Pilot NBS for Proximal Urea Cycle Disorders in Georgia

APHL: New Disorders Workgroup

December 2021

Overview

- Task order to evaluate the feasibility of newborn screening for proximal urea cycle defects (PUCD)
  - PUCD are the 3 urea cycle defects before the production of citrulline (CIT) (localized in the mitochondria):
    - Carbamoylphosphate synthase (CPS) deficiency; autosomal recessive
    - N-acetylglutamine synthase (NAGS) deficiency; autosomal recessive
    - Ornithine transcarbamylase (OTC) deficiency; X-linked (females can manifest)
- Common biochemical marker is a decreased CIT concentration, an amino acid already included in NBS

Newborn Screening for PUCD

- Decreased CIT in isolation is non-specific
  - Measuring decreases is harder than measuring increases (analytically and practically)
  - Can be seen in some mitochondrial disorders, but also in completely normal individuals
- Orotic acid can be elevated in OTC deficiency
  - Can be measured by MS/MS – mixed reports on whether NBS samples from affected individuals have detectable elevations of orotic acid
  - Not elevated in CPS or NAGS
- Publications using glutamine (GLN) and glutamic acid (GLU) ratios to improve discernment
  - Lab developed test derivatized method of measuring amino acids and acylcarnitines

GA Plan

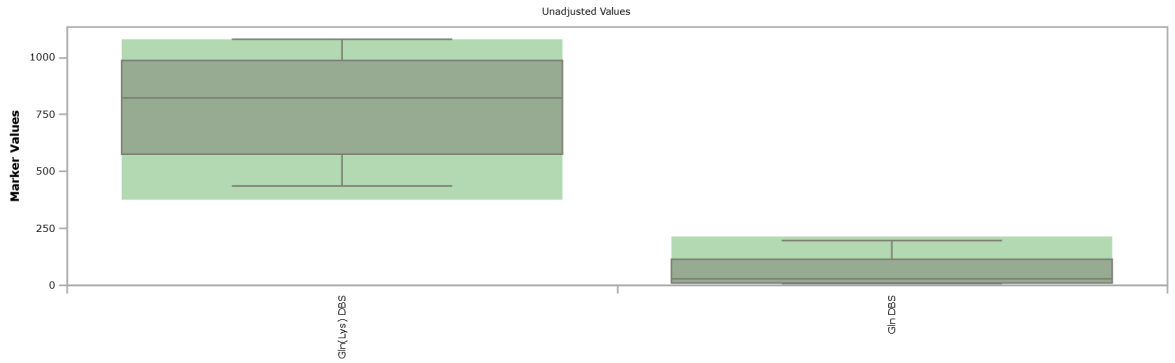
- Use CLIR post-analytical tools to determine if we can screen for PUCD with fewer false positive (FP) results than low citrulline alone
- Initially start with single condition tool based on existing cases in CLIR database
  - Unable to use GLN and GLU due to IT interface issues
- Update to add any FP cases to the database to leverage the use of the DSP
- Expectation based on birth rate (~120 – 130,000 per year) is 1 – 3 cases of OTC deficiency

What we did

- Started 10/31/19 using Neobase 2 kit (had been recently implemented)
- CLIR tool to evaluate NBS results: PUCD Single Condition tool with cumulative percentiles
  - Unable to use glutamine or glutamic acid due to HL7 issues
- Higher risk results – reported as abnormal and received follow up activities
- 11/18/19 We switched to PUCD Single Condition Tool with site specific percentiles, due to downward shift in citrulline concentrations with new method (Neobase 2)
- 11/20/19 We added a dual scatter plot tool (DSP)

What we did, continued

- We paused the program in March 2020
  - COVID – children being referred to ED for ammonia
  - Realized there was a discrepancy with our data and CLIR tool – GLN /LYS (NeoBase2) vs GLN (derivatized)
- We requested samples from California to analyze on Neobase 2
  - allowed for creation of PUCD tools with appropriate GLN / LYS values
- Resumed screening December 2020
- Finished September 2021
- We have screened 138,560 samples



Screening Results

- First Phase
  - Screened 54,452 samples
  - 77 samples were abnormal
  - 0.14% of samples were reported as abnormal
- Second Phase
  - Screened in this phase: 84,108
  - 485 positive screens
  - 0.58% screened positive

- Cumulative Snapshot
  - 244 Female / 318 Male
  - 284 White / 205 Black / 15 Asian / 22 Multiracial / 36 Not Given
  - 57 Hispanic / 501 Not Hispanic / 4 Unknown
  - BW Range: 300 – 6720 g
    - Mean: 3107.7 g
    - Median: 3172.5 g
  - GA Range: 22 – 44 weeks
    - Mean: 36.9
    - Median: 38

Resolution of Cases

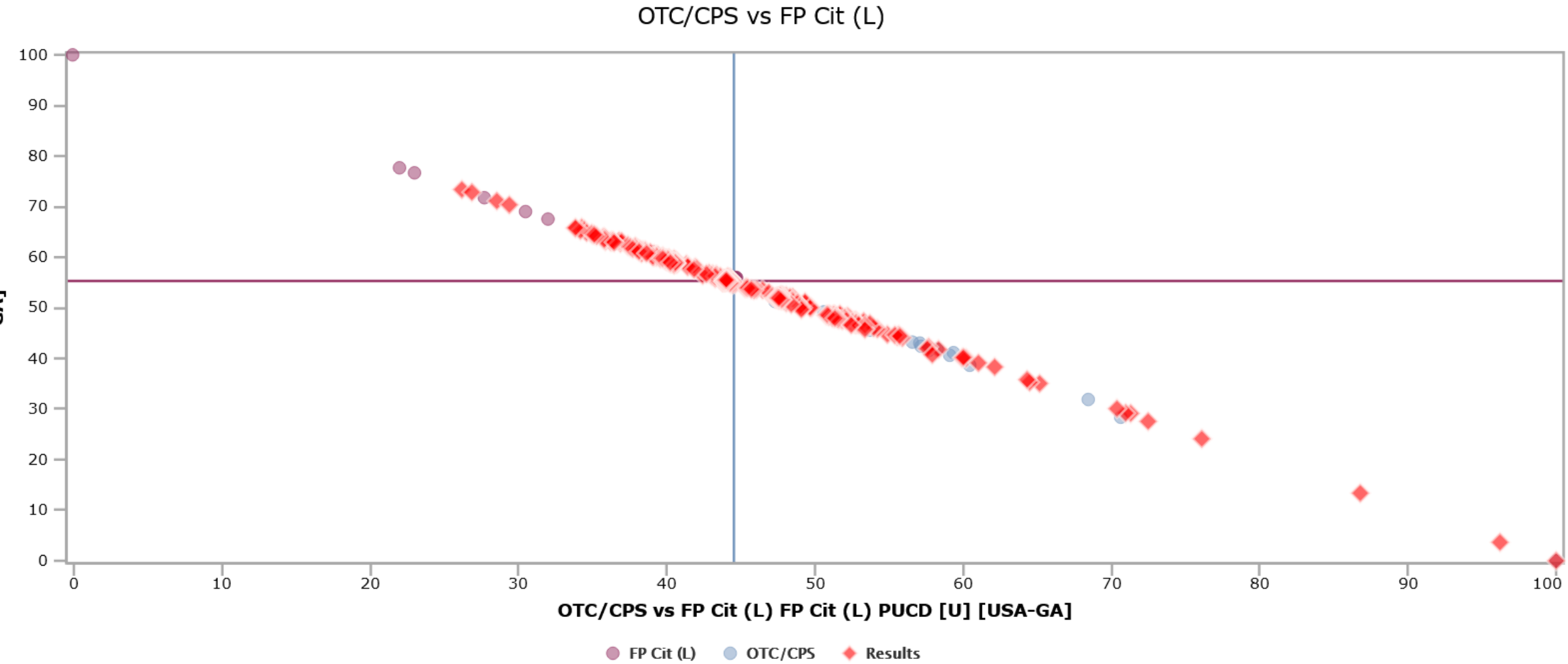
- Initially, all cases were referred for confirmatory testing, including ammonia
- This strategy evolved over the course of the study, due to high number of FP results and challenges with obtaining ammonia
- Resolution pathway (562):
  - 171 by confirmatory testing
  - 262 by subsequent normal NBS
  - 70 by prior normal NBS
  - 42 Closed without testing
  - 17 lost to follow-up

Resolution outcomes (555):

- 528 Normal
- 7 Parent refusal
- 12 unresolved (testing pending)
- 4 expired (all < 1000 g)
- 2 lost to follow-up
- 2 followed in clinic (not PUCD but other relevant findings)

End Project Analysis

- Rudimentary cutoff comparison:
  - Highest CIT concentration in cases obtained from California: 6.7 nmol/mL
  - 99th percentile of disease range in CLIR is 7.01 nmol/mL
  - 1st percentile of initial screens in GA (121,596 consecutive initial screens): 5.42 nmol/mL
    - 3 cases (2 males, 1 female) in the California cohort were > 1st percentile of normal in Georgia
    - 5711 screens had citrulline concentrations lower than the 99th percentile of the disease range in CLIR
- Unified CLIR analysis
  - 84,059 cases analyzed (included in pilot study and full data set available for extraction
    - 616 reportable by algorithm used in study based on single condition tool result
    - 198 informative by DSP (~ 2/3 reduction in FP cases if utilized fully)


OTC/CPS vs FP Cit (L)
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90
100
OTC/CPS vs FP Cit (L) FP Cit (L) PUCD [U] [USA-GA]
FP Cit (L)
OTC/CPS
Results

Study Design Thoughts

- We leaned in favor of reporting and getting outcome data rather than using DSP results to finalize screens due to the exploratory nature of the study
- When combining the population of children screened, plus the population of children born during the pause between the two phases, statistics would say that there should have been children born with PUCD in GA during that time
  - Not aware of any FP cases, it is highly likely any suspected cases would be transferred to Children's Hospital of Atlanta, where geneticists involved in NBS would have been notified
- Not detecting cases makes it difficult to adjust downwards to reduce FP cases, because there is no clear guide for what we are adjusting for

Overall Assessment

- Difficult to judge immediately, as multiple adjustments needed to be made over the course of the study
  - Will analyze all study data with mature tools and algorithm to provide an overall assessment of the potential performance (in progress)
    - As an early adopter with NB2, the study was at times a struggle
- Method variations can limit the usefulness of post-analytical tools and require extra legwork
  - Condition splits are also challenging w/ X-linked condition
- Not having results on the NBS report was a challenge (we have learned this lesson several times)