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Newborn screening for proximal urea cycle disorders: Current evidence supporting recommendations for newborn screening

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ABSTRACT

Current newborn screening (NBS) for urea cycle disorders (UCD) is incomplete as only distal UCDs are included in most NBS programs by measuring elevated amino acid concentrations. NBS for the proximal UCDs involves the detection in NBS spots of low citrulline values, a finding which is often overlooked because it is considered to be inadequate. We retrospectively analyzed NBS blood spots from known UCD patients comparing the utility of the Region 4 Stork (R4S) interpretive tools to conventional cutoff based interpretation. This study shows the utility of R4S tools in detecting all UCDs, and provides evidence to support the nomination to add proximal UCDs to the recommended uniform screening panel.

1. Introduction

Urea cycle disorders (UCDs) are a group of inborn errors of metabolism due to defects in the generation of urea through clearance of waste nitrogen from breakdown of nitrogen-containing molecules. The proximal urea cycle disorders are defined from enzyme deficiencies in the first three steps in urea cycle (*N*-acetylglutamate synthase, NAGS; carbamyl phosphate synthase, CPSI; and ornithine transcarbamylase, OTC) while the distal disorders include the final three steps of the urea cycle (argininosuccinate synthase deficiency, also known as citrullinemia type 1, CIT1; argininosuccinate lyase deficiency, also known as argininosuccinic acidemia, ASA; and arginase deficiency, ARG) [1].

Newborn screening (NBS) for UCD has been incompletely implemented within the last two decades following the introduction of tandem mass spectrometry for NBS [2]. The recommended uniform screening panel (RUSP) adopted by United States Department of Health and Human Services only included primary target screening for CIT1 and ASA while two less common disorders, citrin deficiency (CIT2, also known as citrullinemia type 2) and argininemia (ARG) were labeled as a secondary targets [3]. NBS for CIT1 and ASA is based upon detecting elevated concentrations of citrulline and argininosuccinic acid. At the time of these recommendations, NBS for proximal UCD were not recommended due to the lack of an adequate test, described as measurement of low levels of citrulline, which can result in high false positive rates due to prematurity, low protein intake, and intestinal dysfunction [4]. Low citrulline concentrations have been described in isolated cases of neonatal OTC, CPS1, and NAGS [4–7].

There is a clear and present clinical need for implementation of NBS for the proximal UCDs. All three proximal UCDs may present with neonatal, infant, adolescent, or adult presentations, although the neonatal presentation is considered to be most common. Population estimates for OTC, the most common UCD, has ranged from 1:14,000 to 1:77,000 live births with recent estimates of 1:56,500 [6,8–11]; with CPS1 and NAGS being each > 1:1,000,000 [11]. While presentations in the first week of life, even in the first 2 or 3 days of life, are expected before NBS results are available, presentations after the first week of life are being increasingly recognized, and require treatment to prevent disease morbidity and mortality in all age groups. Advances in current treatments now are available for nearly every clinical presentation, and are clinically available in most children's hospitals. Treatments now include dextrose-containing intravenous fluids, hemodialysis, and FDA-approved ammonia scavengers such as sodium phenylacetate and sodium benzoate via intravenous or two different oral forms. While some advanced first-line treatments such as hemodialysis or intravenous ammonia scavengers may not be available in some smaller hospitals, this only emphasizes the need for early disease recognition so that appropriate management beginning with immediate measurement of ammonia, institution of intravenous fluids with dextrose, and then transfer to the appropriate care center can be done expeditiously.

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Table 1
Clinical Presentation and NBS Amino Acid Results.

Patient ID	Symptoms	Sex	AOC (hours)	Citrulline	Ornithine	Arginine	Argininosuccinic acid	Glutamate	Glutamine
NS004A OTC	Newborn, mild developmental delays	Male	63	3.94	7.73	3.00	0.38	194.38	33.26
NS004B OTC	Duplicate sample		63	3.84	6.21	4.03	0.67	215.97	32.79
NS012A OTC	Newborn	Male	16	3.58	5.65	5.97	0.77	123.55	29.00
NS012B OTC			*336*	*3.18*	*5.34*	*5.06*	*0.83*	*95.71*	*19.85*
NS013A OTC	Child	Male	*336*	*3.96*	*4.00*	*4.25*	*0.80*	*95.34*	*17.59*
NS013B OTC			24	4.48	4.86	4.76	1.05	93.01	23.11
NS003 OTC (het)	Teen	Female	144	10.31	35.08	2.97	0.51	344.55	35.84
NS010A OTC (het)	Asymptomatic	Female	16	4.23	4.04	5.35	0.89	105.12	25.11
NS010B OTC (het)			*1560*	*3.44*	*5.04*	*5.25*	*1.11*	*111.02*	*19.30*
NS010C OTC (het)			*350*	*4.23*	*7.25*	*5.43*	*0.70*	*174.00*	*29.31*
NS002 OTC (het)	Teen/liver transplant	Female	unk	1.58	3.59	3.97	1.58	85.12	17.89
NS007 OTC (het)	Asymptomatic	Female	unk	6.99	–	–	–	–	–
NS015A CIT-I	Newborn/liver transplant	Male	31	98.62	720	9.58	1.07	175.79	45.30
NS015B CIT-I			*244*	*107.84*	*6.29*	*8.84*	*3.84*	*54.96*	*10.66*
NS009A CIT-I	Newborn/liver transplant	Female	39	249.67	7.70	6.02	0.85	229.86	60.04
NS009B CIT-I			*244*	*380.03*	*10.41*	*8.48*	*1.23*	*72.11*	*18.04*
NS016 ASA	Newborn	Female	unk	211.00	35.79	9.45	631.61	355.51	62.26
NS008 ARG	Infant, hyperammonemia	Male	30	15.49	11.29	66.19	2.11	314.95	48.87
Normal Population range (1st to 99th percentile)			-	6–19	35–117	2–18	0.05–0.6	157–407	22–65

Summary of symptoms as self-reported by participants. AOC (Age of Collection in hours of life). All amino acid concentrations in μM. unk = unknown. Rows in italics reflects AOC times > 7 days of life.

The Region 4 Stork (R4S, https://www.clir-r4s.org) collaborative project was developed to facilitate the clinical validation and harmonization of cut-off target ranges [12]. However, upon recognition of the limitations of cutoff-based algorithms, R4S has evolved into a system to generate post-analytical interpretive tools that provide a score and a rank of likelihood of disease, integrated by instruments of differential diagnosis between conditions with overlapping biochemical phenotypes [13]. Ongoing studies have demonstrated the clinical utility and performance improvement of the R4S tools [13–15]. In particular, one study shows the potential for improving specificity and positive predictive rates such as in very long-chain acyl-CoA dehydrogenase deficiency [16]. R4S tools have been designed to improve the detection of the proximal UCD levels comparable to all other conditions [17].

In order to further understand the utility of the R4S tools for UCD, we designed a pilot case series to retrospectively review NBS blood spots from UCD patients, including those with a mutation in the *OTC* gene. This data provides foundational information for using R4S tools in screening of proximal UCDs on NBS.

2. Methods

We designed a retrospective study of NBS in UCDs through identifying existing individuals who have been diagnosed with a proximal and distal UCD and are followed by the Biochemical Genetics clinics at Seattle Children's Hospital. Following Institutional Review Board approval, prospective patients were approached in clinic and informed consent was obtained per protocol. Study data were collected and managed using REDCap electronic data capture tools [18] hosted at the Institute of Translational Health Sciences (ITHS). REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. After consent, patients were given the choice to complete screening questions via a REDCap survey or by paper. Limited clinical information was obtained via REDCap or paper forms included age of presentation, age of diagnosis, symptoms prior to diagnosis, hospitalized at diagnosis, recalled peak ammonia levels, and developmental delays. Patient information was then reviewed and release of the NBS blood spot was requested from their respective state of birth.

NBS blood spots were then de-identified and sent for analysis at the Mayo Clinic Biochemical Genetics Laboratory (Mayo Clinic IRB protocol #09-001709, "Establishment of disease ranges of selected markers and ratios for the detection of OTC deficiency and CPS deficiency using newborn blood spots"). Following analysis, blood spots were then returned and securely stored per protocol. Sample results were then reviewed and analyzed by currently available R4S tools for UCDs. The standard OTC/CPS tool includes glutamine, glutamate, and ornithine in its calculation and was affected by the prolonged storage times so a site specific tool was generated for Washington State that does not include the ratios between citrulline and glutamine, glutamate, or ornithine. After resetting the threshold for an informative result, scores are then stratified into four categories of "very likely", "likely", "possibly", or "not informative" representing transitions across percentile ranks (< 1%ile, 1–10%ile, 10–25%, and > 25%ile, respectively) (Supplemental Table 1).

3. Results

We consented 16 patients of whom 11 patients had NBS spots still available due to storage times in Washington and California, including 7 OTC, 2 CIT1, 1 ASA, and 1 ARG. Five patients did not have NBS spots available as they had been destroyed per their state's NBS policy and so were not further included in our analysis. Neither Washington nor California were screening for proximal UCDs at the time of any of our patients birth and no patient in this case series had an abnormal NBS for any other condition reported (currently California does screen for proximal UCDs). We included information about the CIT1, ASA, and ARG patients in this report for comparison of the utility of the R4S scores to OTC. Our patients included five males and six females with an average birth weight of 3167 g (minimum 2495 g, maximum 4007 g) and normal gestational age (minimum 36 weeks, maximum 40 weeks). Patient's current ages ranged from 3 years to 31 years. Two NBS spots were collected at different time points were available for 5 patients with a third NBS spot available for 1 patient and 1 duplicate NBS spot was run on 1 patient (Table 1).

Clinical presentations as self-reported by participants included neonatal-onset development of symptoms with hyperammonemia in 2