The Uniform Application of Protein Functional Data has an Impressive Potential to Resolve VUS Rates in BRCA2

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Background: Variants of uncertain significance (VUS) identified on clinical genetic tests remain a significant pain point for clinicians and their patients. Many VUS result from a dearth of available information, particularly for rare variants, where population and clinical data are sparse. In these instances, a high-quality, well validated functional study can have a tremendous impact on informing clinically relevant variant interpretation. In this work, we present the impact that the gold-standard homology-directed DNA repair (HDR) assay has on variant classification from one clinical diagnostic laboratory.

Methods: 252 missense variants in the BRCA2 DNA Binding Domain (DBD) were selected for functional analysis as previously described [Guidugli L et al. *Cancer Res.*, 2013 Jan;73:265-75]. The assay was validated for the application of a strong line of evidence towards classification (encoded by the ACMG/AMP guidelines as PS3 and BS3) according to the recommendations from the Sequence Variant Interpretation (SVI) group [Brnich SE et al. *Genome Med*, 2019 12;12:3]. The results of these functional data were incorporated into a modified ACMG/AMP model for variant interpretation for 186 missense variants observed at this laboratory [Pesaran T et al. *Int J Breast Cancer*, 2016 Oct;2016:2469523]. The functional data and final classifications were compared to other publicly available data including other BRCA2 protein functional studies, variant classifications based on a Bayesian model that excludes protein functional data, and ClinVar classifications.

Results: Inclusion of high-quality, clinically validated HDR functional data reduced the VUS rate by 86% (132/154) with over 70% of variants moving from VUS toward benign (93/132) and ~30% moving from VUS toward pathogenic (39/132). The remaining 32 variants were already classified as likely benign/benign (LB/B) or likely pathogenic/pathogenic (LP/P) (Figure 1). Overall, the HDR assay showed 100% concordance with other functional studies with 63 overlapping variants having the same conclusion of functional, or non-functional [Biswas K *et al. Hum. Mol. Genet.* 2012 Sep;21(18):3993-4006; Kuznetsov SG *et al. Nat Med*, 2008 Aug;14:875-81; Mesman RLS *et al. Genet. Med.* 2019 02;21:293-302]. Comparison of 135 variants classified as LP/P or LB/B in this study that overlapped with ClinVar assertions from two-star laboratories (excluding any outlier classifications from this laboratory) revealed that only 27% were in agreement without conflicts (36/135). A further 32% were in partial agreement with some depositors having a conflicting classification (43/135). The remaining 41% of variants (56/135) had VUS assertions in ClinVar (Figure 2). The 73% (99/135) of variants with ClinVar

conflicting interpretations or VUS entries for these now-resolved variants underscores the vast potential that uniform application of PS3 and BS3 weight can have on variant resolution among laboratories.

Interpretation: The high rate of success of VUS resolution achieved by implementing the gold-standard HDR assay into variant interpretation impacted results for over 1900 patients from this clinical laboratory. In extrapolating the 86% VUS resolution rate for this study, 657/764 of the ClinVar deposits with conflicting interpretations or VUS assertions could be resolved leading to improved care and peace-of-mind for countless patients world-wide.

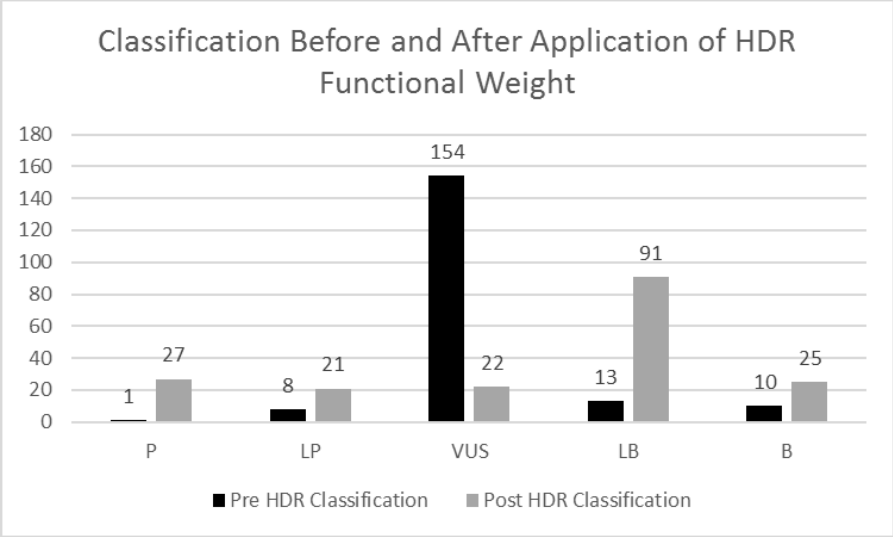

Figure 1: Influence of HDR Functional Data on variant classification using an ACMG-like model. Classification of variants before (black bars) and after (gray bars) the application of protein functional data. Number of variants is indicated above the bar. P-Pathogenic; LP-Likely Pathogenic; VUS-Variant of Uncertain Significance; LB-Likely Benign; B-Benign.

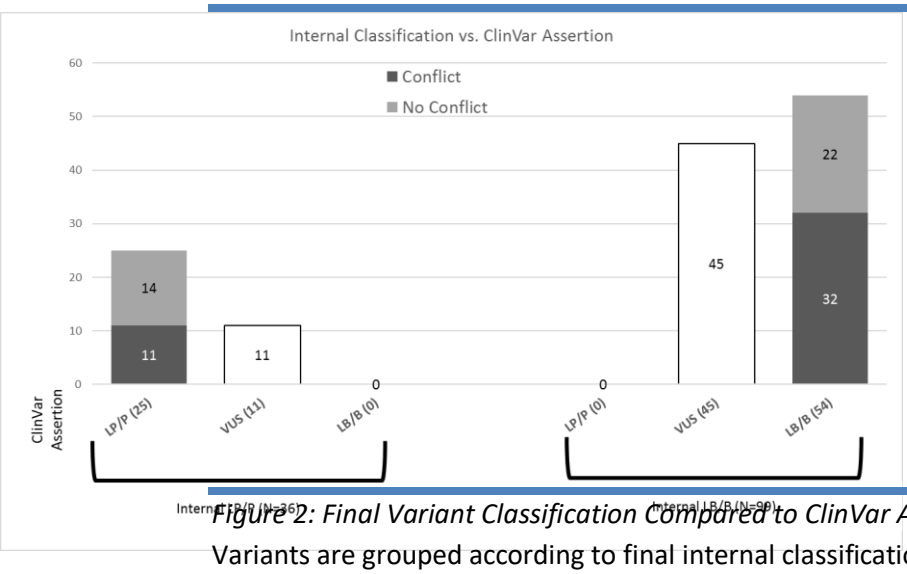

Figure 2: Final Variant Classification Compared to ClinVar Assertions. Variants are grouped according to final internal classification and further subdivided into the general assertion provided by ClinVar (LP/P, VUS, LB/B). Each bar is shaded to indicate a conflicting (dark gray) or non-conflicting (light gray) ClinVar assertion. VUS category is shown as white bars. The total number of internally classified variants in each category is in parentheses and the number of variants designated in ClinVar as conflicting or non-conflicting is indicated within the bar. P-Pathogenic; LP-Likely Pathogenic; VUS-Variant of Uncertain Significance; LB-Likely Benign; B-Benign.