Clinical Impact of Integrating *BRCA2* MAVE Data for Variant Reclassification: A Real-World Evaluation

Kristin Kohler[1], Ashley P.L. Marsh[1], Colin Young[1], Jamie D. Weyandt[1], Sarah A. Dugger[1], Daniel Zimmerman[1], Magan Trottier[1], Steven Harrison[1], Marcy E. Richardson[1]

1. Ambry Genetics, Aliso Viejo, CA, USA Email: kkohler@ambrygen.com

BACKGROUND

- Interpretation of rare missense variants remains a major challenge in clinical genomics, particularly in clinically actionable genes such as *BRCA2.*
- Two *BRCA2* MAVEs functionally characterized nearly all possible single-nucleotide variants (SNVs) across the functionally critical DNA-binding domain (DBD)[1,2], providing functional measurements that can be incorporated into variant classification frameworks.[3]
- However, empirical data demonstrating the utility and impact of integrating MAVE functional evidence into clinical diagnostic workflows remain limited.

Aim: To investigate the real-world impact of integrating *BRCA2* MAVE data into a clinical variant classification workflow.

METHODS

MAVE Integration and Validation

- **Validation**: Two *BRCA2* MAVE studies were validated against multigene panel testing (MGPT) pathogenic/likely pathogenic (P/LP) and benign/likely benign (B/LB) variants from Ambry Genetics.
- **PS3/BS3 Assignment**: OddsPath supported strong evidence. Applied only if both MAVEs showed concordant "Strong" results (excluding exon 25). Discordant or weaker findings received no weight.
- **Re-evaluation**: Eligible variants were re-classified using a modified ACMG/AMP framework combining all evidence.

Assessment of Patient-Level Impact

- **Cohort & Follow-up**: Patients receiving *BRCA2* MAVE-updated reports (Ambry Genetics, 2016–2025) were quantified. Cascade testing was assessed 6 months post-upgrade.
- **Ancestry Analysis**: Compared reclassification, upgrade, and downgrade rates (relative to total *BRCA2* tests) between European (White/Ashkenazi Jewish; reference group) and non-European (Black, Asian, Hispanic, Multiethnic/Other) ancestries. Individuals with unknown ancestry was excluded.
- **Statistical Methods**: Estimated relative rates and 95% CIs; compared proportions using two-sided Fisher's exact tests.

REFERENCES

1. Sahu S, Galloux M, Southon E, et al. Saturation genome editing-based clinical classification of *BRCA2* variants. *Nature*. 2025;638(8050):538-545. doi:10.1038/s41586-024-08349-1
2. Huang H, Hu C, Na J, et al. Functional evaluation and clinical classification of BRCA2 variants. *Nature*. 2025;638(8050):528-537. doi:10.1038/s41586-024-08388-8
3. Brnich SE, Abou Tayoun AN, Couch FJ, et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. Genome Med. 2019;12(1):3. Published 2019 Dec 31. doi:10.1186/s13073-019-0690-2

RESULTS

Integration of MAVE Functional Evidence Resulted in Significant VUS resolution

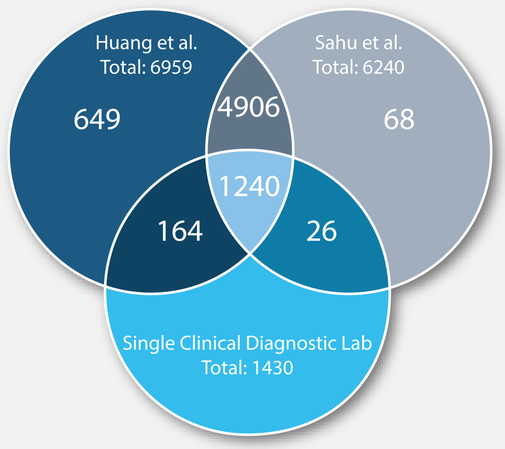

Figure 1. Profile of *BRCA2* Variants Assessed by MAVEs
Overlap between *BRCA2* variants functionally assessed in two MAVE studies and those observed in our clinical diagnostic laboratory. A total of 1,430 *BRCA2* variants assessed by the MAVE studies were observed in our diagnostic laboratory.

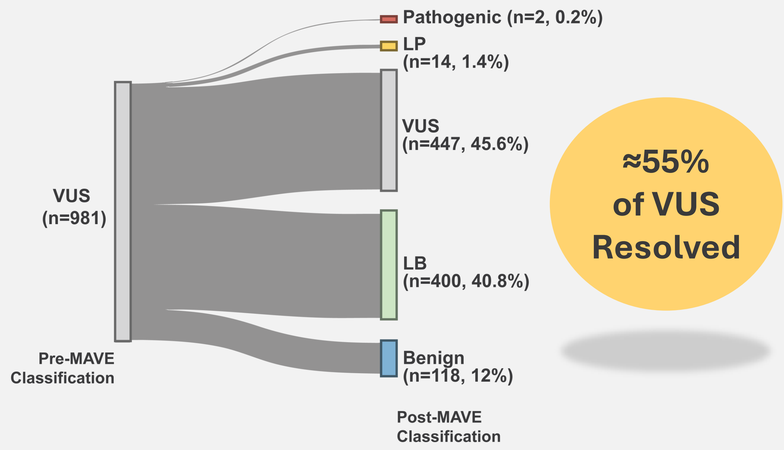

Figure 2. Clinical Classification Outcomes for *BRCA2* VUS After Integration of MAVE Functional Evidence
Flow of *BRCA2* VUS before integration of *BRCA2* functional data and their post-MAVE clinical classification outcome, including pathogenic, likely pathogenic (LP), variants of uncertain significance (VUS) , likely benign (LB), and benign.

Post-MAVE Unresolved VUS

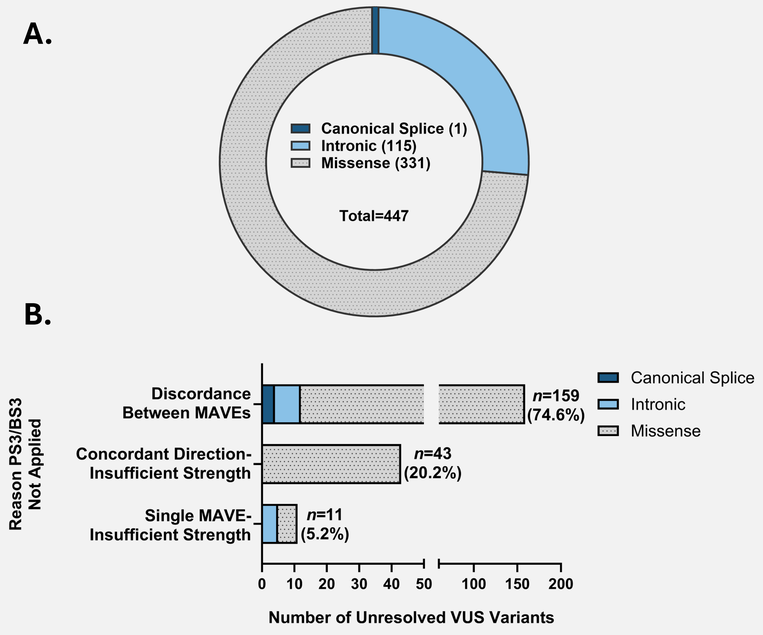

Figure 3. Features of *BRCA2* VUS Unresolved After MAVE Integration
A. Distribution of variant types among *BRCA2* variants that remained classified as VUS after MAVE integration. **B.** Bar graph showing variants for which PS3/BS3 evidence was not applied (*n*=213/447; 47.7%). The remaining variants (n=234/447; 52.3%) had MAVE PS3/BS3 evidence applied; however, the total evidence remained insufficient for reclassification.

Patient-Level Impact & Equity

Table 1: Post-MAVE Reclassification Patient Impact

Reclassification Category	Number of Patients with Updated Reports	Cascade Testing		
		Number of Families Tested	Number of Relatives Tested	Number of Positive Cascade Reports
Pathogenic	4	2	6	4
Likely Pathogenic	88	19	36	19
VUS	-	-	-	-
Likely Benign	2,282	-	-	-
Benign	730	-	-	-
TOTALS	**3,104**	**21**	**42**	**23**

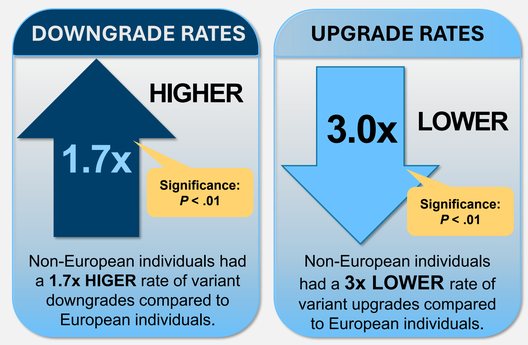

Figure 4. Ancestry-Based Reclassification Rate Comparison

TAKE HOME POINTS

- Integration of two independent *BRCA2* MAVEs resolved **≈55% of VUS within the DBD.**
- Clinical reclassification provided diagnostic clarity for **3,104 individuals**, with an additional 23 individuals identified as *BRCA2* P/LP heterozygotes through family cascade testing.
- Individuals of **non-European ancestry experienced significantly higher VUS downgrade rates and lower VUS upgrade rates** compared to individuals of European ancestry.
- The application of calibrated MAVE functional evidence can **substantially reduce VUS** burden and inform **clinically meaningful reclassification decisions across diverse populations**.