medications.

From a Mother's perspective...

What if?

Just what if my son was diagnosed correctly from newborn screening?

Could the outcome have been better?

Arginase 1 Deficiency was not on newborn screening when my son Josh was born in 1997. There was a red flag though. Josh's newborn screening came back positive for PKU (phenylketonuria) but repeated testing came back negative! We were so scared but relieved my son was healthy. Then things changed and Josh wasn't meeting his milestones and many of us including his doctor, thought he was a late bloomer. His legs were tight and he was showing signs of developmental delay. Then right before Josh's 3rd birthday, he had an onset of seizures and his liver enzymes were elevated. Josh went through numerous tests including MRI's and a spinal tap. Days later, a blood lab showed elevated Arginine. Josh was diagnosed with Arginase 1 Deficiency!

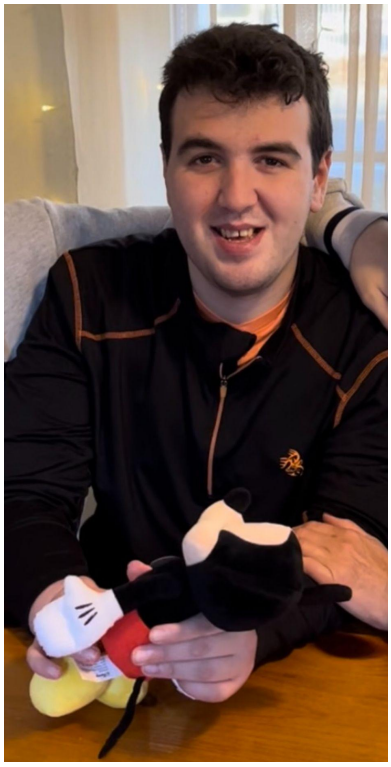
If newborn screening was available for Arginase 1 Deficiency, Josh would've been diagnosed immediately as an infant. Newborn screening and early treatment would've given him the possibility of meeting all his milestones in the first few years of life. Because he didn't have early treatment, his legs became very stiff from spasticity and can only walk for short periods. He needs speech therapy, occupational therapy, physical therapy, leg braces and behavioral therapy. Josh has developmental delay, a seizure disorder and physical disabilities. Early diagnoses and early treatment could've stopped or reduced the effects of this condition and might have made a big difference in Josh's development. It's unfortunate that Josh was left untreated and became severely brain damaged.

As a mother it's heartbreaking to realize that if newborn screening were available Josh could've had early treatment and had the chance of being higher functioning or even mentally capable of living a normal independent life! Early diagnosis of Arginase 1 Deficiency and early treatment could've prevented countless days of seizures, vomiting, hospitalizations, behavioral issues and Josh becoming medically complex needing 24 hour care. The lack of newborn screening robbed Josh the chance to have a typical life and the effects of late diagnosis continues to plague him today at age 25.

What if….this was your child?

Sincerely,

Alexandra Eaton



Hello, my name is Angela Garcia and I'm the mother of Isaiah Lopez.

My son was diagnosed in 2016 with argininemia (Arginase 1 Deficiency) through a newborn screening panel. When Isaiah was first diagnosed, I was very scared of the unknown. I have now accepted that everyday is a learning process. I am thankful that my son was diagnosed before having to go through any kind of crisis. His condition has been controlled since day one. We know he has a special diet. We know what he has to avoid and we know what he can and cannot have on a day-to-day basis. I feel that my son was a miracle and was blessed with being able to be tested through a newborn screening panel. If it weren't for the newborn screening, my son would not be diagnosed on time and would have gone through a medical crisis before we could figure out what was wrong with him. So, therefore, a newborn screening was a lifesaver for my son and gave him a brighter future. I am thankful everyday that I was able to prepare beforehand and able to save my son from a whole lot of unknown crises. I hope that the newborn screening will be approved for everyone's new born baby everywhere.

In March of 2017, my daughter, Briana, was 2.5 years old when she was diagnosed with Arginase Deficiency. Briana had met all her milestones before being diagnosed. The Genetics doctor informed us Briana could have Cerebral palsy symptoms later down the road since Arginase Deficiency was a progressive diagnosis. We were clueless how quickly her mobility would change. After two months of being diagnosed, Briana started to show spasticity in both legs. A few weeks later, she began toe walking and started to lose mobility. She could no longer walk without assistance. Briana began physical therapy 3 months after being diagnosed.

Briana has been taking physical, occupational, and speech therapy for over 5 years. Briana still suffers from spasticity and is extremely flat footed. We have been told by her physical therapist Briana could have hip problems by the time she's in her 20's if footing and posture do not align properly. Briana's enzyme levels have to be constantly monitored when her Arginase levels are high. This is a constant concern we have and monthly labs have to be drawn.

We live in the State of Arkansas and unfortunately Newborn Screening for Arginase Deficiency is not offered. I truly believe if Briana would have been diagnosed at birth, several of her symptoms would not exist or would be minimal. We've had the opportunity to meet other families with Arginase deficiency who had Newborn Screening. These families were able to get their child started on the right diet and medicine and their child has benefited from it. We would love this opportunity for all.

It all started with an ominous, dark grey sky and two of the most vibrant, beautiful double rainbows over a hospital in Temple, Texas. Almost seven years ago this July our lives were changed and not in any way we could have ever imagined. My husband and I watched our siblings have birth after birth that were normal and healthy. So, when we found out we were pregnant, we were anxious like many new parents, but ultimately, we were confident and thrilled for a new adventure.

On July 26, 2016, Lincoln James Istre (pronounced East) was born. During the night while he was being monitored, Lincoln stopped breathing twice and had to be resuscitated, so they moved him into the Neonatal Intensive Care Unit. On July 27th they ran the mandatory Newborn screen 24 hours after birth, via a heel stick, while Lincoln was in the NICU. In addition to low oxygen levels, his bilirubin and white blood cell levels were high, his platelets were low, his red blood cells were all over the place in their levels and size. All we knew at the time was that our newborn son had wires all over his tiny body, required oxygen around the clock or he would stop breathing on his own and that he had to stay in the hospital under the blue light to fix his jaundice. After a week in the hospital, we finally got to go home. We were never told what any of his test results were. The doctors just said, he can breathe on his own, he's not jaundiced any more, and his platelets are normal so we could leave. Three days later, while still adjusting to life with a newborn, we got an urgent phone call while at Lincoln's audiology appointment from a nurse at the state level telling us to go to the ER immediately for testing. We didn't know why. Lincoln was acting fine, we just got out of the hospital after being there for a week and we did not want to go back because the "State of Texas said so." The Newborn Nurse explained what tests were being requested because the Newborn Screen Test "Amino Acidemia" levels were coming back elevated consistently and Lincoln needed to have his Plasma Amino Acids checked. So, at 8 days old, they ran Lincoln's second required Newborn Screen, and we got an official diagnosis of Argininemia.

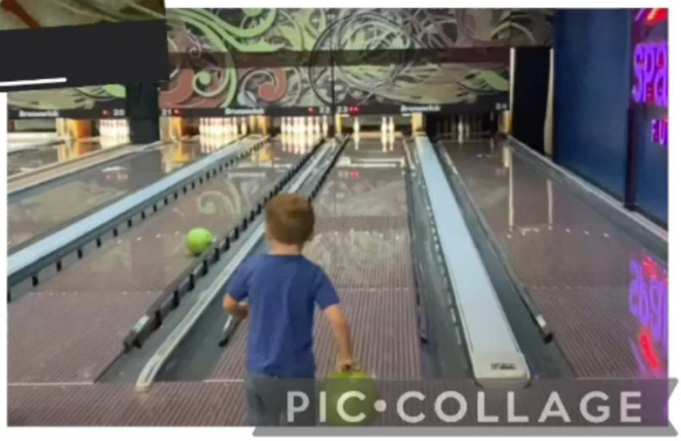
The Texas Newborn Screen tests for 55 rare genetic disorders. On each of Lincoln's test results the "Amino Acidemias" was coded as HIGH. Further testing of his specific amino acids showed that he had elevated Arginine in his blood with a level of 427 at birth. The normal range is anywhere from 40-120. The Newborn Screen changed the course of our life and our son's life. When we met with his specialist we asked for the bottom line. What does this diagnosis mean? "Your son, if not treated, will not be able to walk, talk, could have physical delays, cognitive delays. He could develop seizures, go into a coma, and die." Thanks to the newborn screen, we put Lincoln on the medical/dietary restrictions immediately at **two weeks old!** I am a firm believer that having knowledge is power and with that power, we were able to take measures to set Lincoln up for success which has allowed him to be doing as well as he is. He's better off than most kids his age with the same diagnosis. We were told there could be physical issues-we put him in physical therapy at a month old. We had him in speech therapy at a year to keep his brain sharp. We were told his muscles could get spastic, he could have issues walking- we put him in baseball and encourage all physical exercise. The mandatory newborn screen gave our family important and critical information that has allowed our son to be able to be healthy, happy, and relatively unaffected by his diagnosis of Agininemia. No one in either of our families has this diagnosis, so had we lived anywhere else in the US, we wouldn't have known to check for it, and Lincoln's life could be very different. Instead, in his 7 years, he's only had 2 hospitals stays with hyperammonemic episodes and minimal lingering effects that we can tell. Of the 250 people in the US that have Argininemia, Lincoln is 1 of 5 that are healthy all because of the Newborn Screen.

It takes a lot of work and commitment on our parts as parents and caregivers to love these children, but God gives special children to special people. At least that's what my dad says. Lincoln would not be here or doing as well as he is without the Newborn Screen test. It's literally life altering. He's as healthy as he is because of hard work and dedication to his care by his medical care team and our loved ones. This road has not been easy, and it is not well traveled; but for us, it all began with a heel stick, an ominous grey day, and two of the most beautiful, vibrant double rainbows you've ever seen; promising us a life of challenges, but with double the hope, double the hard work, and double the love we can overcome it all.





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