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

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The success of precision cancer prevention hinges on accurate discrimination between benign and pathogenic germline alleles in cancer susceptibility genes. Variants of uncertain clinical significance (VUS) present a challenge for cancer risk assessment. To address this, we developed a cloud-based environment to collate, curate, integrate and analyze all published functional data related to *BRCA1* and *BRCA2* missense VUS. Variant assessment is guided by the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) classification framework and ClinGen.

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 (Genet Med. 2021;23:306-315) with 14 additional articles. We integrated results from 53 individual instances of functional assays reporting functional data on 3,219 unique missense variants. For *BRCA2*, 28 published articles were identified reporting data from 141 individual instances of functional assays on 4,759 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.

Our study successfully derived unambiguous ACMG/AMP evidence criteria from functional data for 3,040 BRCA1 and 2,704 BRCA2 missense variants. This work underscores the potency of functional data in resolving the majority of BRCA1 and BRCA2 VUS.