A Framework for Prioritizing Germline Variants of Uncertain Significance Using Integrated Computational Evidence in Hereditary Breast Cancer

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COHORT	GENES	VARIANT TYPE	CONSENSUS	STRICT SUPPORT
113 patients • 143 unique VUS	33 genes • BRCA2: 32 VUS	127/143 missense • 88.8%	30 high • 58 intermediate • 55 low	17 VUS • 7 with 6/6 agreement

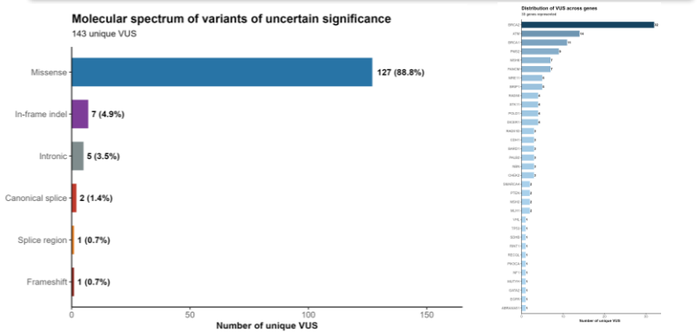
BACKGROUND & AIM

Background: Germline variants of uncertain significance (VUS) remain a challenge in hereditary breast cancer testing, as they cannot directly guide clinical management. Resolving their significance requires multiple lines of evidence, including computational predictions recognized within the ACMG/AMP framework. Integrating in-silico predictors may help prioritize VUS for further functional and clinical investigation, although computational agreement alone does not establish pathogenicity.
Aim: To characterize germline VUS in patients with breast cancer, assess concordance among computational predictors, develop a consensus-based prioritization framework, and examine variant distributions across genes and DNA-repair pathways.

METHODS

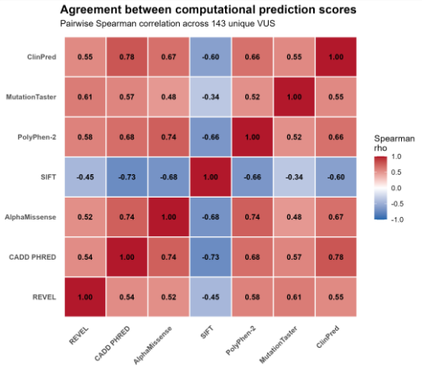
Germline VUS from breast cancer patients were analyzed using one preferred transcript per variant. Six predictors were thresholded: REVEL ≥0.50, CADD ≥20, AlphaMissense ≥0.564, PolyPhen-2 ≥0.85, SIFT ≤0.05, and ClinPred ≥0.50. Consensus was classified as high (≥4 damaging calls from ≥4 available tools), low (≤1 from ≥4 tools), or intermediate/discordant (all others). Missing scores were excluded; strict prioritization required ≥5 damaging calls from ≥5 available tools. MutationTaster contributed to concordance analysis but not consensus classification. Computational evidence was interpreted within ACMG/AMP PP3/BP4 without clinical reclassification. Concordance was assessed using Spearman correlation and categorical agreement; gene/pathway comparisons used non-parametric tests with Benjamini–Hochberg correction.

VUS LANDSCAPE



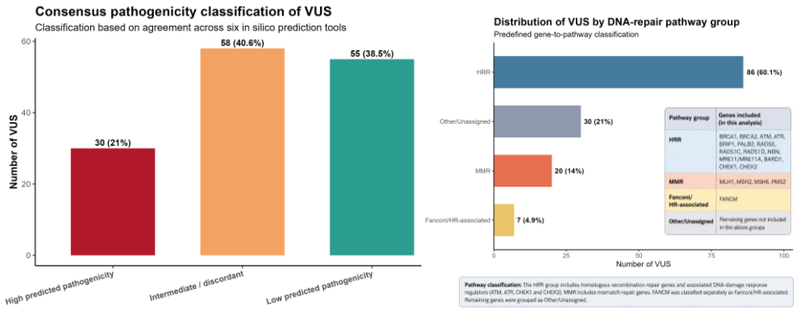
Among 143 unique VUS across 33 genes, 127 (88.8%) were missense. BRCA2 was most represented (32 VUS), followed by ATM (14) and BRCA1 (11). Most patients carried one VUS (73/113, 64.6%); PMS2 c.880C>T, FANCM c.242C>T and BRIP1 c.3431A>G each occurred in three patients.

PREDICTOR CONCORDANCE



Strongest positive correlations: CADD–ClinPred (ρ=0.78) and AlphaMissense–PolyPhen-2 (ρ=0.74). Categorical agreement reached 82.4% and 81.6%, respectively for AlphaMissense–PolyPhen-2 and CADD–SIFT.

CONSENSUS & PATHWAY RESULTS



Consensus classified 30/143 VUS (21.0%) as high predicted pathogenicity, 58 (40.6%) as intermediate/discordant and 55 (38.5%) as low. All 18 strictly prioritized VUS (≥5 damaging predictions among ≥5 available tools) belonged to the high-consensus group; 17 are displayed after excluding GATA2. HRR genes accounted for 86/143 VUS (60.1%); prediction scores and consensus classifications did not differ significantly between HRR and MMR.

STRICT COMPUTATIONALLY SUPPORTED VUS

Computationally prioritized germline VUS

Original six-predictor consensus | GATA2 excluded from focused display

Gene	Original transcript	HGVS c.	HGVS p.	Agreement
ABRAXAS1	ENST00000321945.12	c.308G>A	p.Arg103His	6/6
BARD1	ENST00000260947.9	c.1418A>C	p.His473Pro	6/6
BRCA2	ENST00000380152.8	c.8357C>A	p.Ala2786Asp	6/6
BRIP1	ENST00000259008.7	c.1048T>G	p.Cys350Gly	6/6
BRIP1	ENST00000259008.7	c.2119C>T	p.Arg707Cys	6/6
FANCM	ENST00000267430.10	c.1619T>C	p.Leu540Pro	6/6
STK11	ENST00000326873.12	c.157G>A	p.Asp53Asn	6/6
BRCA1	ENST00000357654.9	c.193A>G	p.Lys65Glu	5/6
BRCA2	ENST00000380152.8	c.7547C>T	p.Ser2516Phe	5/6
BRCA2	ENST00000380152.8	c.8345G>C	p.Ser2782Thr	5/6
BRCA2	ENST00000380152.8	c.93G>C	p.Trp31Cys	5/6
CHEK2	ENST00000404276.6	c.851G>A	p.Cys284Tyr	5/6
CHEK2	ENST00000404276.6	c.937G>A	p.Val313Met	5/6
POLD1	ENST00000440232.7	c.2636C>G	p.Ser879Cys	5/6
STK11	ENST00000326873.12	c.297T>G	p.Ile99Met	5/6
ATM	ENST00000675843.1	c.1009C>A	p.Arg337Ser	5/5
ATM	ENST00000675843.1	c.590G>A	p.Gly197Glu	5/5

Agreement = damaging predictions / available predictor scores. Two ATM variants have 5/5 because one score is missing.
Computational prioritization does not establish pathogenicity.

17 VUS had ≥5 damaging predictions among ≥5 available tools; 7 showed complete 6/6 predictor agreement. Two variants showed 5/5 agreement due to one unavailable predictor score. Gene and protein annotations are shown alongside.

CONCLUSION

Integrating multiple in-silico prediction tools enabled systematic characterization and prioritization of germline VUS in breast cancer-associated genes. Predictor concordance highlighted candidate variants warranting further functional and clinical investigation. This consensus-based approach may support evidence generation for improved hereditary breast cancer variant interpretation; however, computational predictions alone are insufficient for clinical reclassification under ACMG/AMP guidelines.