Rhode Island Metabolic Newborn Screening:

The Effect of Early Identification. A Case Report of Argininosuccinic aciduria (ASA)

Natalie M. Beck, MGC, CGC, Julie Pocost Johnston, RD, LDN, Karen S. Lemke, RN, BSN, Peter Pogacar, MD, and Chanika Phornphutkul, MD

Starting from a single metabolic disorder in 1962, newborn screening (**NBS**) has expanded from identifying persons with phenylketonuria (**PKU**), to a national division of the public health system and includes metabolic conditions, hemoglobinopathies, endocrinopathies, infectious diseases, congenital hearing loss and cystic fibrosis.[1] Modern technological advancements, such as tandem mass spectrometry (**MS/MS**), have allowed for a higher volume of samples to be accessioned for an increasing number of conditions with better accuracy and in a more timely manner. This advanced system still utilizes the original style of specimen, the 'Guthrie specimen' of dried blood spots on filter paper.[2] With MS/MS, a single assay from a sample of this blood spot allows for screening for many inborn errors of metabolism.

Screen positive results can be deemed 'true' or 'false' after additional testing. The majority of positive results are eventually found to be 'false positive NBS' which can result from transient abnormalities due to neonatal physiological analyte variation, transient enzyme immaturity in preterm infants, iatrogenic factors, medications such as antibiotics, or maternal diet or medical conditions. This is usually determined by obtaining a new specimen and running a second MS/MS NBS.[1–3] For more suspicious screen positive results, additional analyte levels can be assessed and second tier testing can be done on the original Guthrie filter paper blood spot, which improves the specificity of NBS and decreases the time for the infant to receive directed follow up care. Examples of these include the automatic molecular testing of common mutations for infants who are screen positive for galactosemia or cystic fibrosis.[2, 4]

To provide more comprehensive and standardized newborn screening throughout the country, the American College of Medical Genetics (**ACMG**) and the United States Health Resources and Services Administration recommended a uniform panel of 29 disorders for what is now called 'expanded NBS' in 2006.[5,6] At present, nearly every infant born in the country is screened for over 30 conditions in a standardized fashion, independent of the state of birth. While each metabolic condition included in NBS is quite rare (ranging in prevalence from one in 5,000 to less than one in 200,000), the incidence of a newborn to have any of these collectively is approximately one in 4,000 births. [5] Early 21st century investigations revealed that even with only some states in the New England region utilizing expanded metabolic NBS, there was already over a 30% increase in affected infants being identified.[7] Over six years later, this number has grown. The state of Rhode Island adopted the expanded NBS in July 2006.

All of the conditions that are part of metabolic NBS are chronic, requiring lifelong treatment and management. NBS allows for early identification of affected infants prior to clinical presentation and for implementation of treatment(s), to prevent or reduce symptoms of these disorders, which can include, but are not limited to: developmental delay, mental retardation, alopecia, ataxia, seizures, memory problems, metabolic acidosis, hyperammonemia, coma, and in some cases, death.[2, 3] In this article, we report a newborn with a urea cycle defect which was detected by NBS. Diagnosis was confirmed and treatment was instituted prior to the infant becoming symptomatic.

Case Report

A boy was born at 40 weeks by vaginal delivery to a G1P0 mother following a pregnancy complicated by intrauterine growth restriction (**IUGR**), bilateral hydronephrosis and mild hypospadias, weighing 5lbs 12oz (2.6kg). NBS specimen collection was obtained on day two of life. Four days after specimen collection, the NBS co-coordinators were notified of a sample showing increased argininosuccinic acid of 47.64 and an increased citrulline level of 73.39. This pattern was suggestive of argininosuccinic aciduria (**ASA**), a disorder of the urea cycle.

On day of life seven, the patient was alert and had a normal examination, however his plasma ammonia was elevated at 134 µmol per liter. The patient was treated for hyperammonemia using a combination of sodium benzoate and sodium phenylacetate (Ammonul®) to reduce ammonia levels, arginine hydrochloride, IV dextrose solution and was made n.p.o. Metabolic laboratory evaluations for the biochemical diagnosis of ASA included plasma amino acids, urine organic acids, plasma ammonia level, electrolytes and glucose level. The results confirmed a biochemical diagnosis of ASA. Due to initial insufficient oral intake during hospital admission, a nasogastric (**NG**) tube was inserted to ensure appropriate feeds until the infant could maintain proper oral intake. NG tube was discontinued at around two weeks of age. The infant continues to be well-managed at home on a calculated low protein diet of medical formula supplemented by pumped breast milk and arginine supplement. Molecular diagnosis confirmed the biochemical diagnosis of ASA with two previously reported disease-causing mutations: c.1045_1057del13 (p.V349fs) and c.1135C>T (p.R379C). The patient is growing adequately, and is developmentally appropriate for his age.

Overview of ASA and the Urea Cycle

ASA, also known as argininosuccinate lyase deficiency (OMIM#207900), is a rare autosomal recessive disorder of the urea cycle. The homozygous loss of function of the argininosuccinate lyase (ASL) gene at 7cen-q11.2 leads to insufficient enzyme activity for proper metabolism of excess

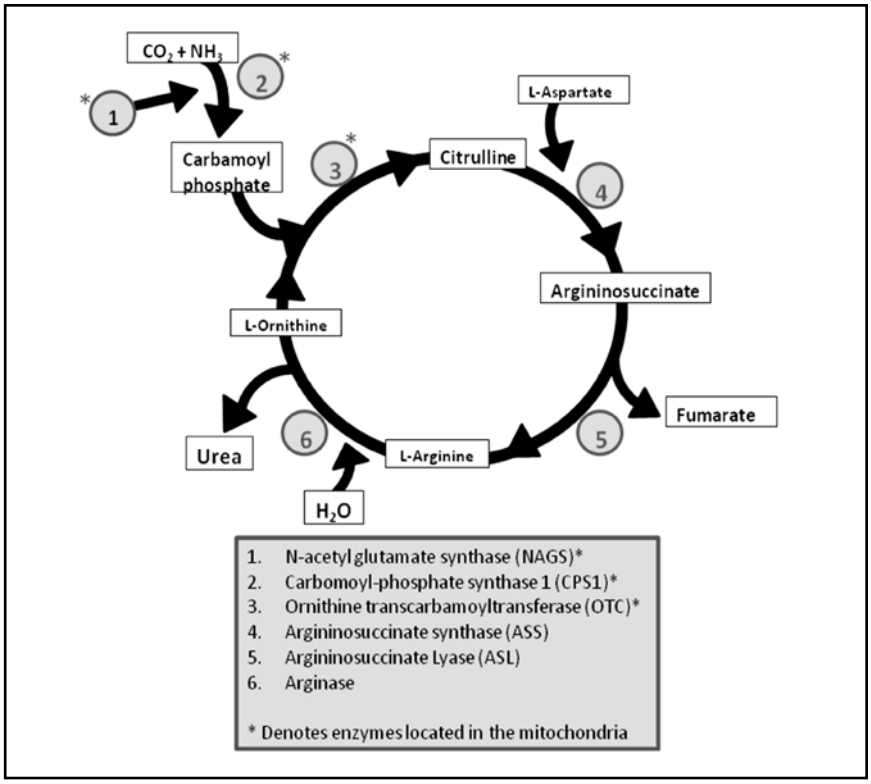

Figure 1. The Urea Cycle

nitrogen, in the form of ammonia, to be converted into urea for excretion.[8,9] There are five main enzymes of the urea cycle with an important sixth that provides a necessary co-factor for the initial reaction of the cycle (Figure 1). As depicted in the figure, ASL is the fifth enzymatic step in the cycle. With insufficient or absent function of the argininosuccinate lyase enzyme, argininosuccinic acid and citrulline accumulate in the blood and argininosuccinic acid is excreted in the urine. In addition to nitrogen metabolism, the urea cycle is responsible for the synthesis of arginine to supplement dietary arginine.[9] In persons with ASA, this synthesis is prevented; causing arginine to become an essential amino acid.

Clinical presentation of ASA patients can vary greatly. Severe presentations often result in neonatal death, while others with ASA may only have symptoms during illness or other times of environmental stressors. The clinical features of ASA can include: lethargy, vomiting, hypothermia, hyperventilation, hepatomegaly, progressive encephalopathy, seizures, coma, and death. These symptoms all stem from rising ammonia levels in the blood and the brain.[9] Since there is no alternative pathway for ammonia in the body, it can elevate in concentration rapidly in patients with urea cycle defects. During hyperammonemic episodes, ammonia climbs to toxic levels, causing central nervous dysfunction and disturbances in cerebral metabolism, neurotransmitter systems, membrane potentials, pH, protein expression, calcium signaling, and mitochondrial functions, among other problems. [8,9]

Studies have examined the difference in outcome for patients with metabolic conditions identified by NBS compared to those who present clinically. Significant differences were found in favor of those identified pre-symptomatically by NBS, with lower instances of cognitive, behavioral and motor deficits.[10,11] Other factors that significantly influenced long term outcome include the prevention of hyperammonemic crises and the age at first hyperammonemic episode.[11,12]

Dietary management for patients with ASA is similar to those with other urea cycle defects. A protein-restricted diet will prevent excess nitrogen from entering the urea cycle and therefore prevent the resulting elevated ammonia. A standardized level of protein prescribed for this diet is based on weight and age.[13] Protein intake should allow for proper growth and prevent the body from entering catabolism—which produces nitrogen from stored protein. In children who frequently refuse feedings, are recurrently ill or have decreased intake, the placement of NG or gastrointestinal tubes may be necessary for proper intake for a period of time. Arginine supplementation is the second component of the medical diet for persons with ASA, as their bodies are unable to synthesize de novo arginine.[14] Patients who maintain their prescribed medical diet and seek medical attention in times of illness to prevent metabolic crises are expected to have normal outcomes.

Newborn Screening in Rhode Island

In the US, every state performs metabolic NBS, however until recently the panel varied greatly. Rhode Island implemented the ACMG-recommended expanded panel in July 2006, and it remains the standard metabolic screen. The state of Rhode Island benefits from its concentrated and multidisciplinary professionals who respond to positive metabolic NBS results. This team includes newborn screening co-coordinators, metabolic and genetic specialists, nursing staff, a genetic counselor, and a dietician specializing in the dietary needs of individuals with metabolic conditions. Our state's metabolic screening co-coordinators notify the primary care provider of the positive screen and the necessary follow up testing, and then notify the patient's parents/caregivers of the same. Based on the screen and/or follow up testing, patients are referred for further evaluation and management. A larger group of providers participate in the management of patients with true positive NBS results, including early intervention programs, developmental pediatricians, neurologists, gastroenterologists, physical/occupational/speech and language therapists, case managers, pharmacists, social workers and language interpreters.[14] A key component of this multidisciplinary team is the patient's pediatrician, who must be familiar with the conditions included in metabolic NBS, as well as their presenting clinical features.[15, 16] For newborns discharged home soon after birth, the pediatrician is most likely to respond to early symptoms of metabolic disease in the first days of life, before NBS results are available. Some resources that may benefit pediatricians and other aforementioned providers are listed in Figure 2.

Resource:	Online Access:
ACTion sheets for immediate steps following positive screen by ACMG	www.acmg.net/resources/policies/ACT/condition-analyte-links.htm
Acute illness protocols from New England Consortium of Metabolic Programs	newenglandconsortium.org/for-professionals/acute-illness-protocols/
Newborn screening Information by March of Dimes	www.marchofdimes.com/professionals/24279.asp
Screening, Technology and Research in Genetics (STAR-G) Newborn screening Factsheets	www.newbornscreening.info/
Peer-Reviewed Literature:	
Waisbren SE. Expanded Newborn Screening: Information and Resources for the Family Physician. *Am Fam Physician*, 2008;77(7): 987-994.	PubMed ID#: 18441864
Saudubray JM, Sedel F, and Walter JH. Clinical approach to treatable inborn metabolic disease: An introduction. *J Inherit Metab Dis*. 2006;29: 261-274.	PubMed ID#: 16763886

Figure 2.

Conclusions

The nationwide standardized expanded NBS affords physicians and families the opportunity to identify individuals affected by rare inborn errors of metabolism and intervene before clinical symptoms develop. A well-designed and specific protocol in each state/region allows for notification of positive newborn screen results and the necessary follow up to occur appropriately. After identification, infants with these metabolic disorders require specific treatments and life-long multidisciplinary medical management. This case exemplifies the benefits that a timely and well-coordinated metabolic NBS program provides to infants with positive newborn screen results.

References

1. Sahai I, Marsden, D. Newborn screening. *Crit Rev Clin Lab Sci*. 2009;46(2):55-82.
2. Marsden D, Larson C, Levy H. Newborn screening for metabolic disorders. *J Pediatr*. 2006 May;148(5):577-84.
3. Garg U, Dasouki, M. Expanded newborn screening of inherited metabolic disorders by tandem mass spectrometry: clinical and laboratory aspects. *Clin Biochem*. 2006 Apr;39(4):315-32.
4. Chace DH. Mass spectrometry in newborn and metabolic screening: historical perspective and future directions. *J Mass Spectrom*. 2009 Feb;44(2):163-70.
5. Dietzen DJ, Rinaldo P, Whitley RJ, et al. National Academy of Clinical Biochemistry Laboratory Medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary. *Clin Chem*. 2009 Sep;55(9):1615-26.
6. Center for Disease Control and Prevention (CDC). Impact of expanded newborn screening—United States, 2006. *MMWR Morb Mortal Wkly Rep*. 2008 Sep 19;57(37):1012-5.
7. Comeau AM, Larson C, Easton RB. Integration of new genetic diseases into statewide newborn screening: New England experience. *Am J Med Genet C Semin Med Genet*. 2004 Feb 15;125C(1):35-41.
8. Ficicioglu C, Mandell R, Shih VE. Argininosuccinate lyase deficiency: long term outcome of 13 patients detected by newborn screening. *Mol Genet Metab*. 2009 Nov;98(3):273-7.
9. Mitchell S, Ellingson C, Coyne T, et al. Genetic variation in the urea cycle: a model recourse for investigating key candidate genes for common diseases. *Hum Mutat*. 2009 Jan;30(1):56-60.
10. Waisbren SE, Albers S, Amato S, et al. Effect of expanded newborn screening for biochemical genetic disorders on child outcomes and parental stress. *JAMA*. 2003;290(19):2564-72.
11. Shih VE. Alternative-Pathway Therapy for Hyperammonemia. NEJM. 2007;356(22):2321-2322.
12. Enns GM, Berry SA, Berry GT, et al. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *NEJM*. 2007;356(22):2282-92.
13. Leonard JV. The nutritional management of urea cycle disorders. *J Pediatr*. 2001 Jan;138(1 Suppl):S40-4;discussion S44-5.
14. Kemper AR, Boyle CA, Aceves J, et al. Long-term follow-up after diagnosis resulting from newborn screening: statement of the US Secretary of Health and Human Services' Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children. *Genet Med*. 2008 Apr;10(4):259-61.
15. Waisbren S. Expanded newborn screening: information and resources for the family physician. *Am Fam Physician*. 2008 Apr 1;77(7):987-94.
16. Saudubray JM, Sedel F, Walter JH. Clinical approach to treatable inborn metabolic diseases: an introduction. *J Inherit Metab Dis*. 2006 Apr-Jun;29(2-3):261-74.

Natalie M. Beck, MGC, CGC is Clinical Genetic Counselor, Rhode Island Hospital & Hasbro Children's Hospital.

Julie Pocost Johnston, RD, LDN, is a Metabolic Specialist at the Children's Neurodevelopment Center, Inborn Errors of Metabolism Clinic at Rhode Island Hospital/Hasbro Children's Hospital.

Karen S. Lemke, RN, BSN, is a graduate of the University of Rhode Island ('97).

Peter Pogacar, MD, is a pediatrician at East Greenwich Pediatrics and staff physician at Hasbro Children's Hospital and Women and Infants' Hospital.

Chanika Phornphutkul, MD is Associate Professor of Pediatrics, Warren Alpert School of Medicine at Brown University and Hasbro Children's hospital.

Disclosure of Financial Interests

The authors and/or their spouses/significant others have no financial interests to disclose.

Correspondence

Chanika Phornphutkul, M.D.
Department of Pediatrics
593 Eddy Street
Providence, RI 02903
phone: (401) 444-5504
fax: (401) 444-2534
e-mail: Chanika_Phornphutkul@brown.edu