The ClinGen ENIGMA BRCA1/2 Expert Panel: a dynamic framework for evidence-based recommendations to improve classification criteria for variants in BRCA1/2

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Category: Cancer genetics and therapeutics

600 words – currently 600 (excluding title, authors)

Introduction: The role of *BRCA1* and *BRCA2* in Hereditary Breast and Ovarian Cancer (HBOC) has long been recognized, with clinical testing initiated soon after research discovery of these genes in the 1990s. The ENIGMA international research consortium (https://enigmaconsortium.org/) focuses on development and application of methods to determine the clinical significance of variants in HBOC genes. At the request of ClinGen, ENIGMA steering-committee members formed an external *BRCA1/2* variant curation expert panel in 2016. Documented classification criteria captured qualitative criteria generally adopted clinically (e.g. most premature termination codon variants assumed to be pathogenic) and quantitative multifactorial likelihood analysis methods developed in the research setting. A total of 7456 expert curations for variants in these genes were submitted to ClinVar. In 2020 this external expert panel sought to become an internal ClinGen Variant Curation Expert Panel (VCEP), to align with FDA-recognized processes for expert panel contributions to ClinVar.

Methods: The original ENIGMA expert panel membership was extended to include representatives from several major US diagnostic testing laboratories, and additional research experts. VCEP members met approximately monthly to review the baseline ACMG/AMP sequence variant classification guidelines

(PMID: 25741868) in order to determine whether each classification criterion should be adopted, modified, or omitted for *BRCA1* and *BRCA2* variant interpretation. A critical aspect of VCEP activities was to convert the multiple evidence types and strength outlined in pre-existing ENIGMA external panel classification guidelines to ACMG/AMP codes. Statistical methods were used to calibrate strength of evidence for different data types, using likelihood ratio estimates to assign code weights as per Tavtigian *et al*. (PMID: 29300386). Alongside, key members of the ClinGen Sequence Variant Interpretation (SVI) group were consulted to ensure adequate use of information types and processes that do not strictly conform to the ACMG/AMP codes/guidelines. With input from biocurators, pilot specifications were tested on 40 variants, and documentation revised for clarity and ease-of-use. Variants with inter-biocurator differences in classification were re-reviewed by additional biocurators using the revised documentation.

Results: After initial review of ACMG/AMP criteria for relevance to interpretation of *BRCA1* and *BRCA2* variants, 11 codes were considered non-applicable or overlapping (non-independent) with other criteria. Extensive analysis was used to inform weights relevant for 8 codes (spanning use of protein functional, population frequency, segregation, and bioinformatic data). Considering empirical knowledge of HBOC-related clinical features, the use-case was extended or re-purposed for another 7 codes. Pre-existing ClinVar classification of the 40 pilot variants was as follows: 13 VUS/conflicting, 11 P, 3 LP/P, 1 LB, 1 LB/B, and 11 B. Review using the refined specifications resolved classification for 8 VUS/conflicting variants (3P, 2LP, 1LB, 2B), and retained or improved classification for the remainder. All variants with pre-existing ClinVar class P or B retained class. Of the remainder, 3 LP/P variants were upgraded to P, the LB variant retained class, and the LB/B variant was classified as B.

Conclusion: Alignment of existing ENIGMA external expert panel *BRCA1/2* classification criteria with ACMG/AMP classification criteria highlighted several gaps between pre-existing external expert panel criteria and the baseline ACMG/AMP criteria. These included use of calibrated functional evidence (lacking from the external panel criteria), and the need for ACMG/AMP criteria to be adapted or repurposed to capture more evidence types (and weights) against pathogenicity, and also pre-existing diagnostic laboratory classification practices for premature termination codon variants. Calibration of evidence types using statistical approaches was key to both VCEP member and SVI acceptance (or rejection) of the utility of different ACMG/AMP evidence codes for classification. The *BRCA1/2* VCEP is now poised to move to Step 4 of the ClinGen VCEP approval process, permitting ongoing ACMG/AMP-aligned review of *BRCA1* and *BRCA2* variants in ClinVar.