Newborn screening second tier molecular testing: What are we learning from MPS I and Pompe disease?

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ABSTRACT

Newborn screening (NBS) has significantly improved the clinical outcome for many inherited metabolic disorders through early detection. As state NBS panels expand to include more disorders, such as lysosomal storage diseases, second tier molecular testing has become increasingly important for accurate detection and confirmation, increasing the positive predictive value (PPV) and reducing the burden on both physicians and families. Two lysosomal storage diseases recently added to the NBS panel in some states are mucopolysaccharidosis type I (MPS I) and Pompe disease. MPS I is a multisystem disorder that can have characteristic facial features with growth retardation, neurologic and cardiovascular involvement and premature death. It is caused by biallelic pathogenic variants in the *IDUA* gene, which encodes the α-L-iduronidase enzyme. Pompe disease presents with cardiomyopathy, muscle weakness and respiratory insufficiency due to deficiency in the acid alpha-glucosidase enzyme. Patients with Pompe disease carry biallelic pathogenic variants in the *GAA* gene. Here we summarize the first 13 months of NBS second tier molecular testing results for MPS I and 21 months for Pompe disease from the state of Pennsylvania and first 8 months from the state of Tennessee.

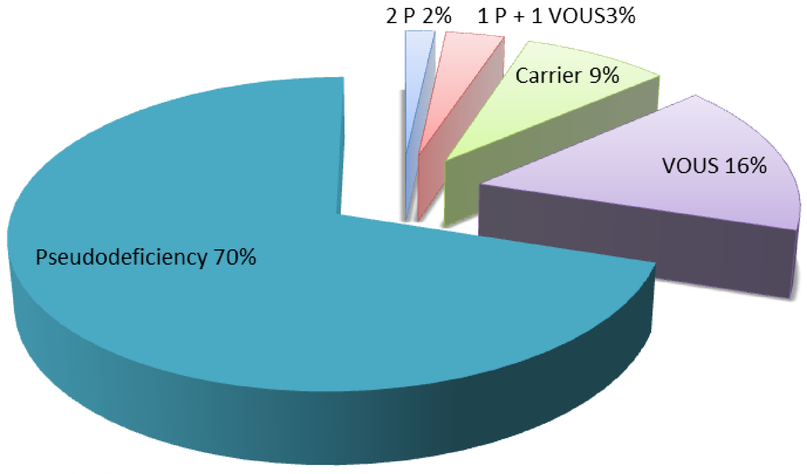
From February 2017 to March 2018, there were 117 NBS positive dry blood spot (DBS) specimens sent to PerkinElmer Genomics Laboratory for second tier molecular testing of *IDUA* by Sanger sequencing and/or next generation sequencing (NGS). Two specimens (2%) were confirmed to harbor two pathogenic variants indicating these individuals are potentially affected with disease. An additional four specimens (3%) contained one pathogenic variant and one variant of unknown significance (VOUS). One pathogenic variant was detected in ten specimens (9%), indicating a likely carrier status of these individuals. Of the remaining samples, 106 (91%) contained at least one pseudodeficiency allele that could explain the reduced enzyme activity. Further, 72 specimens (62%) contained two or more pseudodeficiency alleles. These results suggest that the high population frequency of pseudodeficiency alleles contributes to the high NBS positive rate and low PPV, and illustrates the necessity of molecular confirmation of positive NBS results.

From July 2016 to March 2018, there were 67 NBS positive dry blood spot (DBS) samples sent to PerkinElmer Genomics Laboratory for second tier molecular testing for *GAA* by Sanger sequencing and/or NGS. Unlike *IDUA*, 34% of specimens (23) were identified with two pathogenic/likely pathogenic variants, indicating a high disease frequency and high PPV. However, almost all of the pathogenic variants detected were known to be late-onset variants, including the common c.-32-13T>G variant which was detected in 39% of affected samples (10 homozygous and 16 compound heterozygous). There were another 25% of samples (17) containing one pathogenic/likely pathogenic variant with one VOUS. Ten specimens (15%) were found to contain one pathogenic variant, indicating likely carrier status. In contrast to the high pseudodeficiency allele prevalence in *IDUA*, 16% of specimens (11) contained pseudodeficiency alleles without pathogenic or likely pathogenic alleles. There was a single sample in which no reportable variants were detected. These results suggest a higher disease frequency and lower population frequency of pseudodeficiency alleles for Pompe disease than for MPS I.

In summary, these sequencing results demonstrate the clinical necessity of NBS second tier molecular testing on the lysosomal storage diseases MPS I and Pompe disease. Accurately identifying potential pathogenic variants in these diseases significantly increases the PPV, especially in MPS I, to avoid unnecessary clinical visits and thus reduce the cost and stress on patients and families.

Mucopolysaccharidosis type I (MPS I)

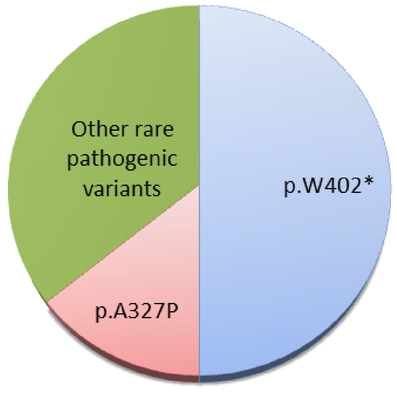
IDUA sequencing results



N=117

- 2 Pathogenic
- 1 Pathogenic + 1 VOUS
- Carrier (1 Pathogenic w/o VOUS)
- VOUS
- Pseudodeficiency allele only

IDUA Pathogenic Variants

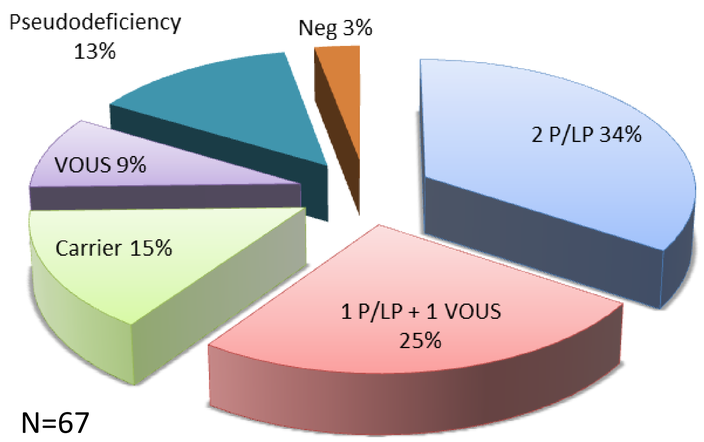


Genotype-phenotype correlation:
p.W402* and p.A327P are both common pathogenic variants in the European descendants and associated with severe phenotype

- High population frequency of pseudodeficiency alleles in the *IDUA* gene contributes to the high MPSI NBS positive rate and low PPV

Pompe disease

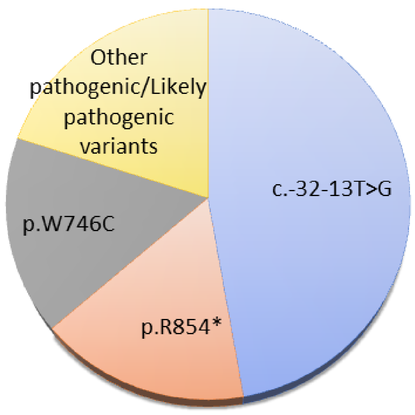
GAA sequencing results



N=67

- 2 Pathogenic/Likely Pathogenic
- 1 Pathogenic/Likely Pathogenic + 1 VOUS
- Carrier (1 Pathogenic/Likely Pathogenic w/o VOUS)
- VOUS
- Pseudodeficiency allele only
- Negative

GAA Pathogenic Variants



Genotype-phenotype correlation:

- c.-32-13T>G late-onset Pompe (common in all ethnic background)
- p.W746C late-onset Pompe (common in Chinese population)
- p.R854* Infantile-onset Pompe (common in African and African American)

- High Pompe disease frequency (mostly late-onset pathogenic variant) accounts for the high NBS positive rate
- Low population frequency of pseudodeficiency alleles in the *GAA* gene