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A three-tier algorithm for guanidinoacetate methyltransferase (GAMT) deficiency newborn screening☆

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ABSTRACT

Background: Guanidinoacetate methyltransferase (GAMT) deficiency is a rare disorder of creatine biosynthesis presenting with epilepsy and developmental delay in infancy. Excellent developmental outcomes have been reported for infants treated from birth due to a family history. The BC Newborn Screening Program initiated a 3 year pilot screening study for GAMT deficiency to evaluate the performance of a novel three-tiered screening approach.
Methods: Over 36 months all bloodspots submitted for routine newborn screening were included in the pilot study (de-identified). Initial GAA measurement was integrated into the standard acylcarnitine/amino acid first-tier assay. All samples with elevated GAA were subjected to second-tier GAA analysis by LC-MS/MS integrated into an existing branched-chain amino acid (MSUD) method. GAMT gene sequencing was completed on the original bloodspot for all specimens with elevated GAA on the second-tier test. The protocol allowed for re-identification for treatment of any specimen with one or two likely pathogenic GAMT mutations.
Results: Over the study period 135,372 specimens were tested with 259 (0.19%) over the first-tier GAA cut-off. The second-tier assay removed an interference falsely elevating GAA levels, and only 3 samples required genotyping. No mutations were identified in any samples, all were deemed negative screens and no follow-up was initiated.
Conclusions: A three-tier algorithm for GAMT newborn screening showed excellent test performance with zero false positives. No cases were detected, supporting a low incidence for this disorder. Given the low incremental costs and evidence of positive outcomes with early intervention, GAMT deficiency remains an excellent candidate for newborn screening.

1. Introduction

Guanidinoacetate methyltransferase (GAMT) deficiency is an autosomal recessive condition due to bi-allelic mutations in *GAMT* (MIM 602140), and one of three known inherited cerebral creatine deficiency disorders [1]. Creatine is synthesized from arginine and glycine through an intermediate, guanidinoacetate (GAA), by the sequential activities of arginine:glycine amidinotransferase (AGAT) and GAMT enzymes. Deficiencies in either of these enzymes or the X-linked creatine transporter (*SLC6A8*) lead to a deficiency in cerebral creatine levels. This deficiency results in early global developmental delay with progressive neurodegeneration and epilepsy if untreated. In GAMT deficiency, GAA toxicity is also implicated in the pathophysiology of disease [1].

Over 80 cases of GAMT deficiency have been reported in the literature since the discovery of this disorder in 1994, and although some small scale carrier detection studies have been completed, the true incidence of the disease remains unclear [2,3,4]. Selective screening for creatine deficiency disorders in a cohort of French patients with unexplained neurological dysfunction identified GAMT deficiency in

Abbreviations: GAMT, guanidinoacetate methyltransferase; BC, British Columbia; GAA, guanidinoacetate; LC-MS/MS, liquid chromatography tandem mass spectrometry; MSUD, maple syrup urine disease; AGAT, arginine:glycine amidinotransferase; ddH_20, distilled deionized water; ACN, acetonitrile; ACMG, American college of medical genetics; *Human gene*: *GAMT* (HGNC:416), guanidinoacetate *N*-methyltransferase; *Human gene*: *SLC6A8* (*HGNC:11055*), solute carrier family 6 (neurotransmitter transporter), member 8.

☆ **Previous Presentation**: Preliminary results were presented at the 2014 Association for Public Health Laboratories (APHL) Newborn Screening and Genetic Testing Symposium (Anaheim, CA).

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~1/1000 individuals; however this was a highly selected group and does not represent a population incidence [5]. Although a founder effect in Portugal has led to an incidence of 1/60,000 in that country, several pilot molecular screening studies in other populations have calculated highly variable, but significantly lower carrier frequencies suggesting a low overall disease incidence [3,4,6].

Given the underlying biochemistry of GAMT deficiency, a number of rational treatment approaches have been employed. A recent review of 48 treated patients from around the world has shown improved outcomes with combinations of creatine and ornithine supplementation, dietary arginine restriction, and in some cases, the addition of sodium benzoate as a glycine scavenger [7]. Such approaches have been shown to normalize CSF creatine levels and reduce toxic accumulations of GAA in both CSF and plasma. Despite the biochemical improvements, however, clinical improvements have been variable, correlating strongly with age at initiation of therapy. Older patients have shown reductions in seizure activity and a halting of disease progression, but few improvements in existing intellectual disabilities. In contrast, those infants treated from birth due to a previous family history have shown normal or near-normal intellectual development. Although there are only a handful of such cases worldwide, these positive outcomes have lead authors to argue strongly for newborn screening for this treatable intellectual disability, GAMT deficiency [8,9].

Newborn screening for GAMT deficiency has been trialed in a number of jurisdictions with variable outcomes. An initial trial in Austria suffered from a high false positive rate and was terminated (Stoeckler S, personal communication). Similarly high false positive rates also affected a trial in Portugal, although a successful long-term screening program in Australia has recently been reported [10]. More recently, a variety of multi-tiered approaches to screening have been proposed and trialed in British Columbia (BC), Utah, Italy, Netherlands, and Texas [3,11,12]. Adding a second-tier LC-MS/MS assay for GAA quantitation from bloodspots removes the interference seen in standard flow injection assays for some newborns, greatly improving test performance. Utah went live with state-wide GAMT screening using such a two-tiered approach in July 2015 [13]. Despite these analytical improvements, implementation of routine GAMT screening remains limited and to the best of our knowledge, no affected infants have been identified through newborn screening. Given the apparent low incidence, very high test-specificity would be required to maintain a low false positive rate and high positive predictive value for GAMT deficiency screening. In October 2012, the BC Newborn Screening Program initiated a 3-year pilot screening study for GAMT deficiency to evaluate the performance of a novel three-tiered screening assay for this apparently rare but highly treatable disorder.

2. Materials and methods

2.1. Ethics

All bloodspot samples submitted for routine newborn screening in BC were included in the pilot study as de-identified but linkable specimens. This was a non-consented pilot but families were informed of the study through a newborn screening pamphlet provided at the time of sample collection and information on the program website. The samples were de-identified for testing but were linkable to patient identifiers if the screen result was deemed positive after the third-tier of the screening algorithm. Approval for this approach was granted by the UBC C&W Research Ethics Board and the BC Newborn Screening Advisory and Research Review committees to allow for therapeutic intervention should an affected infant be identified during the pilot.

2.2. Screening algorithm

All submitted bloodspot cards were tested for GAA on the first tier assay, integrated into our existing flow injection tandem mass spectrometry (FIA-MS/MS) method for amino acids and acylcarnitines. All samples above the screening cutoff for the first-tier assay (set initially

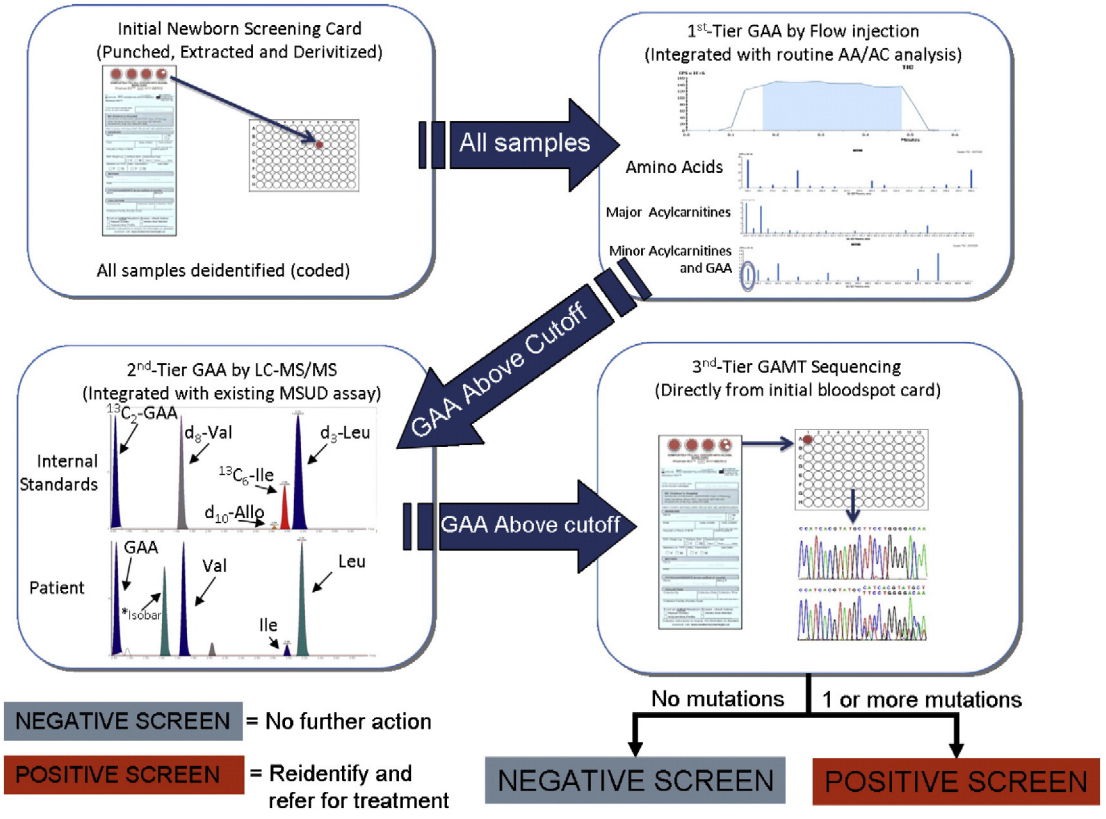

Fig. 1. Three-tiered GAMT screening algorithm.

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