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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076

BACKGROUND

- *BRCA1* and *BRCA2* are two of the most sequenced hereditary cancer susceptibility genes in clinical practice, since their discovery in the mid 1990's
- Despite the extensive study of these two genes, classification of variants and the burden of variants of uncertain significance (VUS) remains a significant issue
- The ENIGMA Expert Panel has been operating as a ClinGen External Expert Panel since 2015, submitting 7456 variant classifications to the ClinVar database
- Updates to the ClinGen Expert Panel process and wide adoption of the ACMG/AMP criteria[1] has led to the need to update the classification criteria and processes of the ENIGMA Expert Panel

METHODS

- ENIGMA EP was expanded to include additional experts, primarily from diagnostic laboratories in the USA
- Monthly meetings were held to discuss conversion of existing classification criteria to ACMG/AMP codes
- The ClinGen Sequence Variant Interpretation (SVI) group leadership was consulted multiple times by email and through meetings about existing classification data types not captured in the original ACMG/AMP guidelines[1], and need to align with existing clinical practice.
- Calibration of evidence types was performed using Likelihood Ratio (LR) based methods[2], and LRs converted to evidence weights as proposed by Tavtigian et al[3]
- Pilot specifications were tested on 40 variants, selected to cover different variant types and classifications
- Specifications were updated based on biocurator feedback to improve usability, and re-tested

RESULTS

- After aligning evidence types documented in the external expert panel rules with the baseline ACMG/AMP criteria, 8 codes had weights informed by statistical analysis, use of 7 codes was extended or repurposed, and 11 codes were deemed not applicable or overlapping
- After applying defined LR ranges, continuous outputs from statistical models were weighted from supporting to very strong evidence (e.g. cosegregation)
- Bioinformatic predictions did not add information to missense variants outside of critical functional domains

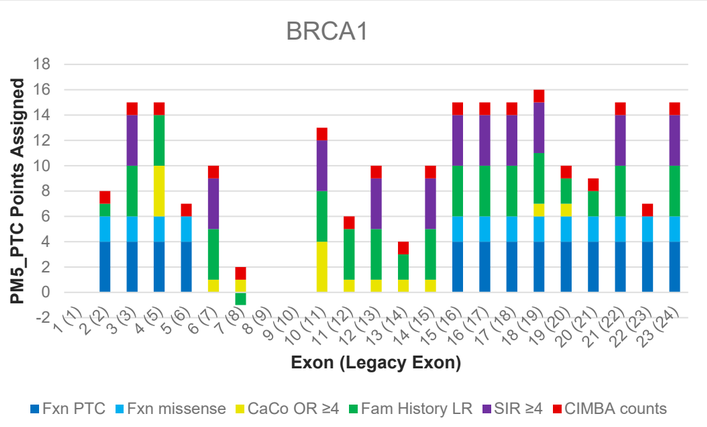

FIGURE 1: PM5_PTC Code Weights Applicable per Exon

- It was noted that, although protein termination codon (PTC) BRCA1/2 variants are classically treated as pathogenic, using baseline ACMG/AMP criteria would downgrade many PTC - inconsistent with clinical practice
- In consultation with the SVI, the PM5 code was repurposed to provide additional evidence for PTC variants under the rationale that PTC variants in the same exon are likely to have the same molecular effect (PM5_PTC)
- Evidence to support PM5_PTC exon-specific weights was derived from functional assays, case-control studies, family history likelihood ratio models, presence in highly selected BRCA families from CIMBA (Figure 1)
- This approach highlighted a previously known exception documented in the ENIGMA external expert panel rules, which is that PTC variants in exons 8/9 (formerly 9/10) require additional information before classification
- A searchable excel table was created to accompany the BRCA1/2 VCEP Specifications to aid in application of PVS1 and PM5_PTC codes
- 40 variants were chosen for the pilot phase, spread across various variant types (e.g. PTC, splicing, missense, silent, intronic)
- 14 variants were VUS or conflicting classifications in ClinVar
- Biocurator feedback highlighted areas for clarification in the documentation, including interpretation of mRNA splicing data
- A second pilot phase using the updated Specifications showed improved concordance between biocurators
- Use of BRCA1/2 VCEP specifications maintained or improved classification for 35/40 variants (5 remained VUS) (Figure 2)

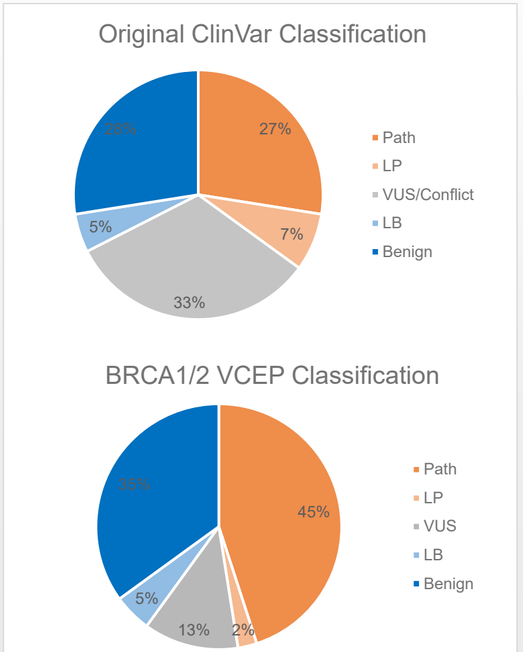

FIGURE 2: PILOT VARIANT CLASSIFICATIONS

DISCUSSION

- Alignment with ACMG/AMP criteria plus gene-specific knowledge results resulted in improved classification relative to original ClinVar classification
- Calibration of evidence types using statistical approaches was key to justify acceptance (or rejection) of the utility of different ACMG/AMP evidence codes for classification
- Calibration of criteria aided in specifying appropriate weights to be applied for evidence codes
- The multi-stage pilot phase helped to improve usability of specifications and improve consistency between biocurators
- Assessment of mRNA splicing data remains challenging

FUTURE DIRECTIONS

- On-going curation of *BRCA1* and *BRCA2* variants, initially focusing on re-classification of previous ENIGMA EP submissions and ClinVar discrepancies
- Improvements to specifications, informed by ENIGMA research

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