# Challenges in newborn sequencing: classification of variants with measurable biological dysfunction but limited clinical significance

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## 1 Introduction

Variant interpretation is a cornerstone of clinical laboratory genomics, guided by the American College of Medical Genetics and Genomics (ACMG) framework, which classifies variants into five categories: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. However, the expansion of newborn genomic sequencing reveals a subset of variants that challenge this framework—those with impaired biochemical function or reduced enzymatic activity (often identified by newborn screening laboratory testing) but without clear evidence of clinical disease. These functionally abnormal yet clinically silent variants are not benign, given measurable biological impairment, yet they lack overt phenotypic consequences. The detection of these variants in asymptomatic newborns raises critical questions:

- How should variants demonstrating abnormal function but resulting in an unaffected phenotype be classified?
- Should these types of variants be reported in asymptomatic newborns?
- What are the implications for counseling, follow-up, and long-term monitoring?

## 2 Methods

A retrospective review of the laboratory's internal variant database identified variants meeting the following criteria:

- Documented functional impairment, including abnormal newborn screening analytes, reduced enzyme activity, or *in vitro* functional studies.
- Minimal or absent clinical symptoms in both published literature and tested individuals.
- Review of literature for pseudo-deficiency to distinguish true biological impairment from benign assay artifacts.

## 3 Functional impairment does not always predict disease

Across several genes, variants were identified that produced abnormal biochemical results yet showed no associated clinical phenotype, even in longitudinal follow-up.

Examples include:

- *ABCD1* c.1448C>T (p.Thr483Met) Identified repeatedly in X- ALD newborn screening cohorts and shown to impair protein function in fibroblasts, yet no clinical features or family history have been reported.
- *ACADS* c.625G>A (p.Gly209Ser) A common SCAD variant producing reduced enzyme activity and elevated metabolites, but no evidence of SCADD-related disease.
- *GALE* c.770A>G (p.Lys257Arg) A well-characterized peripheral variant causing reduced epimerase activity only in RBCs and leukocytes, with normal activity in other tissues and no clinical galactosemia.

These examples illustrate the interpretive challenge: variants that clearly alter biochemical pathways but do not cause disease *in vivo*.

**Why these variants don't easily fit ACMG categories:**

- Not Benign → measurable biochemical dysfunction
- Not Pathogenic → no disease or penetrance evidence
- Not Uncertain (VUS) → functional impact is known
- Not Pseudo-deficiency → impairment is real, not an assay artifact

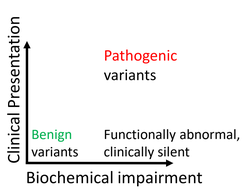


**Table 1. Functional abnormal, clinically silent variants.**

| Gene-Associated Disorder | Inheritance | Gene | Variant | Genomic Position | Interpretation Summary | ClinVar Classification (# of labs) |
|---|---|---|---|---|---|---|
| X-Linked Adrenoleukodystrophy (X-ALD) | X-Linked | ***ABCD1*** | NM_000033.4: c.1448C>T (p.Thr483Met) | X:153002665 | Identified multiple times in X-ALD newborn screening (PMID: 33920672, 34946879, 35466195, 39803877). The variant affects protein function in fibroblasts (PMID: 34946879) but has not yet been associated with overt clinical features of disease. Uncertain significance for clinical features but could explain abnormal newborn screening biochemical results. Limited clinical significance in asymptomatic individuals. | Pathogenic (1); Uncertain significance (3) |
| Short-Chain Acyl-CoA Dehydrogenase Deficiency (SCADD) | AR | ***ACADS*** | NM_000017.4: c.511C>T (p.Arg171Trp) | 12:121175678 | Common variant found in positive newborn screens that result in reduced SCAD enzyme activity and elevated blood and urine metabolites, but no clinical evidence of disease has been reported (PMID: 21938826, 32802992). Unlikely to cause clinical features but could explain abnormal newborn screening biochemical results. Limited clinical significance in asymptomatic individuals. | Benign (6); Likely Benign (2) |
| Short-Chain Acyl-CoA Dehydrogenase Deficiency (SCADD) | AR | ***ACADS*** | NM_000017.4: c.625G>A (p.Gly209Ser) | 12:121176083 | Common variant found in positive newborn screens that result in reduced SCAD enzyme activity and elevated blood and urine metabolites, but no clinical evidence of disease has been reported (PMID: 21938826, 32802992). Unlikely to cause clinical features but could explain abnormal newborn screening biochemical results. Limited clinical significance in asymptomatic individuals. | Benign (8); Likely Benign (4) |
| Epimerase Deficiency Galactosemia | AR | ***GALE*** | NM_000403.4: c.770A>G (p.Lys257Arg) | 1:24123212 | This variant is a known peripheral variant, which only results in deficient epimerase activity in red blood cells and circulating white blood cells but normal or near normal in all other tissues (PMID: 21290786). Individuals with the peripheral epimerase deficiency generally remain clinically asymptomatic regardless of diet. | Uncertain significance(3); Benign(3); Likely benign(3) |
| Epimerase Deficiency Galactosemia | AR | ***GALE*** | NM_000403.4: c.956G>A (p.Gly319Glu) | 1:24122673 | This variant is a known peripheral variant, which only results in deficient epimerase activity in red blood cells and circulating white blood cells but normal or near normal in all other tissues (PMID: 21290786). Individuals with the peripheral epimerase deficiency generally remain clinically asymptomatic regardless of diet. | Uncertain significance(3); Likely benign(2); Other (1) |
| Galactosemia | AR | ***GALT*** | NM_000155.4: c.-129_-126delCAGT (Duarte) | 9:34646573-34646576 | Promoter deletion characteristic of the Duarte (D2) allele. Individuals with the Duarte variant allele have reduced enzyme activity but are not expected to present clinically with classic galactosemia. Variant unlikely to cause clinical features but may explain abnormal newborn screening biochemical results. No dietary restrictions needed in asymptomatic individuals. | Pathogenic(10); Likely pathogenic(3); Uncertain significance(1); Other(1) |
| Galactosemia | AR | ***GALT*** | NM_000155.4: c.940A>G (p.Asn314Asp) (Duarte) | 9:34649442 | Hallmark Duarte allele variant. Individuals with the Duarte variant allele (D2) have reduced enzyme activity but are not expected to present clinically with classic galactosemia. Variants unlikely to cause clinical features but may explain abnormal newborn screening biochemical results. No dietary restrictions needed in asymptomatic individuals. | Pathogenic (5); Likely pathogenic (2); Uncertain significance (1); Benign (3); Likely benign (3) |
| Homocystinuria due to MTHFR Deficiency | AR | ***MTHFR*** | NM_005957.5: c.1286A>C (p.Glu429Ala) | 1:11854476 | Common polymorphism with reduced enzyme activity but limited clinical utility. Not associated with actionable risk. May be detected during genomic testing or newborn screening evaluations, but it does not explain clinically significant metabolic disease and is not associated with classical MTHFR deficiency. | Benign (10); Likely benign (1); Other (1) |
| Elliptocytosis-2; Pyropoikilocytosis; Spherocytosis, type 3 | AR; AD | ***SPTA1*** | NM_003126.4: c.5572C>G (p.Leu1858Val) (Alpha Lely) | 1:158597507 | Component of the Alpha Lely low-expression allele. Acts as a modifier but not disease-causing alone. In asymptomatic individuals with normal hematologic parameters, this variant is considered to have limited clinical significance and is not expected to contribute to disease without a second pathogenic *SPTA1* allele (e.g., α-LELY) or additional membrane-related variants. | Pathogenic (1); Benign (13); Likely benign (1) |
| Elliptocytosis-2; Pyropoikilocytosis; Spherocytosis, type 3 | AR; AD | ***SPTA1*** | NM_003126.4: c.6531-12C>T (Alpha Lely) | 1:158587858 | Intronic Alpha Lely component. Low-expression modifier allele without independent clinical significance. In individuals without clinical or laboratory evidence of hemolysis, this variant is considered to have limited clinical significance. | Likely pathogenic (1); Uncertain significance (2); Benign (3); Likely benign (4) |
| Oculocutaneous Albinism | AR; AD | ***TYR*** | NM_000372.5: c.1205G>A (p.Arg402Gln) | 11:89017961 | Common reduced-function allele. Hypomorphic but not sufficient to cause albinism without a second pathogenic TYR variant. No clinical impact expected in isolation. In asymptomatic individuals or in the absence of a second pathogenic *TYR* allele, this variant is considered to have limited clinical significance. | Pathogenic (2); Likely pathogenic (1); Uncertain significance (9); Benign(2); Likely benign (2) |

## 4 Classification and reporting are inconsistent across laboratories

ClinVar submissions for these variants span Benign to Pathogenic, reflecting the lack of consensus. For example:

- *ABCD1* p.Thr483Met: Pathogenic (1), VUS (3)
- *GALE* p.Lys257Arg: VUS (3), Benign (3), Likely benign (3)
- *GALT* p.Asn314Asp: Pathogenic (5); Likely pathogenic (2); Uncertain significance (1); Benign (3); Likely benign (3)

Classification inconsistencies directly affect newborn genome programs, many of which return only pathogenic or likely pathogenic variants, meaning classification determines whether a family receives a result.

## 5 Program-level implications for newborn sequencing

Newborn genome programs operate at the intersection of early detection and uncertain penetrance. Consideration is needed for:

**Age-dependent expression**
- Some disorders manifest later in childhood or adulthood, complicating interpretation in asymptomatic newborns.

**Risk of over-calling disease**
- Reporting variants without proven clinical impact may increase anxiety and unnecessary follow-up.

**Need for biochemical correlation**
- Functional assays, enzyme activity, and newborn screening analytes remain essential for contextualizing genomic findings.

**Importance of defining returnable findings**
- Programs must establish clear criteria at both the gene and variant level.

**Longitudinal value**
- Even clinically silent variants may inform future risk or accelerate diagnosis if symptoms emerge later.

## 6 Discussion

**Functionally abnormal, but clinically silent variants** challenge the binary nature of current classification systems. They are:

- Not Benign, because they produce measurable biological dysfunction
- Not Pathogenic, because they lack clinical consequences
- Not Uncertain (VUS) in the traditional sense, because functional impact is known
- Not Pseudo-deficient, because the biochemical impairment is real

This suggests a need for **expanded classification terminology** or **context-specific modifiers** for newborn screening and sequencing programs.

Potential approaches include:
- A new subclassification (e.g., "biochemical variant with limited clinical significance")
- Reporting modifiers indicating functional impact without disease association
- Program-specific reporting categories for screen-positive, phenotype-negative variants

## 7 Conclusions

Functionally abnormal but (presumed) clinically silent variants represent a growing interpretive challenge in genomic medicine, particularly in the context of newborn screening. Their presence may explain abnormal biochemical findings without corresponding disease, complicating decisions around disclosure, follow-up, and parental counseling. The lack of consensus for these variants risks inconsistent reporting and unnecessary anxiety. To address these variants, a consensus is needed amongst the newborn screening community on how to manage variants that result in abnormal function but lack clear clinical outcomes. Longitudinal studies are also essential for evaluating potential late-onset effects and refining classification systems to accommodate this emerging category of genomic findings.

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