Impact of Structural Biology Assessment on Variant Interpretation and Patient Outcomes Over Ten Years in a Clinical Diagnostic Laboratory

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BACKGROUND

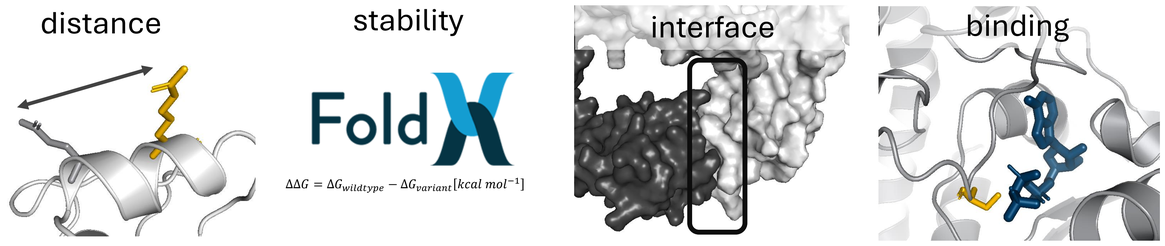
The clinical interpretation of genetic variants, particularly variants of uncertain significance (VUS), remains a significant challenge for diagnostic laboratories. One way of overcoming this obstacle is by using knowledge of a protein's three-dimensional (3D) structure to help inform variant interpretation. Ambry Genetics has used an expert panel of structural biologists since 2015 to help provide evidence for select cases. This manual workflow combines high-resolution structural data with a deep dive into primary literature and the strategic application of in silico tools. This approach provides a level of interpretive accuracy that fully automated systems cannot currently replicate (9,10).

While structural biology offers a powerful tool for assessing a variant's impact on protein function, its routine quantitative impact on clinical classifications is not well characterized (1). This raises the question of how, and to what extent, structural assessment influences variant interpretation and patient outcomes in a large-scale diagnostic setting. To answer this, we undertook a large-scale retrospective study to quantify the impact of structural biology assessment on variant interpretation at a single diagnostic laboratory.

METHODS

We retrospectively reviewed internally classified all variants that had associated notes at our institution from 2015 to 2025. We identified a subset that underwent **structural biology assessment**. Structural biology assessment evaluates a variant based on a variety of features related to the protein's 3D shape and stability.

The Anatomy of a Structural Biology Assessment



Proximity of the variant of interest to classified **pathogenic variants**

Calculation of ΔΔG to quantify variant-induced destabilization

Determination of variant location within an intermolecular **interface**

Observation of variant participation in **small molecule** binding

Structural assessment yields 4 different lines of evidence: (strong disruptive [1B/+2], weaker disruptive [1C/+1], stabilizing/benign [1E/-1] or inconclusive) under two different evidence codes (PM1 and BP8). This evidence is combined with other evidence codes to reach a final classification based on ACMG/AMP guidelines: Pathogenic (P), Likely Pathogenic (VLP), Variant of Uncertain Significance (VUS), Likely Benign (VLB) or Benign (B).

Structure Evidence Scores

PM1	Points	Meaning	BP8	Points	Meaning
1B	+2	Strong disruptive	1E	-1	Stabilizing/benign
1C	+1	Weaker disruptive			
inconclusive	0	Unknown			

Variant Classifications

Classification	Combination Rules	Meaning
Pathogenic	≥ 10pts	Disease-causing
VLP	9 to 9 pts	Likely pathogenic
VLB	-2 to -3 pts	Likely benign
Benign	≤ -4 pts	Normal Variation

RESULTS

Structural Biology Impact

3,700 variants received structural biology assessment from 2015 to 2025.

325 genes assessed for structural features, binding interactions and structure destabilization.

76,000 patients impacted directly by structural biology assessment, having received a definitive classification featuring structural evidence.

Structural Assessment Impact & Evidence Distribution

- Structural evidence was conclusive for over 50% of all variants reviewed.
- Evidence Tier Distribution:
  - 35% Strong Pathogenic (Tier 1B / +2)
  - 18% Supporting Pathogenic (Tier 1C / +1)
  - 4% Supporting Benign (Tier 1E / -1)
  - 43% Inconclusive (Tier 0)

VUS Resolution: Variants subjected to structural assessment achieved a higher proportion of definitive (not uncertain significance) clinical classifications compared to the control group (variants meeting the same criteria but lacking structural review).

Expert Review Success: When expert structural review provided conclusive evidence, 78% of variants were successfully reclassified with a non-VUS clinical status.

Structure-Assessed Variants by Structure Evidence and Final Classification

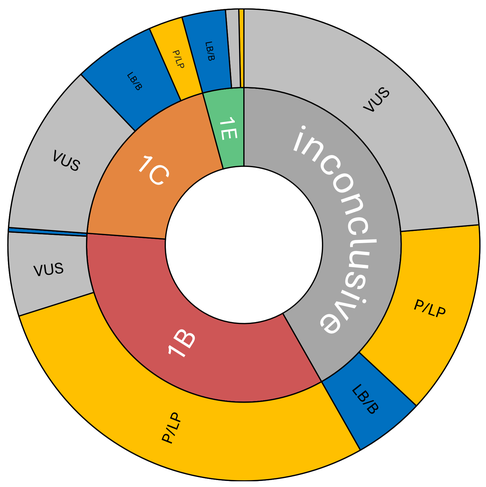
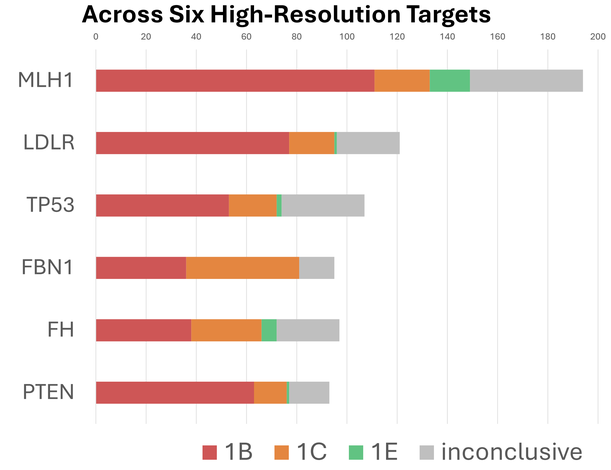


Fig.1 – A sunburst diagram showing the distribution of structural evidence assigned to all variants assessed on the inner ring, and the breakdown of final classification for each line of evidence on the outer ring.

The Best Genes for Structural Assessment

Furthermore, our analysis of frequently assessed genes identified a subset of six genes (*MLH1*, *LDLR*, *TP53*, *FBN1*, *FH* and *PTEN*) that had high rates of both conclusive structural scores and high final rates of definitive classification. This means these six genes consistently receive conclusive structural evidence (1B/1C/1E) and go on to be classified as P/VLP/B/VLB. These genes feature high-resolution structures for FoldX calculations and modeling, as well as well-annotated, discrete domains of known function (2).

Summary of Structural Biology Review Outcomes Across Six High-Resolution Targets



All Genes Assessed by Structural Biology

Fig. 2, (left) – Stacked bar chart detailing the number of variants assessed by structure for each of the ideal structural candidate genes, each subdivided by the evidence received from structure assessment.

Fig. 3 (above) – Word cloud illustrating the distribution of genes reviewed by the structural biology team. The scale of each gene name corresponds to the total number of variants assessed (N=325).

Structure Spotlight: p53

More than 50% of human tumors carry *TP53* gene mutations, and as a result, more than 45,000 somatic and germline mutations have been recorded in the UMD TP53 database (3). Six residues essential for DNA binding have been highlighted "hotspot" residues due to their critical role in p53 function (4). Variants in this region are often eligible for structural evidence due to this binding interaction, which can provide the necessary data to support reclassification to a Likely Pathogenic or Pathogenic status. Structural biology has assessed a total of 107 *TP53* variants, with 14% of these falling in the DNA-binding region and receiving Pathogenic classification.

This structural feature is not limited to p53— DNA-binding evidence is just as powerful in cases featuring variants in *BRCA1*, *PAX6*, *HNF1A* and *GATA1-6* (5, 6, 7, 8).

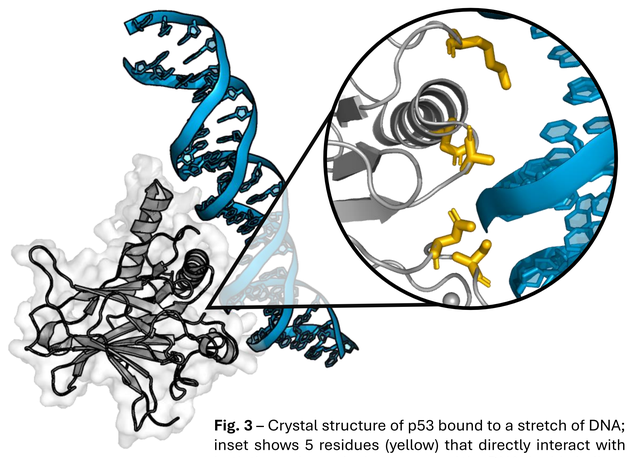


Fig. 3 – Crystal structure of p53 bound to a stretch of DNA; inset shows 5 residues (yellow) that directly interact with both the major and minor groove of the DNA double helix.

TAKE HOME POINTS

- ❑ At Ambry, **expert structural biologists contribute to variant interpretation** by evaluating protein structure, stability and binding interactions.
- ❑ When conclusive evidence from structural biology is available, **78% of variants receive non-VUS classifications.**
- ❑ Structural biology evidence is especially effective for *MLH1*, *LDLR*, *TP53*, *FBN1*, *FH* and *PTEN*—genes that have **high-resolution, well-documented and functionally-tested structures**.

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