**An In Silico Assessment of Functional Assay Impact on Clinical Variant Interpretation**

S.E. Brnich¹,², E.A. Rivera-Muñoz¹, J.S. Berg¹,²
¹Department of Genetics, School of Medicine, University of North Carolina at Chapel Hill
²Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill

**Introduction**

- Clinical and presymptomatic screening applications of genomic sequencing require additional tools for clinical variant interpretation.
- Variant interpretation guidelines provide a general framework for this process, but significant gaps exist in the ability to utilize evidence such as functional assays.

**Methods**

We performed an in silico analysis of the ACMG/AMP framework by generating all possible rule combinations (Figure 2) applicable to missense variants. For this purpose, we assumed that each piece of evidence considered was independent and either met/not met.

- We excluded rules that are not applicable to missense variants (e.g., PV5 is meant strictly for LOF/truncating variants).
- We filtered out “unrealistic” combinations (e.g. meeting more than 1 allele frequency criteria).
- Since ACMG/AMP criteria do not provide a method to resolve conflicting VUS, we excluded combinations where conflicting benign and pathogenic criteria exceeded minimal supporting evidence.

**Results**

- **How much would a well-validated functional assay help?**
  - Focus on VUS due to insufficient information (typically, rare missense variants).
  - How could availability of “strong” functional evidence improve the ability to make a pathogenic or benign assertion?

**Curated Variant Examples**

**RA3opathy Expert Panel Curation:**
- Curated 103 variants total from 9 different genes.
- Only 35/103 met PS3, all classified as pathogenic.
- 1/103 met BS3, classified as likely benign.
- 6 non-conflicting missense VUS.
- 5/6 would be reclassified with PS3 or BS3 criteria.

**MYH7-Associated Cardiomyopathies Expert Panel Curation:**
- Curated 60 variants.
- Only 4/60 met strong functional criteria, all were pathogenic.
- 11 non-conflicting missense VUS.
- 5 would be reclassified as LP with evidence from a well-validated functional assay demonstrating a damaging effect on the gene.
- 3 only satisfied PP3 criteria and 3 met no criteria for clinical interpretation and thus would remain VUS even with the addition of strong functional evidence.

**Conclusions**

- Well-validated functional assays could improve missense VUS interpretation within the ACMG/AMP framework.
- Investigators should focus on developing methods of generating data types that provide “strong” evidence (ex. functional assays).

**Future Directions**

- Evidence-based prioritization method for assay development and variant assessment.
- Increase capacity for high-throughput functional assay development and variant assessment.
- Reduce the burden of VUS complicating patient care.

**Acknowledgements & References**

We would like to acknowledge funding from ClinGen Grant U41 HG009650 and the UNC School of Medicine Yang Family Biomedical Scholars Award. Sarah was also supported in part by the UNC MSTP Training Grant 2T32GM008719-6, and a grant from the National Institute of General Medical Sciences under award ST32 GM007092.

1 Adapted from Figure 1 in Richards, et al., Interpretation of sequence variants, Genet Med (2015).
2 Adapted from Table 3 in Richards, et al., Interpretation of sequence variants, Genet Med (2015).