Integration of functional data to classify BRCA1/2 missense variants: an ENIGMA project

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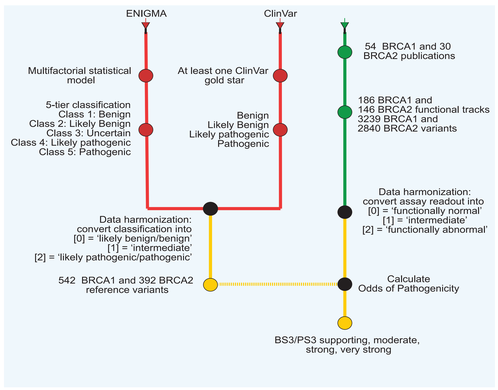
INTRODUCTION

The success of precision cancer medicine is predicated on the accurate discrimination between benign and pathogenic germline alleles in susceptibility genes. Variants of uncertain clinical significance (VUS) pose a significant challenge in cancer risk assessment. Functional data derived from validated functional assays have become an important resource to assess the pathogenicity of VUS. We developed a cloud-based environment to integrate and analyze all published functional data for BRCA2 missense VUS according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) classification framework[1].

We expect that functional data will be critical for the classification of rare variants. Previously, we compiled all functional data for missense variants in BRCA1[2]. The ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium has been working on updating the BRCA1 database and expanding the database of functional assays for BRCA2 missense variants.

METHOD



Our approach involved selecting published articles reporting functional analyses of BRCA1/2 missense variants to assess their impact on various biochemical and cell biological assays. Functional results were harmonized using original authors' thresholds and classifications, then converted to ordinal variables: [0 = no functional impact], [1 = intermediate impact], and [2 = functional impact]. For BRCA1, we updated our previous published integration[2] with 14 additional articles. We integrated results from 53 individual instances of functional assays reporting functional data on 3,239 unique missense variants. For BRCA2, 28 published articles were identified reporting data from 141 individual instances of functional assays on 2,840 unique missense variants.

Utilizing a panel of 542 and 396 known reference variants for BRCA1 and BRCA2, respectively, we determined the sensitivity, specificity, and ACMG/AMP odds of pathogenicity for each individual instance of the assay. Variants were assigned ACMG/AMP criteria based on the level of evidence[3].

CONCLUSION

Our study successfully derived unambiguous evidence criteria from functional data for 2,463 BRCA1 and 2,326 BRCA2 missense variants, excluding discordant results that could not be assigned a functional code. This work underscores the potency of functional data in resolving the majority of BRCA1 and BRCA2 VUS.

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RESULTS

Figure 1 - Distribution of ACMG/AMP criteria assignments for BRCA1 (186 assays) and BRCA2 (146 assays) datasets. a) Percentage of assays assigned PS3 criteria in BRCA1 dataset. b) Percentage of assays assigned BS3 criteria in BRCA1 dataset. c) Percentage of assays assigned PS3 criteria in BRCA2 dataset. d) Percentage of assays assigned BS3 criteria in BRCA2 dataset.

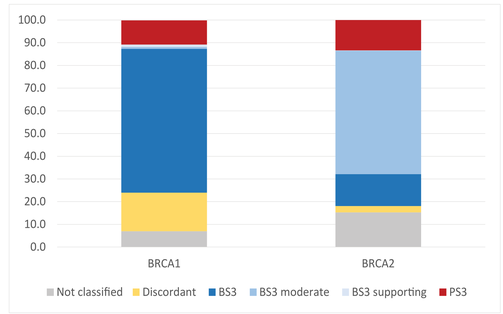
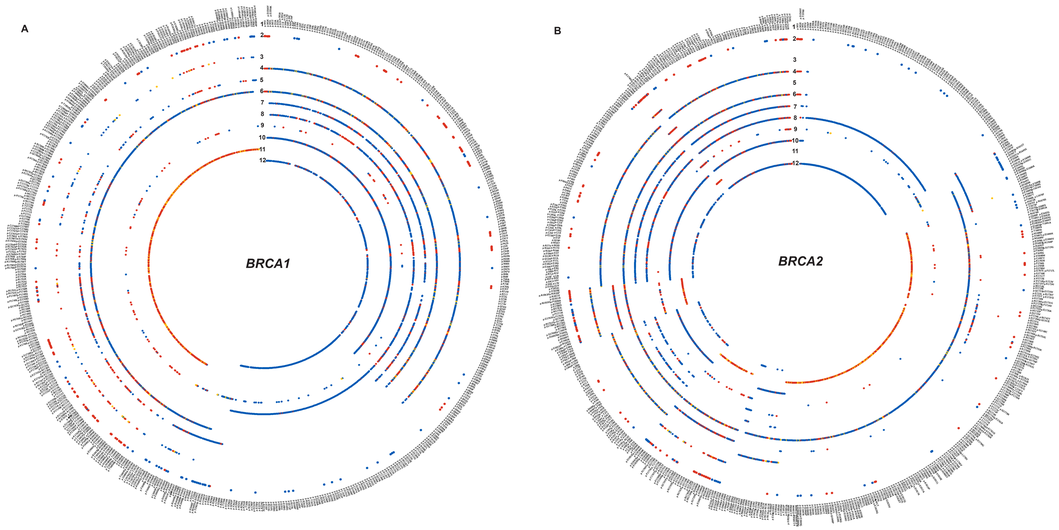
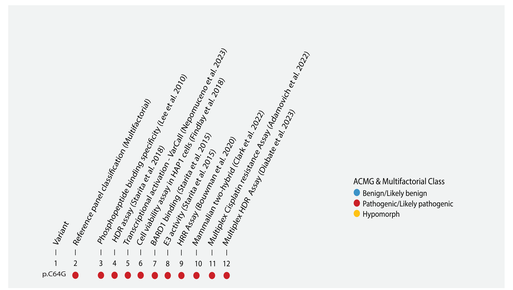


Figure 2 - Comparison of evidence criteria assignment for all variants tested. Graphs show the fraction of each evidence criteria variant assigned under the two datasets (BRCA1 and BRCA2).





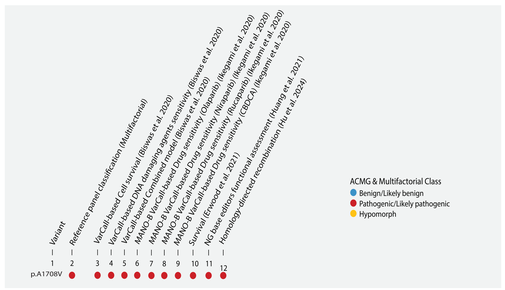


Figure 3 - Hi-set classification for BRCA1 and BRCA2. Illustration of 10 selected tracks, which assign hundreds of variants with ACMG/AMP classification for BRCA1 and BRCA2.