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

Authors: Rachel Jones, Caitlin Reid, Holly LaDuca, Amanda Jacquart, Matthew Varga

Introduction: The clinical interpretation of genetic variants, particularly variants of uncertain significance (VUS), remains a significant challenge for diagnostic laboratories. While structural biology offers a powerful lens for assessing a variant's impact on protein function, its routine quantitative impact on clinical classifications is not well characterized. 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 variants within oncology and cardiovascular genes that had associated notes at our institution from 2015 to 2025. We identified a subset that underwent structural biology assessment. We analyzed the distribution of the assigned structural evidence from this study group, which was recorded as 'inconclusive' or 'conclusive' (subdivided into strong pathogenic [1B], weaker pathogenic [1C], or benign [1E]).

To determine patient-level impact, we calculated the total number of individuals impacted by a final non-VUS clinical classification (i.e., Pathogenic, Likely Pathogenic, Likely Benign, or Benign) that had been informed by structural evidence.

A secondary analysis was performed to identify genes most frequently assessed by structural biology. We filtered for genes in the 75th percentile for assessment frequency ($n > 78$, where n is the number of times a variant from the gene received structural assessment) and analyzed this group to identify candidates with high rates of 'conclusive' structural evidence and high final rates of Pathogenic, Likely Pathogenic, Likely Benign, or Benign classification.

Results: A total of 3,695 unique variants across 325 genes assessed by structural biology made up the cohort for this study. Over 76,000 patients received conclusive structural evidence that contributed to a final, non-VUS classification. Structural evidence was conclusive for over half of the variants reviewed. The overall distribution of evidence was

34% strong pathogenic (1B), 18% weak pathogenic (1C), 5% benign (1E), and 43% inconclusive.

A higher proportion of variants received a clinical non-VUS classification after structural assessment compared to those that met the same filtering criteria for this retrospective study but did not receive structural assessment. Specifically, 78% of variants received a non-VUS clinical classification when expert structure review provided conclusive evidence. Furthermore, our analysis of frequently assessed genes identified a subset of six genes (LDLR, PTEN, FH, MLH1, TP53, and FBN1) that had high rates of both conclusive structural scores and high final rates of non-VUS resolution.

Conclusions: Our findings demonstrate that structural biology assessment is a high-impact tool that directly contributes to the resolution of variants into final, non-VUS clinical classifications. The identification of six "structurally amenable" genes provides insight into how a more targeted, proactive workflow can be developed to systematically increase the rate of non-VUS classification. This work provides a model for optimizing the integration of structural biologists into routine variant interpretation to improve diagnostic resolution. Most importantly, this system impacts thousands of patients precisely because our unique approach relies on the irreplaceable judgment of expert scientists.