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WHITE PAPER

The Path to *Classifi*-cation: Translating MAVE Outputs into Clinical Results

Introduction to MAVEs

Functional studies are a valued source of evidence for variant classification, providing direct insight into the impact of a given variant on protein function. Historically, functional studies required time-intensive, manual workflows and therefore were conducted on a limited, per-variant basis.

Multiplex assays of variant effect (MAVEs) are high-throughput, functional studies which are often designed to include all potential single nucleotide variants in a clinically significant gene or protein domain. This approach is ancestry-agnostic, helping reduce racial and ethnic disparities in variant classification. The number of variants with MAVE-based functional data has increased significantly over the past five years. In the most recent report from MaveDB, there were over 7 million variants with measurement effects—a six-fold increase from their seminal publication.[1]

MAVE experiments involve two major components—generating massive libraries of mutant DNA and assessing protein function for all the respective variants in parallel. Common types of MAVE approaches include deep mutational scanning, massively parallel reporter assays and saturation genome editing. However,

MAVEs have transformed the approach to functional studies. They enable scientists to analyze thousands of variants in a single experiment and have significantly improved variant resolution for numerous genetic diseases.

generating functional scores is not the end of the story (Figure 1). MAVE outputs need to be translated into clinically meaningful evidence, and the evidence needs to be applied for variant (re)classification. In a recent review, McEwen et al.[2] identified 30 MAVE datasets that had been clinically calibrated and/or applied in variant reclassification. Ambry has systematically integrated MAVEs into Classifi,® our comprehensive program designed for diagnostic resolution. The remainder of this white paper focuses on the translational steps that occur within the Classifi program.

Translating Functional Scores into Evidence

Before MAVE outputs (or any other functional data) can be utilized in variant classification, they need to be translated into an interpretable piece of evidence.[3]

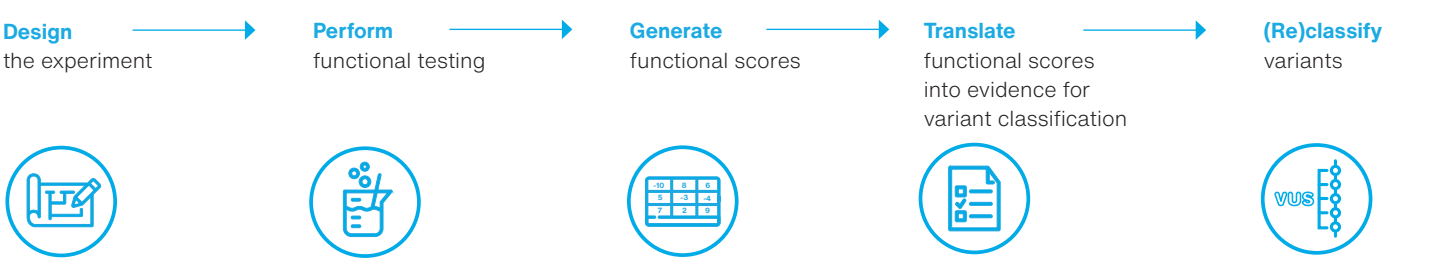


Figure 1. MAVE process

At minimum, this requires calibrating functional scores with a truth set of variants and weighting the strength of functional evidence. There is also value in correlating functional scores with clinical phenotypes to increase confidence that the functional evidence will translate to clinically meaningful variant classifications. The MAVE process is optimized when there is close collaboration between the experimentalists generating the MAVEs and the variant scientists who interpret variants at clinical testing laboratories. Broader collaborations, such as the Atlas of Variant Effects (AVE) Alliance,[4] are also imperative in the development and refinement of standards for the clinical translation of MAVE evidence.

Calibrating functional scores to establish cutoffs

An ideal assay will have a high dynamic range, meaning there is a clear separation between expected benign and pathogenic variants. For example, the functional assay shown is well calibrated, as it clearly distinguishes between nonsense and synonymous variants (Figure 2).

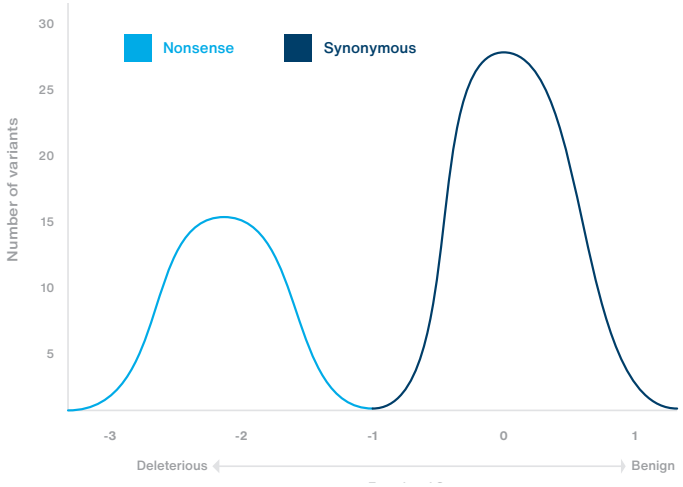


Figure 2. Calibration Graph Example

Correlating functional scores with clinical phenotypes

An important aspect of MAVE validation is the correlation of functional score with the clinical phenotypes associated with the gene of interest. Ideally, neutral functional scores have low or no association with a given phenotype while deleterious functional scores show strong association with a given phenotype (Figure 3).

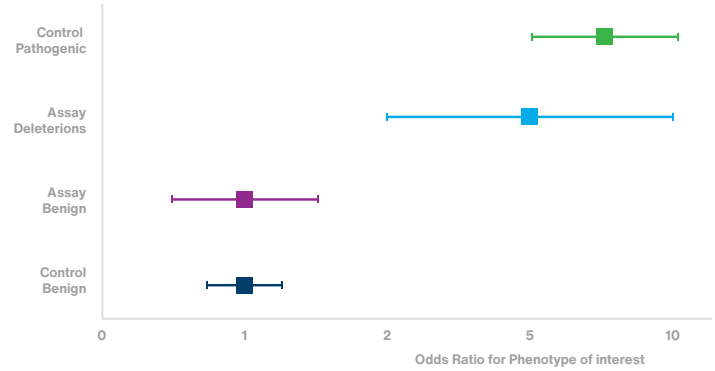


Figure 3. Correlation Graph Example

Calibration of functional evidence weight

The final aspect of implementing a MAVE assay is to determine how well the functional data matches known classifications. Assays with strong concordance between pathogenic and benign variants may receive stronger weight than assays that have discordance. Based on the statistical method implemented, strengths may be discrete (pathogenic or benign strong) or continuous (some variants receive supporting, moderate or strong weight) (Figure 4).

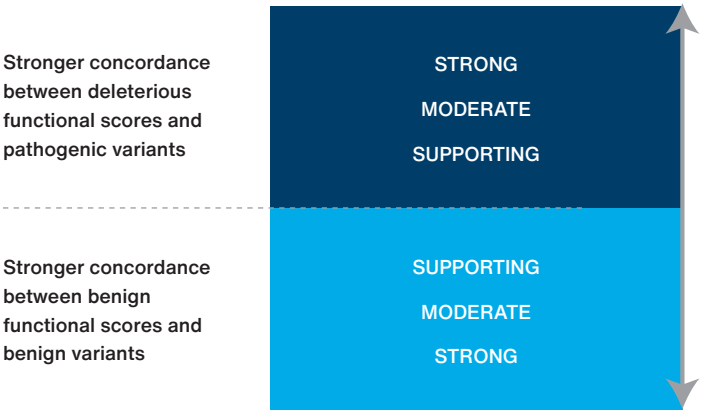


Figure 4. Weighting Functional Evidence

Applying Functional Evidence to Re-*Classifi* Variants

After taking the appropriate steps to transform MAVE outputs, variant-level functional evidence is finally ready to be incorporated into variant classification. Variant classification integrates multiple lines of evidence to determine the association of a variant with a certain phenotype (Figure 5). Current variant classification standards rely on a 5-tier scheme, where pathogenic and likely pathogenic variants are considered disease-causing, benign and likely benign variants are not disease-causing, and variants of uncertain significance are inconclusive with respect to disease association.[5,6] With few exceptions, multiple lines of evidence are required to classify a variant as anything other than a Variant of Uncertain Significance (VUS).

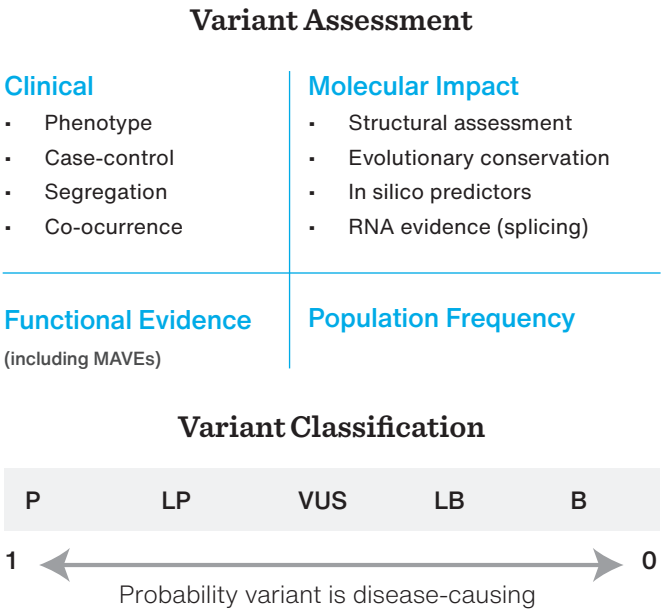


Figure 5. Variant Assessment & Classification

Functional evidence from MAVEs have drastically reduced VUS rates for the respective genes. However, not all VUS will be resolved with data from MAVEs. Functional evidence, while powerful, is just one line of evidence utilized in variant assessment (Figure 6). Depending on how the functional evidence is weighted for a given assay and how much additional information is available, there still may not be sufficient data assign a non-VUS classification to a variant. There also may be conflicting lines of evidence that prevent a VUS from being resolved. Generally speaking, a majority of MAVE-related variant reclassifications are VUS downgrades to likely benign or benign. The proportion of variants reclassified varies, depending on the MAVE and how the outputs are weighted.

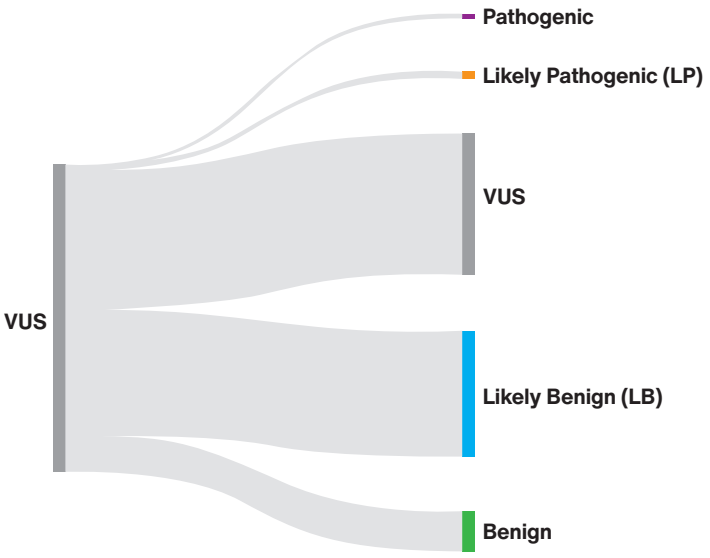
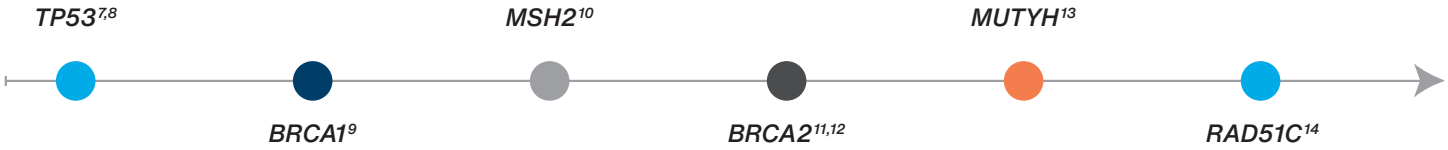


Figure 6. Post-MAVE Variant Reclassification

The impact of MAVEs extends beyond variant reclassification. A major benefit of MAVEs is access to functional evidence on variants before they are observed in a patient. Having functional evidence available at the outset of variant classification reduces the likelihood of a patient initially receiving a VUS result.

Spotlight on MAVEs in Hereditary Cancer

Ambry's variant scientists have been actively translating hereditary cancer MAVE data into variant assessment and classification for years, beginning with *TP53* in 2019.



Real-World Impact

Each of these translation efforts involved close collaboration with MAVE researchers and ultimately resulted in clinically significant variant reclassifications.

Over 200 VUS have been ***upgraded*** to Pathogenic/Likely Pathogenic.

About 2,000 VUS have been ***downgraded*** to Benign/Likely Benign.

Over 18,000 Ambry patients have received greater clarity due to VUS upgrades or downgrades from these MAVEs! These reclassifications also ***reduce diagnostic uncertainty*** for new Ambry patients.

Take Home Points

- **MAVEs are large-scale studies that generate functional scores on all theoretical variants in a gene.**
- **Several critical steps are needed to incorporate MAVE outputs into variant assessment and classification.**
- **Collaboration between researchers performing MAVEs and laboratories performing variant classification ensures the most accurate utilization of a powerful tool.**
- **MAVEs provide functional evidence on variants before they are observed in patients, reducing the likelihood of an initial VUS result and improving clarity in genetic testing.**

References

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