Improved diagnostic confirmation of newborn screening conditions by next-generation sequencing: A collaboration between laboratory and clinician

INVITAE

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BACKGROUND

- Newborn screening (NBS) identifies patients at risk for potentially actionable genetic disorders, and molecular analysis of positive NBS cases is a vital step in diagnostic confirmation.
- Next-generation sequencing (NGS) has enabled clinicians to sequence larger gene panels at lower costs. This practice has the downside of identifying variants of uncertain significance (VUS), which lack sufficient evidence needed to confirm a diagnosis.
- We reviewed cases referred for positive NBS clarification and focused on uncertain cases that have the potential to be reinterpreted as a positive molecular diagnosis with additional evidence. We demonstrate how the laboratory and clinician working together can contribute to increasing the number of positive diagnoses.

METHODS

- Relevant genes, based on referral indication, were sequenced by NGS in patients referred for positive NBS. Variant classification was performed using an ACMG guidelines-based evidence system (PMID: 28492532).
  - Our cohort of 464 probands included 201 females and 263 males with an age range of 0-49 years; 65% were less than 1 year old. Indications included positive NBS for inborn errors of metabolism, cystic fibrosis, or immune deficiency disorders.
- Patient results were classified as Positive when the variants confirmed a genetic diagnosis, including homozygous or compound heterozygous pathogenic (P) or likely pathogenic (LP) variants in an autosomal recessive (AR) or X-linked (XL) gene or Uncertain when the variants identified could not lead to a diagnosis. Negative results (only benign or likely benign variants) were not reviewed in detail in this study.
- Uncertain results containing a VUS with potential for reclassification, where the result could lead to a positive diagnosis (such as a Pathogenic variant + VUS in an AR gene), were evaluated to determine if the VUS could be reclassified if additional evidence were obtained. Examples of additional evidence criteria include PP1, PP4, PM2, and PM3.[1]

RESULTS

- 126 cases were positive for a molecular diagnosis (27%), 123 were uncertain (26.5%), 80 (17%) were carriers of a single pathogenic AR variant, and 134 cases were negative (**Figure 1**).
  - Positive diagnoses included 43 patients with PKU/hyperPhe, 30 with fatty acid oxidation disorders, and 8 with biotinidase deficiency. A number of additional metabolic disorders were identified (**Figure 2**).
- 64 of the Uncertain cases had either a Pathogenic or Likely Pathogenic plus VUS, or two homozygous VUS in autosomal recessive genes, or one single VUS in an autosomal dominant or X-linked gene (**Figure 3**). Of these cases, 22 (34%) had VUS with the potential to reclassify to Pathogenic or Likely Pathogenic, leading to positive diagnoses. **This would increase the number of cases with positive diagnoses to 148 (32%)**.
  - The VUS could be upgraded with the addition of the following evidence: confirmatory lab data (PP4), parental testing for phase/segregation (PM2 + PM3 or PP1), and development of pathognomonic criteria for the specific gene/disease. Condition-specific case examples are outlined in **Table 1**.
    - Pathognomonic criteria incorporates the patient's condition-specific data and is defined as specific phenotypic, laboratory, or ancillary test results in which published literature demonstrates the diagnostic yield for a disorder is >75% by this method.

Figure 1. Distribution of result types

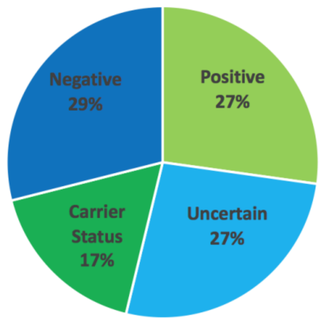


Figure 2. Diagnoses in patients with abnormal newborn screen and positive molecular results

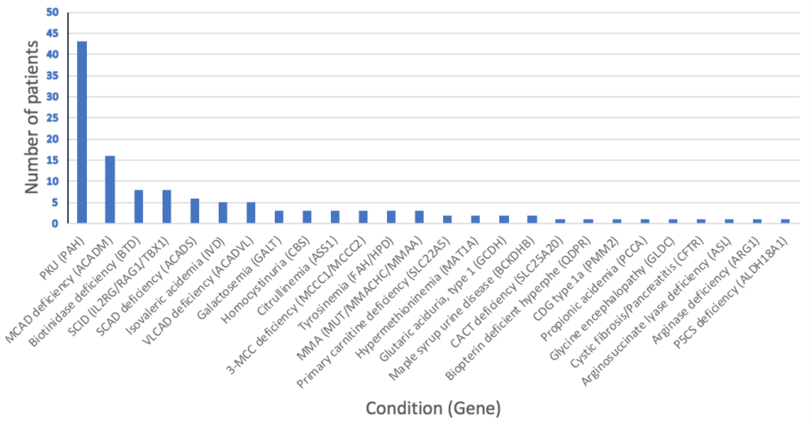
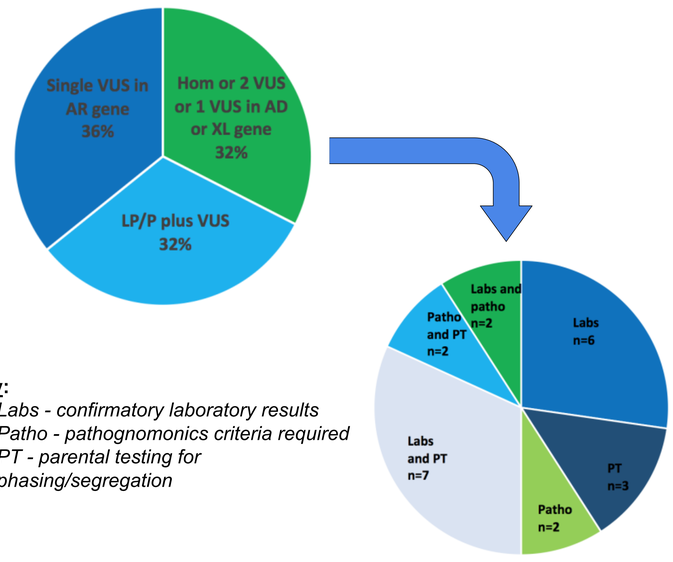


Figure 3. Variant classification in uncertain cases and additional data required to reclassify VUS to Pathogenic



Key:
- *Labs - confirmatory laboratory results*
- *Patho - pathognomonics criteria required*
- *PT - parental testing for phasing/segregation*

Table 1. Case examples of variants in which additional data would upgrade classification

Gene	Variant	Condition	Additional data	Interpretation change
CBS	c.430G>C (p.Glu144Gln) [second variant: c.770C>T (p.Thr257Met)]	*Homocystinuria*	Parental testing - in trans with path variant or co-segregation (PM2 + PM3 or PP1) Labs - apply pathognomic criteria (PP4)	VUS → Likely Pathogenic
MCCC2	c.455A>C (p.Lys152Thr) [second variant: c.1216+2T>C (Splice donor)	*3-MCC deficiency*	Parental testing - in trans with LP/P variant or co-segregation (PM2 + PM3 or PP1) Labs - apply pathognomic criteria (PP4)	VUS → Pathogenic
ASS1	c.262C>A (p.Leu88Ile) (homozygous)	*Citrullinemia*	Labs - apply pathognomonic criteria (PP4)	VUS → Pathogenic

CONCLUSIONS

- We determined that with more evidence in the form of additional labs and/or clinical information, phasing/segregation, and/or development of pathognomonic criteria, a significant number of additional positive molecular diagnoses could be confirmed. With minor effort from the ordering clinician, the data can make a large difference in variant interpretation and a potential positive diagnosis for the patient.
- Aiming at enhancing NBS confirmation, we recommend these additional steps for the clinician and lab as collaborators:
  - The clinician can submit comprehensive clinical information (clinic notes, pedigree, medical records, biochemical lab results) at the time of the initial test order.
  - The provider's team can discuss the expectation for possible parental testing with the family during the at-risk individual's genetic counseling sessions, both before and after the proband's testing. We recognize this can be overwhelming for a family facing a positive NBS result, but it will introduce the option and expectation to the parents.
  - The diagnostic laboratory can develop additional pathognomonic criteria for more genetic conditions.
  - The diagnostic laboratory can provide outreach and education to the clinical community about the process of variant interpretation and classification, and types of evidence used.

1. PP1 = cosegregation with disease in multiple affected family members in a gene definitely known to cause the disease; PP4 = patient's phenotype or family history is highly specific for a disease with a single genetic etiology; PM2 = absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or ExAC ; PM3 = for recessive disorders, detected in trans with a pathogenic variant (PMID: 25741868)

Disclosures: All authors are stockholders and employees of Invitae.