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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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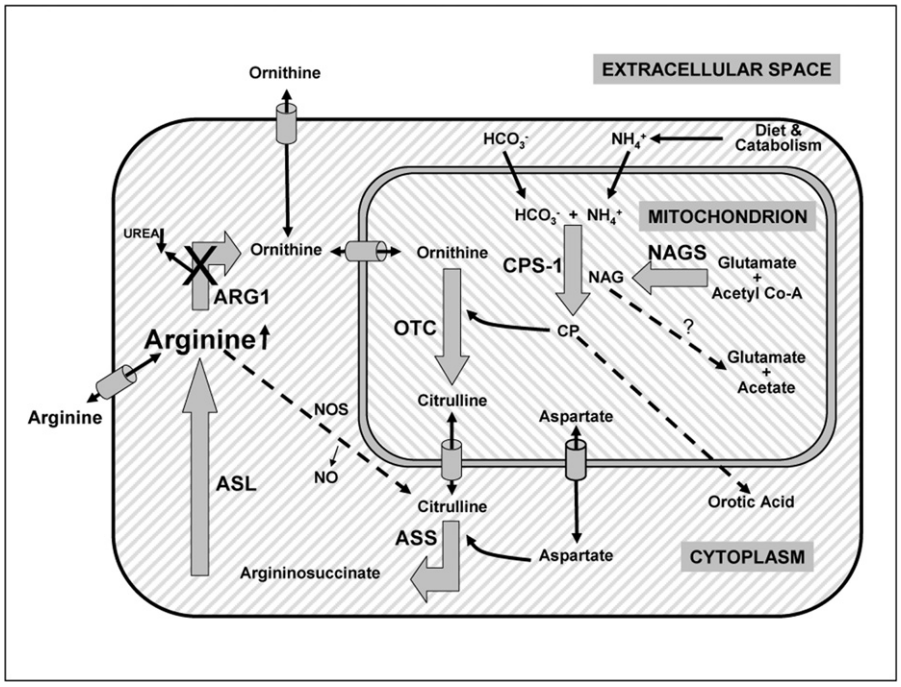


**Fig. 1.** The Complete Urea Cycle: Focusing on the left side of the figure, the consequences of Arg1 deficiency are illustrated. Arginine accumulates both intra- and extracellularly and urea production is diminished. Ornithine production should be diminished, but neither this nor lowered ornithine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