Evaluation of Genetic Variant Interpretation Guidelines in the New York State Newborn Screening Program

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Background

- In 2015, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) published guidelines for variant interpretation and classification. However, application of the guidelines lacks specificity, particularly for rare diseases and disorders.[1]
- The New York State (NYS) Newborn Screening (NBS) Program screens babies for more than 50 different conditions to identify and treat potential affected newborns before the onset of symptoms.
- The NYS NBS Program currently uses molecular testing and DNA sequencing for 9 conditions after a positive first-tier biochemical screen to reduce the number of false positive results and guide clinical decision-making (**Table 1**). Pathogenic, Likely Pathogenic, and Variants of Uncertain Significance are reported to clinical providers as information to guide further testing and follow-up.
- Variant interpretation involves assessing 31 types of clinical and molecular evidence. Some metrics utilize variant population allele frequency, but thresholds for defining common and rare variants are lacking. Implementing condition-specific allele frequency thresholds will improve accuracy and reduce subjectivity in variant interpretation.

Table 1. Genes Sequenced by NYS NBS Subsequent to a Positive Biochemical First-Tier Screen

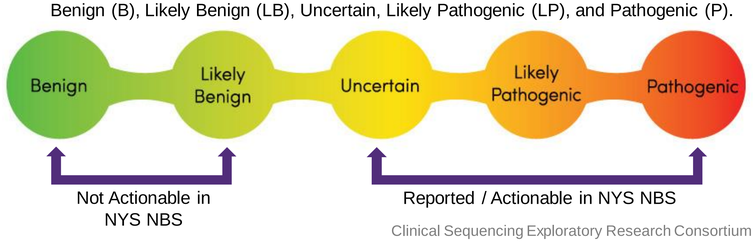
Gene	Condition
CFTR	Cystic Fibrosis
GALC	Krabbe Disease
GAA	Pompe Disease
IDUA	Mucopolysaccharidosis Type I
****ABCD1***	X-linked Adrenoleukodystrophy
****GAMT***	Guanidinoacetate Methyltransferase Deficiency
****GALT***	Galactosemia
****ACADM***	Medium-Chain Acyl-CoA Dehydrogenase Deficiency
****ACADVL***	Very Long-Chain Acyl-CoA Dehydrogenase Deficiency

*Referral Regardless of Detected Variants

Objectives

- To review the literature and existing curation programs to identify condition-specific thresholds and rules (BA1, BS1, PM2, BS2) to implement in variant interpretation performed by the NYS NBS Program.

The ACMG-AMP criteria and rules are used to classify each variant as one of the five categories: Benign (B), Likely Benign (LB), Uncertain, Likely Pathogenic (LP), and Pathogenic (P).



Clinical Sequencing Exploratory Research Consortium

Methods

Table 2. American College of Medical Genetics and Genomics-Association for Molecular Pathology Definitions for Variant Interpretation Criteria

Criteria (4 out of 31)	ACMG-AMP Definition
BA1 (Benign Stand-Alone)	Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, Exome Aggregation Consortium, or gnomAD.
BS1 (Benign Strong)	Allele frequency is greater than expected for disorder.
PM2 (Pathogenic Moderate)	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, Exome Aggregation Consortium, or gnomAD.
BS2 (Benign Strong)	Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age.

- Databases, datasets, variant curation programs, and literature were evaluated to assess the spectrum of thresholds used by clinical laboratories and NBS programs.
- The maximum population minor allele frequencies of *CFTR* variants classified by NYS NBS and CFTR2 were plotted in R.

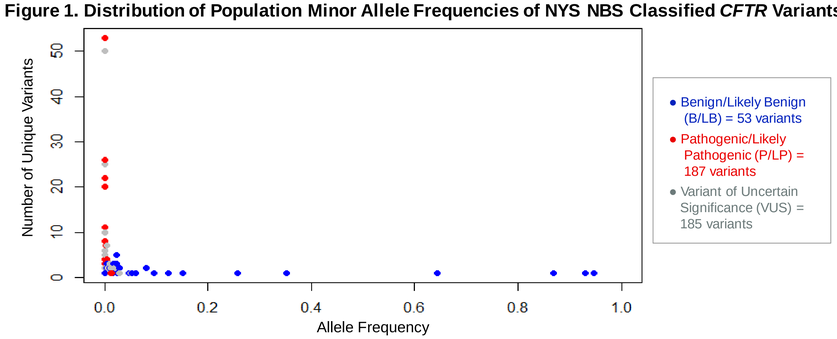
Results

Table 3. Proposed* Thresholds for Each Population Data Criteria Evaluated

Gene	BA1 (MAF high, stand-alone)	BS1 (MAF higher than expected)	PM2 (MAF absent or rare)	BS2 (Number of homozygotes)
CFTR	≥ 0.030	≥ 0.015	< 0.0028	≥ 4
GALC	≥ 0.015	≥ 0.003	< 0.0015	≥ 2
GAA	≥ 0.010	≥ 0.005	< 0.0010	≥ 2
IDUA	≥ 0.019	≥ 0.003	< 0.0027	≥ 2
ABCD1	≥ 0.008	≥ 0.0008	< 0.0005	≥ 1
GAMT	≥ 0.003	≥ 0.001	< 0.0004	≥ 1
GALT	≥ 0.027	≥ 0.0055	< 0.0027	≥ 2
ACADM	≥ 0.023	≥ 0.005	< 0.0036	≥ 1
ACADVL	≥ 0.007	≥ 0.0035	< 0.0013	≥ 3

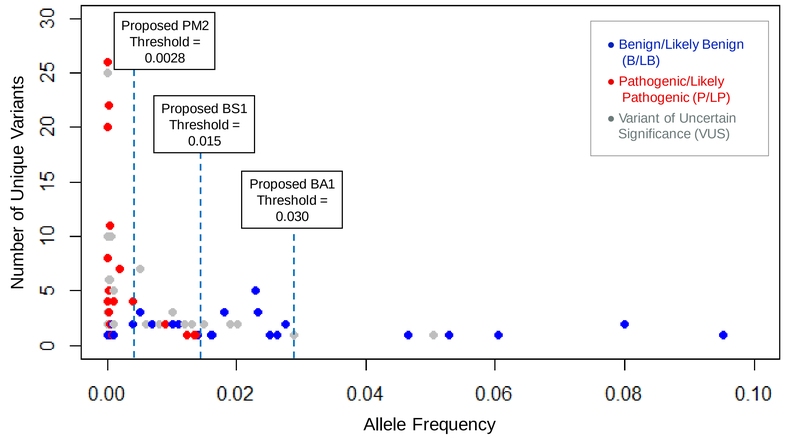
*Undergoing Review and Discussion

Figure 1. Distribution of Population Minor Allele Frequencies of NYS NBS Classified *CFTR* Variants



Results

Figure 2. Distribution of Population Minor Allele Frequencies of NYS NBS Classified *CFTR* Variants and Proposed Thresholds



* Each dot represents the number of unique *CFTR* variants at a given minor allele frequency

Conclusions and Future Directions

- Condition-specific thresholds vary depending on factors such as disease prevalence, penetrance, and genetic heterogeneity.[2]
- gnomAD v2.1.1 is recommended as the reference population database, but has limitations due to rare, private variants and limited populations studied.
- Proposed thresholds are consistent with the recommendation by the Clinical Genome Resource Sequence Variant Interpretation Working Group that BA1>BS1>PM2.[3]
- Implementation is expected to guide and support the standardization of variant interpretation.
- Future directions include validation and discussion to review the proposed thresholds, and evaluation of additional criteria, such as functional domains and missense variation to guide variant classification.

Acknowledgements and References

The authors thank the NYS Newborn Screening Program staff and the New York State Public Health Corps Fellowship.

[1]Richards S, *et al*. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the ACMG-AMP. *Genetics in Medicine*. 2015;17(5).

[2]Whiffin N, *et al*. Using High-Resolution Variant Frequencies Empowers Clinical Genome Interpretation and Enables Investigation of Genetic Architecture. *Am J Hum Genet*. 2019;104(1).

[3]Ghosh R, *et al*. Updated Recommendation for the Benign Stand-Alone ACMG/AMP Criterion. *Hum Mutat*. 2018;39(11).