Low Citrulline in Newborn Screening Specimens: The Proximal Urea Cycle Defects and Beyond
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Abstract

Background: The New England Newborn Screening Program (NENSP) has used low citrulline (Cit) as a biomarker to screen for the proximal UCDs [ornithine transcarbamylase (OTC), carbamylphosphate synthase (CPS) deficiency, and N-acetylglutamate synthase (NAGS)] since 2004. Low plasma Cit has been reported in other metabolic disorders (pyrroline-5-carboxylate synthetase deficiency -P5CS) and Cit is used as a marker of enterocytes mass and function. We performed a retrospective analysis to evaluate the (1) determine the etiology of the false positives (FP) and (2) possibility of screening for other disorders when using low Cit as a marker in screening.

Methods: The study cohort is all 1.2 M newborns screened for the proximal UCD’s in the region from Aug 2004 to Aug 2013. Cit is analyzed as part of the amino acid and acylcarnitine markers analyzed by MS/MS. A positive screen for proximal UCDs were specimens with 1) Cit < 3uM or, 2) Cit > 3uM to < 3.8 in conjunction with Cit/[Tyr x Met] < 0.002.

Results: In 20 infants the initial specimen collected by day of life 7 screened positive for a proximal UCD. Of these infants 8 were confirmed to have a proximal UCD (5 OTC, 2 CPS, and 1 NAGS). The other 12 were concluded to be FP. Nine FP were from neonates in intensive care units (NICU) on total parenteral nutrition (TPN).The 3 neonates with FP screens who were not in the NICU showed various plasma and/or clinical anomalies on follow-up. An additional 5 infants with in-range Cit values on an initial screen and a positive screen on a subsequent screen were identified; these were all infants who had had a bowel resection and were on TPN. The newborn screening data of the only infant with P5CS deficiency in the cohort was reviewed and was noted to have a low Cit (4.2 uM), albeit above our threshold. One case of a late onset OTC had a false negative screen (Cit 5.02 uM).

Conclusions: Screening using low Cit as a marker can detect the proximal UCDs and it may be feasible to screen for additional metabolic disorders (e.g. P5CS) using low Cit as a marker; although some adjustments in cut-offs maybe required. A large portion of the FP appear to be as a result of a decreased enterocyte mass and function.

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Missouri’s Full Population Pilot Screening for Fabry Disease and the Implications for Families
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Abstract

Introduction: In January, 2013, Missouri began a full population pilot/implementation phase to screen for four lysosomal storage disorders, one of which was Fabry disease. Fabry disease follows an X-linked recessive pattern of inheritance, meaning the gene is carried on the X sex chromosome and males are affected more often than females. It is estimated that Fabry disease affects one in every 40,000 to 60,000 males. Fabry disease can cause episodes of pain, hearing loss, progressive kidney damage, heart attack, and stroke. Milder or later onset forms of Fabry disease are likely more common, may appear later in life, and affect only the heart or kidneys.

Methodology: Full population pilot screening for Fabry disease began on January 11, 2013 utilizing the digital microfluidics multiplex enzymatic assay technology provided by Advanced Liquid Logic, Inc., an Illumina Company (ALL). All positive Fabry screens were referred to one of four contracted genetic tertiary centers for further evaluation and confirmatory testing.

Results: During the first full year of screening, 90,682 newborn samples were screened at the Missouri State Public Health Laboratory. There were a total of 27 newborns confirmed positive with Fabry genotypes, 6 of which were later onset, 1 unknown onset, and 1 genotype of unknown significance. Approximately 80% of the confirmed positive Fabry genotypes were the A143T mutation. Each newborn was referred to genetic specialists for evaluation and confirmation as well as genetic counseling for the family. As part of the genetic counseling, family histories were obtained and testing was offered to the at risk family members of the confirmed positive newborns. Of those families that pursued testing, a total of 58 family members have been diagnosed with Fabry disease. Some of these family members were found to be symptomatic and have since begun treatment for Fabry disease.

Implication: The number of family members identified with Fabry disease as a result of newborn screening emphasizes the importance of genetic counseling and familial testing in the follow up of newborns identified with Fabry disease.

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Summary

Introduction: Fabry disease is an X-linked lysosomal storage disorder caused by deficient activity of the enzyme alpha-galactosidase A (GLA). As a result of the deficient enzyme activity, there is a progressive buildup of a specific type of fat called globotriaosylceramide (GL-3) in cells, most often affecting the cells that line the blood vessels in the skin and cells in the kidneys, heart, and nervous system. Characteristic symptoms of classical Fabry disease include: neuropathic pain, acroparasthesias, angiookeratomas, altered temperature sensitivity, hypohidrosis, ophthalmological problems, gastrointestinal problems, and auditory problems. Fabry disease can also lead to life threatening renal, cardiac, or neurological complications. In affected males, symptoms typically begin in childhood or adolescence, however some
affected individuals may have milder forms of Fabry disease that can appear later in life and may affect only the heart or kidneys.

It has been estimated that Fabry disease affects approximately 1 in 40,000 to 60,000 males. Fabry disease is found in all ethnic, racial, and demographic groups. Fabry disease can be transmitted by both males and females, and is considered to be an X-linked dominant disease with variable expressivity in males and females with hemizygous males typically being more severe than heterozygous females. Females may be as severely affected as males, have less severe symptoms with a later onset than males, or be completely asymptomatic.

Individuals with Fabry disease may benefit from certain medications and treatments to help reduce signs and symptoms related to this condition and to prevent future complications. In males with classic Fabry disease, it is recommended that enzyme replacement therapy (ERT) be initiated in early childhood (age 4-6 years) in order to improve signs and symptoms and stabilize organ function. Heterozygous females who are symptomatic with signs of Fabry disease may benefit from starting treatment early as well but typically are recommended to start treatment between 16-18 years of age.

Methodology: On January 11, 2013, Missouri began an IRB approved full population pilot/implementation phase to screen newborns for four lysosomal storage disorders, one of which was Fabry disease. The Missouri State Public Health Laboratory utilized the digital microfluidics multiplex enzymatic assay technology provided by Advanced Liquid Logic, Inc., which is now Baebies, Inc. All positive Fabry screens were referred to one of four contracted genetic tertiary centers for further evaluation and confirmatory testing via enzyme analysis and mutation studies. The results of the evaluations and confirmatory testing along with the confirmatory diagnoses were then reported back to the Missouri Department of Health and Senior Services.

Results: During the first full year of screening, 90,682 newborn samples were screened at the Missouri State Public Health Laboratory. There were a total of 30 newborns confirmed positive with reduced enzyme activity and a mutation identified in the GLA gene. Of those 30 newborns, there were 3 newborns with variants of unknown significance and there were 2 females. Twenty, or approximately 66%, of the confirmed positive Fabry genotypes were the A143T mutation.

Based on the data collected from the first full year of screening, the incidence of Fabry disease in Missouri is approximately 1 in 2,500 live births. If the number of live births is broken down even further to differentiate between males and females, the incidence for Fabry disease in Missouri is approximately 1 in 1,300 males and 1 in 19,000 females. In particular, the A143T mutation has been found to be surprisingly prevalent throughout the state. The incidence for this mutation alone in Missouri is approximately 1 in 3,800 live births. If patients with the A143T mutation and variants of unknown significance are excluded, the incidence would still be higher than previous estimates at approximately 1 in 11,000 live births or 1 in 5,300 males. These numbers are a great deal higher than estimates found in the literature. However, this is only data for one year; therefore the incidence will need to be reevaluated in the future.

The table below provides further information on the specific Fabry mutations detected during the first year of screening.

As part of the Missouri newborn screening follow-up process, each newborn was referred to one of four contracted genetic tertiary centers for evaluation and confirmation as well as genetic counseling for the family. Genetic counseling plays a vital role in most all newborn screening disorders, as more often than not; there is no known family history of the disorders detected through newborn screening. Genetic counseling provides families with much needed information and support during an extremely stressful time period.

As part of the genetic services provided, family histories were obtained and testing was offered to at-risk family members of the confirmed positive newborns. Of those family members that have pursued testing, a total of 55 relatives have been identified to carry the same mutation as seen in the affected proband within their family. Parents, siblings, aunts, uncles, cousins, and grandparents have all been identified with Fabry mutations as a result of assessments following an abnormal newborn screening. Some of these family members were found to be symptomatic and a few have begun treatment for Fabry disease. Reported symptoms have ranged anywhere from renal or heart disease to asymptomatic males and females. Complete formal evaluations of many of these affected relatives are still pending.

Implications: Missouri's first full year of screening for Fabry disease has provided invaluable information for the Newborn Screening Program and detecting these babies early will allow for careful monitoring and the initiation of treatment as soon as clinically appropriate, allowing them to grow and develop as healthy as possible with the ultimate goal of preventing or lessening the complications typically associated with this disorder. However, screening does not come without challenges. Parents often express frustration due to the fact that there are no established surveillance and treatment protocols for newborns. In addition, the lack of available information on some mutations can be frustrating to both the families and the genetic counselors that are providing support and guidance. For the A143T mutation and late-onset mutations, there is little evidence to indicate if or when symptoms will occur or when treatment should be initiated.

Despite the challenges, newborn screening for Fabry disease has the potential to make a positive impact on the lives of newborns and their families. The number of newborns and subsequent family members identified with Fabry disease as a result of newborn screening emphasizes the great importance of genetic counseling in short-term follow-up for Fabry disease. Genetic counseling helps families cope with this unexpected diagnosis, provides families with appropriate educational materials, helps identify other affected relatives, and provides information regarding recurrence risks and family planning. With appropriate genetic counseling, newborn screening for Fabry disease will not only make a difference in the lives of newborns, but will also make a difference in the lives of numerous family members.

<table>
<thead>
<tr>
<th>Number of Cases</th>
<th>Mutation</th>
<th>Classification</th>
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<tr>
<td>20</td>
<td>A143T</td>
<td>Pathogenic¹</td>
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<tr>
<td>3</td>
<td>I198T</td>
<td>Likely Pathogenic¹</td>
</tr>
<tr>
<td>2</td>
<td>R118C</td>
<td>Likely Pathogenic¹</td>
</tr>
<tr>
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<td>L120P/L121T</td>
<td>Pathogenic (mother is a known Fabry patient)²</td>
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<td>1</td>
<td>c.369+5G&gt;T</td>
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<tr>
<td>1</td>
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</tr>
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<td>1</td>
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Developing a Newborn Screening Follow-up Framework for Pompe Disease

Abstract

In June of 2013, the Discretionary Advisory Committee on Heritable Disorders in Newborns and Children recommended that the Recommended Uniform Screening Panel (RUSP) be expanded to include Pompe disease. New York, as well as other states, are now preparing for newborn screening for this disorder. Short-term and long-term follow-up guidelines were developed and reviewed with a meeting of metabolic geneticists from the New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services (NYMAC), as well as with an expert on the disorder, in November, 2013. With feedback from this group, a diagnostic algorithm and medical management guidelines were refined until consensus was reached. Case definitions were also created. These documents establish a framework for New York’s future follow-up efforts for newborn screening for Pompe disease, and may serve as a resource for other states in the early stages of development. We will present and review the algorithm, guidelines, and case definitions.

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Summary

Pompe disease is a genetic disorder caused by mutations in the GAA gene. It is a progressive metabolic condition that causes muscle weakness. The GAA gene codes for an enzyme called acid alpha-glucosidase (GAA) which is necessary for the degradation of glycogen in the lysosome. Mutations in GAA result in an accumulation of glycogen in the lysosomes. It is therefore considered a Lysosomal Storage Disorder. The inheritance of Pompe disease is autosomal recessive, it has an incidence of approximately 1 in 17,000, and it is panethnic.

There are two primary Pompe disease phenotypes. The early-onset form of Pompe disease results from complete or near absence of GAA enzyme activity. Symptoms begin at birth or shortly thereafter, with hypotonia, hypertrophic cardiomyopathy, failure to thrive, and respiratory insufficiency. Without treatment progression is rapid and most babies die from cardiac or respiratory complications before a year of age. The late-onset form of Pompe disease results from partial deficiency of GAA enzyme. The age of onset is variable; symptoms may appear as early as the first few months of life, or as late as adulthood. The primary symptom is a slowly progressive myopathy primarily involving skeletal muscle. There is not usually cardiac involvement with the late onset form of Pompe disease.

References
- EmVClass, Emory Genetics Laboratory’s Variant Classification Catalog, http://genetics.emory.edu/egl/emvclass/emvclass.php
- As reported by contracted genetic tertiary center.
In June of 2013, the Discretionary Advisory Committee on Heritable Disorders in Newborns and Children recommended that the Recommended Uniform Screening Panel (RUSP) be expanded to include Pompe disease. The New York State Newborn Screening Program has been planning and preparing for the initiation of Pompe disease screening. Pompe screening in NYS will be accomplished using a two-tier algorithm. The first tier will be MS/MS of the GAA enzyme. The second tier involves sequencing of the GAA gene.

The New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services (NYMAC) sponsored a Pompe disease symposium November 1-2, 2013 in Valhalla, NY. Participants at this meeting included metabolic geneticists from the mid-Atlantic region, genetic counselors, and newborn screening personnel from several states, as well as an expert on Pompe disease, Dr. Priya Kishnani from Duke University. Through discussion with this group, and subsequent follow-up through email and phone calls, the New York State Newborn Screening Program developed a Pompe disease diagnostic algorithm, medical management recommendations and case definitions.

The diagnostic algorithm was developed first. The goal of the diagnostic protocol was to determine if the newborn has Pompe disease, and to recommend the minimum confirmatory testing needed to determine this. The algorithm will be reviewed during the presentation.

Management recommendations and considerations were also created through feedback from the metabolic geneticists, as well as Dr. Kishnani, who attended the NYMAC Pompe symposium. The goal of these were to provide medical management recommendations for infants and children with Pompe disease identified on newborn screening, with the understanding that they include evaluations that were believed to be minimally needed, and are not a substitute for good clinical judgment. The
reasoning behind the creation of this document is that the most recently published management guidelines on Pompe disease were from 2006 (Kishnani PS, Steiner RD, Bali D et al. (2006)) when the diagnosis most often occurred because an infant or child was symptomatic, and when enzyme replacement therapy was still considered an emerging treatment. There were no published recommendations in the scientific literature for medical management following a positive newborn screen.

The management recommendations cover four topics: determining Cross Reactive Immunologic Material (CRIM) status, recommendations and considerations for initiating ERT, recommended evaluations for monitoring of asymptomatic patients with Pompe disease, and recommended evaluations for monitoring of symptomatic patients with Pompe disease. These will be presented in more detail during the presentation.

Lastly, Newborn Screening Program staff developed Pompe disease case definitions that will be used by follow-up staff, as well as the metabolic geneticists at the specialty care centers, to classify diagnostic end points. These will aid in short and long term data collection. These will be briefly presented during the presentation.

Developing Short- and Long-term Follow-up for X-linked Adrenoleukodystrophy
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Abstract

New York State was mandated by legislation to screen newborns for X-linked adrenoleukodystrophy by December 31, 2013. Short-term and long-term follow-up guidelines were developed during a series of conference calls with metabolic specialists from nine specialty care centers and a disorder expert. A diagnostic algorithm, case definitions, medical management guidelines, long-term follow-up data elements and educational materials were developed to prepare for the initiation of testing. The materials were reviewed with neurologists, endocrinologists and genetic counselors from the metabolic specialty care centers in New York. A diagnostic algorithm and case definitions were developed to account for all anticipated outcomes. Thus, newborns with a positive screen have a differential diagnosis of peroxisomal disorders including Zellweger spectrum disorders. Medical management guidelines incorporate scheduled surveillance. This schedule is needed due to the delayed onset of adrenoleukodystrophy. Because New York was the first newborn screening laboratory to include X-ALD on its panel, and symptoms may not develop for years, long-term follow-up infrastructure was built by determining the 40 most relevant data elements before screening started. Educational materials are available to parents and providers to explain X-linked inheritance and the implications of a positive screen for this condition for other family members.

Presenter: Beth Vogel, MS, New York State Department of Health, Wadsworth Center, Newborn Screening, Albany, NY, Phone: 518.474.7945, Email: bmh06@health.state.ny.us
Summary

X-linked adrenoleukodystrophy is a metabolic disorder affecting the adrenal glands, neurological system and testes (Loes DJ, Fatemi A, Melhem ER, Gupte N, Bezman L, Moser HW, 2003) occurring in 1 in 17,000 births (Dubey et al., 2005; Raymond, Jones, & Moser, 2007; Schaller, Moser, Begleiter, & Edwards, 2007) and 1 in 20,000 males (Mahmood et al., 2007; McKinney AM, Nascene D, Miller WP, Eisengart J, Loes D, Benson M, Tolar J, Orchard PJ, Ziegler RS, Zhang L, 2013). There are two main phenotypes: childhood cerebral ALD and the adult onset adrenomyeloneuropathy (AMN) (Kemp), which can occur within the same family (Berger J, Molzer B, Faé I, 1994). New York State began newborn screening for X-linked adrenoleukodystrophy on December 30, 2013.

The New York State Newborn Screening Program developed a workgroup in June 2013 to determine diagnostic guidelines, surveillance protocols, guidance initiating treatment, parental educational materials and long-term follow-up data elements for adrenoleukodystrophy newborn screening. The group met over several conference calls and consisted of a director from each metabolic center specialty care center in NYS as well as disorder expert Dr. Gerald Raymond, NYS NBS Program Director Dr. Michele Caggana, NYS NBS Director of Operations Dr. Joseph Orsini, NYS NBS Inherited Metabolic Disease Laboratory Supervisor Dr. Mark Morrissey, NYS NBS DNA Laboratory Supervisor Dr. Carlos Saavedra and NYS NBS Genetic Counselor Beth Vogel.

ALD NBS in NYS is accomplished using a three-tier algorithm. The first tier is MS/MS of C26:0 and the second tier is the C26:0 LPC using HPLC MS/MS. The third tier involves sequencing of the ABCD1 gene.

The diagnostic protocol was developed first. The goal of the diagnostic protocol was to determine if the newborn has one of the differential diagnoses: adrenoleukodystrophy or another peroxisomal disorder. The protocol differs based on the outcome of the third tier of ALD NBS and the newborn’s gender. Three categories were created: a male with a mutation, a female with a mutation, or no mutation identified.

For males with a mutation, confirmatory VLCFA to an independent laboratory and a repeat specimen to NYS NBS is done. If VLCFA are elevated at an independent laboratory, it is consistent with a diagnosis of X-linked ALD. Each mother of a male is offered carrier testing at the NBS Program. A female with a gene mutation is likely only a carrier of x-linked ALD. If there is no clinical evidence of peroxisomal disease, then the newborn is considered a carrier of ALD only and genetic counseling is recommended. Both parents are offered gene mutation testing at NYS NBS Program.

If a gene mutation is not identified, regardless of gender, then VLCFA and plasmalogen are ordered to evaluate for a peroxisomal disorder including Zellweger spectrum disorder, acyl-CoA oxidase deficiency and D-bifunctional protein deficiency.

Surveillance protocols were also developed for asymptomatic boys diagnosed with ALD. A neurologist and an endocrinologist from each institution with a metabolic specialty care center were consulted via conference calls. Guidelines were developed for asymptomatic boys at the time of diagnosis, during childhood and during adulthood.
Upon diagnosis, it is recommended that newborn boys establish with an endocrinology practice and have a baseline serum ACTH and cortisol level. It is also recommended that they establish with a neurologist and the family should be referred for genetic counseling.

Childhood is the time of the most frequent monitoring. For monitoring of adrenal involvement, an annual clinical evaluation is recommended along with serum ACTH and cortisol every six months. An annual neurology evaluation is also recommended. Brain MRI without contrast is recommended annually from 6 months until 30 months of age. From 36 months to 10 years of age, brain MRI without contrast is recommended every six months. From age 10 until age 18 annual brain MRI is recommended. Cerebral involvement can be detected by brain MRI. (Loes DJ, Hite S, Moser H, Stillman AE, Shapiro E, Lockman L, Latchaw RE, 1994) If there are suspicious findings on MRI, the group decided that it should be repeated with contrast as soon as possible. Each brain MRI will be evaluated using the ALD MRI severity scoring system, which was designed to compare serial MR exams (Loes DJ, Hite S, Moser H, Stillman AE, Shapiro E, Lockman L, Latchaw RE, 1994). Clinical Genetics evaluation is also recommended. The development of these guidelines were based on recommendations in the literature that boys should have increased monitoring of neurological and adrenal function from age 3 until the end of the first decade. (Mahmood et al., 2007; Peters et al., 2004)

Recommendations were also developed for monitoring of asymptomatic men in adulthood. Starting at age 18, serum ACTH, cortisol and a brain MRI without contrast should be done annually. Transition to adult practices for endocrinology, neurology and genetics should also be considered, depending on the policies and practices of each specialist.

Adrenal monitoring recommendations are for individuals without adrenal insufficiency (Addison disease). Monitoring of adrenal function for individuals with primary adrenal insufficiency should be appropriate for their clinical situation. If ACTH or cortisol abnormalities develop, adrenal hormone therapy should be instituted and is lifesaving. (Raymond et al., 2007)

Considerations for referral of a boy with ALD to HCT were discussed by the group. A boy should be evaluated further for HCT if the ALD MR severity score is greater than one and less than 9 with a performance IQ of greater than 80 (Mahmood et al., 2007; McKinney AM, Nascene D, Miller WP, Eisengart J, Loes D, Benson M, Tolar J, Orchard PJ, Ziegler RS, Zhang L, 2013; Peters et al., 2004).

The group also had a conference call with a genetic counselor from each specialty care center to discuss genetic counseling considerations of ALD NBS. Given the X-linked inheritance of ALD (Kemp et al., 2012) and the potential for multiple individuals in the family at risk for ALD, genetic counselors have an important role in ALD NBS in the identification and counseling of potentially affected family members. (Raymond et al., 2007; Schaller et al., 2007)

Long-term follow-up was also discussed and data elements were developed. Data will be collected on growth and development, ALD symptoms and outcome of monitoring for adrenal and cerebral involvement, prenatal history and family history. Long-term follow-up is critical for ongoing evaluation of the protocols and recommendations.

References:


Diagnostic Follow-up of 41 Infants with a Positive Newborn Screen for Hurler Syndrome (MPSI): Identification of four recurrent IDUA Sequence Changes that Significantly Reduce Enzyme Activity

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Abstract

Pseudo-deficiency has been reported for several lysosomal enzymes in which a clinically unaffected individual's cells demonstrate decreased enzyme activity using an artificial substrate in vitro. Examples of this are rare because they are typically identified during familial testing. Newborn screening has been initiated for several lysosomal storage disorders, resulting in large numbers of unaffected individuals being evaluated. Our laboratory has received samples from 41 infants with an abnormal newborn screen for alpha-iduronidase, the deficiency of which causes Hurler syndrome (MPS I), for diagnostic enzyme analysis using a 4-methylumbelliferone (4-MU) substrate in leukocytes. Of these 41 cases, 15 (37%) had normal alpha-iduronidase activity (> 6 nmol/hr/mg) and three (7%) had activity within our affected range (< 1 nmol/hr/mg). The remaining 23 infants (56%) had alpha-iduronidase activities below normal, but above the affected range. Decreased enzyme activity (below normal) was confirmed in 25/26 patients using an MS/MS substrate, suggesting the results are not 4-MU substrate specific. Molecular analysis of 25 patients with enzyme activities in the affected range or “gray zone” (1-6 Proceedings of the 2014 APHL Newborn Screening and Genetic Testing Symposium, Anaheim, CA, October 27-30, 2014
nmol/hr/mg) revealed four recurring sequence alterations in the IDUA gene: c.235G>A (p.A79T), c.246C>G (p.H82Q), c.667G>A (p.D223N) and c.965T>A (p.V322E), all of which have been reported in the dbSNP database. p.A79T, which has an allele frequency of 2.8% in African Americans, was the most common (44% alleles; 6 homozygotes). These findings indicate that pseudo-deficiency is a common phenomenon for alpha-iduronidase, and both biochemical and molecular characterization of infants with a positive newborn screen for MPSI is strongly recommended.

**Presenter:** Laura Pollard, Greenwood Genetic Center, Greenwood, SC, Phone: 864.388.1070, Email: lpollard@ggc.org

**Summary**

Pseudo-deficiency has been reported for at least eight different lysosomal storage disorders, whereby a clinically unaffected individual’s cells demonstrate significantly decreased enzyme activity using an artificial substrate in vitro. In some cases, it is predicted that the decreased enzyme activity is specific towards the artificial substrate, while the patient has normal enzyme activity towards the natural substrate in vivo. And in other cases, it is predicted that the decreased enzyme activity observed in vitro also occurs towards the natural substrate; however, enough residual activity exists to prevent any clinical effects. In either case, pseudo-deficiency is likely caused by one or more specific amino acid substitutions in the enzyme. Examples of such pseudo-deficiency alleles have been published for several lysosomal enzymes. However, this phenomenon has historically been considered rare, detected only when testing unaffected family members for carrier status or when an apparent enzyme deficiency is not compatible with the patient’s phenotype.

Newborn screening for several lysosomal storage disorders has already been initiated in a small number of states by measuring enzymatic activity in dried blood spots (DBS), and it is likely to expand in the near future. Both fluorescent (4-methyl-umbelliferone (4-MU) conjugated) substrates that use a digital microfluidics platform and substrates designed by Genzyme and provided by the Centers for Disease Control (CDC) for use on the tandem mass spectrometer (MS/MS) are available. A positive newborn screening result must be followed by confirmatory diagnostic testing, which typically involves measuring the activity of the enzyme of interest in leukocytes isolated from peripheral blood.

Our diagnostic laboratory has received samples from 47 infants with a positive newborn screen for deficient alpha-iduronidase activity, which causes Hurler syndrome (Mucopolysaccharidosis type I), from the state of Missouri, which utilizes the fluorometric microfluidics platform. We measured alpha-iduronidase activity in peripheral leukocytes from 46/47 of these individuals using the same 4-MU conjugated substrate used by the Missouri NBS laboratory. Of these 46 cases, 17 (37%) had alpha-iduronidase activity within our normal reference range (> 6 nmol/hr/mg protein) and three (6.5%) had activity within our affected range (< 1 nmol/hr/mg protein), which was developed using results from patients with a confirmed molecular diagnosis of Hurler syndrome. The remaining 26 (56.5%) cases had activity below our normal range, but above our affected range (1 – 6 nmol/hr/mg protein). Patients with activity in this “gray zone” created a diagnostic conundrum. Dried blood spot (DBS) cards had been made using the same peripheral blood sample that was used for leukocyte extraction for 26/29 patients who had alpha-iduronidase activity below the normal reference range (< 6 nmol/hr/mg). Alpha-iduronidase activity was measured in these 26 DBS samples using the tandem mass spectrometer (MS/MS) substrate, and 25/26 (96%) also had enzyme activity below the normal reference range.
previously established in our laboratory using this methodology. Therefore, the unexpectedly high number of samples with decreased enzyme activity was not specific to the 4-MU conjugated substrate that is used by both the Missouri newborn screening laboratory and in our diagnostic assay in leukocytes. Urine glycosaminoglycan results are known for 23/29 of the infants with decreased alpha-iduronidase activity in leukocytes, and none of the results are suggestive of a diagnosis of Hurler syndrome.

Molecular analysis of the IDUA gene was performed for 28 patients with alpha-iduronidase activity below the normal range in leukocytes (< 6 nmol/hr/mg), plus one patient for whom leukocyte extraction failed, to further investigate the cause for the decreased enzyme activity. None of these patients were homozygous or compound heterozygous for previously reported pathogenic mutations. However, we identified four missense changes (p.A79T, p.D223N, p.V322E, p.H82Q) that we had not previously observed in any patients submitted for testing to our laboratory based on clinical suspicion for Hurler syndrome, that were observed at least four times within this cohort of 29 patients. One alteration (p.A79T) was observed in 29/58 (50%) alleles, with nine patients homozygous for this change. This alteration is reported in the dbSNP database and is specific to African Americans, with an allele frequency of 2.8% in this population according to the Exome Variant Server (EVS). The significantly higher frequency of this change within our cohort of patients with reduced alpha-iduronidase activity compared to its frequency within the general African American population suggests that it is contributing to the reduced enzyme activity. However, the frequency of this change within the general population is high enough that it is very unlikely to cause Hurler syndrome. Furthermore, a parent of one of these infants was found to be homozygous for the p.A79T alteration and is clinically unaffected, despite significantly reduced alpha-iduronidase activity (1.15 nmol/hr/mg protein).

The other three recurrent sequence alterations identified in the IDUA gene in patients with reduced alpha-iduronidase activity are p.D223N (7 alleles; 12%), p.V322E (5 alleles; 8.6%) and p.H82Q (4 alleles; 6.9%). All three of these changes have allele frequencies too low (< 1%) to be classified as a polymorphism (0.53% in African Americans, 0.64% in African Americans, and 0.74% in European Americans, respectively), but likely too high to be pathogenic mutations considering none has been reported in association with a clinically affected patient in the past. In comparison, the common p.Q70X mutation that is associated with Hurler syndrome only has an allele frequency of 0.077% in the general population. However, the frequency of these three changes within our patient cohort is significantly higher than that in the general population, suggesting that they contribute to the reduced enzyme activity.

Homozygosity or compound heterozygosity was detected for these four missense changes in 20/29 (69%) patients with reduced leukocyte alpha-iduronidase activity, all of whom had activity within the “gray-zone” (1 – 6 nmol/hr/mg). Of the remaining nine patients, two were compound heterozygous for one of these four changes and one previously reported pathogenic mutation (one with activity in the affected range), four were compound heterozygous for one of these four changes and a variant of unknown significance (VUS) (one with activity in affected range), one was compound heterozygous for a pathogenic mutation and a VUS, one was homozygous for two VUS (activity in affected range), and one had only one VUS detected.

We propose that p.A79T, p.D223N, p.V322E and p.H82Q are pseudo-deficiency alleles in the IDUA gene that can result in decreased alpha-iduronidase activity when found in the homozygous state or in the compound heterozygous state with another pseudo-deficiency allele, a pathogenic mutation, or a VUS.
Pseudo-deficiency for alpha-iduronidase appears to be much more common than previously thought, representing approximately 50% of cases with a positive NBS, and this phenomenon certainly complicates the interpretation of confirmatory testing results. Pseudo-deficiency appears to be especially prevalent in the African American population, with 25/29 (86%) patients with decreased alpha-iduronidase activity in leukocytes being African American or biracial. Molecular analysis of the IDUA gene is strongly suggested for all cases in which alpha-iduronidase activity is below the normal reference range in leukocytes. And consideration must be given as to how to manage patients identified by newborn screening that have alpha-iduronidase activity within the affected range, but without two previously reported or predicted pathogenic mutations in the IDUA gene, especially if they have normal urine glycosaminoglycan results.