UMass Chan MEDICAL SCHOOL

New England Newborn Screening Program
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Project Summary

Development of Reference Ranges for Additional Newborn Screening Markers for Early Detection of the Homocystinurias: Classical Homocystinuria and Remethylation Disorders

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Background: Newborn screening (NBS) for the homocystinurias (HCU), a group of disorders, has traditionally relied upon abnormal levels of the primary markers methionine and propionylcarnitine (C3) in dried blood spots (DBS) of newborns. These markers are not specific for these conditions because the abnormal result can also be due to secondary dietary interventions, and other diseases, thus causing false-positive results. This leads to unnecessary follow-up of unaffected babies, parental anxiety, and unneeded medical tests. The NBS programs attempt to minimize the number of false-positive test results by using more specific markers and/or second tier confirmatory testing.

More specific markers for HCU are total homocysteine (tHcy), methylmalonic acid (MMA), and methylcitric acid (MCA). Analysis of additional markers such as cystathionine (cysta) and cysteine (cys), along with tHcy, MMA and MCA in the DBS may further improve the differentiation amongst the various forms of HCU. However, NBS for HCU using these markers as primary and/or 2nd tier test (2TT) is still not widely established due to the cost of instrumentation, complexity of test, limited capacity, and resources. We embarked on a pilot project to develop and validate a simple, robust, and high throughput liquid chromatography tandem mass spectrometry (LC-MS/MS) 2TT test in particular, measurement of tHcy, cystathionine, cysteine and MMA.

The next step is to determine a reference range and cutoff value for the target markers. A reference range for a screening test is a range of values that is considered normal for a particular test in a healthy population. The reference range helps healthcare professionals determine whether an individual's test results fall within expected values, and if a newborn is at risk for a disorder. NBS programs, including New England Newborn Screening (NENSP), have implemented different cutoffs for some metabolic disorders detectable by tandem mass spectrometry (MS/MS) depending on the infant's age (in hours) at blood collection. In addition, co-variates such as prematurity, birth weight, transfusions, neonatal jaundice, type of feed and parenteral nutrition can all potentially influence NBS results and need to be considered when establishing cut-off values and interpreting results.

Goal and Objectives: The overall goal of this project is to develop, optimize and implement new 2TT algorithms in order to assess the applicability of this approach in lowering the number of false positives while increasing the likelihood of identifying newborns with classical homocystinuria (HCU) and HCU-Remethylation disorders. We received funding from HCU.org to accomplish the following objectives:

- Determine normal reference ranges for total homocysteine (tHcy), cystathionine (cysta), total cysteine (tCys) and methylmalonic (MMA) by analyzing >200 DBSs collected between 24-48 hours of age, from babies thought to be negative for these conditions, using the newly developed method.
- Retrospectively analyze confirmed classical HCU and HCU-Remethylation (Remet) disorders DBS.
- Analyze data, determine reference ranges, and publish findings.

Results: We have developed and validated a high throughput 2TT LC-MS/MS assay to simultaneously measure elevated tCys, tHcy, MMA and Cysta using a single DBS for screening HCU disorders. This

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method was used to determine reference ranges of >400 DBSs collected between 24-48 hours of age from babies thought to be negative for these conditions. The preliminary cutoffs were determined at the 99th percentile for tCys, tHcy, MMA and Cysta by comparing the control group with confirmed classical HCU and HCU-ReMet disorders DBS. This assay clearly differentiated biochemical patterns between confirmed cases of classical homocystinuria, methylmalonic acidemia, Methylene tetrahydrofolate reductase deficiency, cobalamin C deficiency and propionic acidemia.

Future Plans: The analyses performed by NENSP thus far have been on retrieved specimens. We will re-evaluate and adjust the reference ranges and cutoffs when the method is implemented and the sample size increases. We anticipate that implementation of 2TT will also help us assess and refine our current cutoffs for primary markers and the metabolic profile. Further, the two-tier strategy will offer the advantage of reducing the burden of both false positives and false negative results.