

# Newborn Screening Second Tier Molecular Testing for Lysosomal Storage Diseases and X-linked

# Adrenoleukodystrophy is Critical for Identifying True Positives

Yang Wang, Ruby Liu, Bethany Sgroi Gaita, Meredith Patik, PJ Borandi, Alice K. Tanner, Christin Collins, Akanchha Kesari, Babi Nallamilli, Jing Xie, Megan Landsverk, Madhuri Hegde. Revvity Omics, Pittsburgh, PA

#### **BACKGROUND & METHODS**

Newborn screening (NBS) focuses on infant or childhood onset disorders for which early interventions can significantly improve the clinical outcome.

Lysosomal storage diseases (LSD) are multisystem disorders often involving cardiovascular and neurological systems and can lead to irreversible damage and even premature death without treatment. Starting 2016, we performed second tier full gene sequencing on 868 dry blood spot (DBS) specimens that are positive or inconclusive for NBS of mucopolysaccharidosis type I (MPS I, *IDUA* gene), Pompe disease (*GAA* gene) and Krabbe disease (*GALC* gene) submitted by states pf Pennsylvania, California, Tennessee, Nebraska, Florida, District of Columbia and Illinois.

X-linked Adrenoleukodystrophy (X-ALD) is the most common leukodystrophy characterized by the progressive deterioration of the adrenal glands, spinal cord, white matter of the nervous system. Phenotypes range from adrenocortical insufficiency (Addison disease), to a progressive adult on-set paraparesis with adrenal gland dysfunction (adrenomyeloneuropathy, AMN), to the most severe and fatal cerebral form of X-ALD. Our laboratory have tested 319 DBS samples for NBS second tier *ABCD1* full gene sequencing since 2017 from states of California, Pennsylvania, Tennessee, Florida, Nebraska and District of Columbia.

Here we analyzed sequencing data reflexed from positive NBS of MPS I, Pompe disease and Krabbe disease and X-ALD, calculated positive rate of different genotypes including affected, carrier, pseudodeficiency and uncertain significance.

## **RESULTS – XALD**

- Among all 319 NBS positives, 43% are males and 57% are females.
- Approximately 29% NBS positives cases yield a pathogenic/likely pathogenic (P/LP) variant.
- Approximately 35% NBS positives/inclusive cases do not have any reportable variant.
- Three males were confirmed to be positive for partial gene deletions.
- Among those DBS specimens with available parental testing, 26% cases occurred *de* novo; and 26% cases with pathogenic variants were maternally inherited; and two cases with a pathogenic variant (females) were inherited from reportedly unaffected father.



#### **RESULTS - LSDS**

- MPS I: Among 488 NBS positive DBS specimens, only 2% are truly affected with two pathogenic/likely pathogenic variants. The majority (86%) of NBS positives contain pseudodeficiency allele or VOUS.
- Pompe disease: Among 265 NBS positive DBSs, true positive rate is much higher; 19% were confirmed with two pathogenic/likely pathogenic GAA variants. Another 9% harbor one pathogenic/likely pathogenic variant with a VOUS. However, majority affected individuals (>78%) possess at least one late-onset pathogenic variant c.-32-13T>G.
- Krabbe disease: Among 115 NBS positives, only 3% are truly affected with two pathogenic/likely pathogenic GALC variants. As high as 76% NBS positives have pseudodeficiency allele or VOUS.



## PATHOGENIC VARIANTS IN IDUA, GAA & GALC

- Majority of MPS I patients/carriers harbor p.Trp402Ter and/or p.GIn70Ter common IDUA pathogenic variants that associated with severe disease phenotype
- Almost half Pompe patients/carriers harbor c.-32-13T>G and/or p.Trp746Cys lateonset GAA pathogenic variants; Only 9% was identified with p.Arg854Ter and/or p.Trp367Arg Infantile-onset GAA pathogenic variant
- There are 39% Krabbe patients/carriers harbor at least one of the three common *GALC* pathogenic variants; majority carry at least one rare pathogenic variant



#### DISCUSSIONS

- High detection rate of pseudodeficiency allele makes it difficult to discern and adjust the cut off values on the biochemical screening. Second tier molecular confirmation is necessary to improve positive predicted value of NBS for MPS I and Krabbe diseases.
- Biochemical screening for XALD has a high false positive rate that requires molecular confirmation to rule out.
- As the cost of sequencing reduces, NBS second tier molecular testing is becoming one of the most economic and efficient approach to improve clinical outcomes.