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Reclassification of Variants of Uncertain Significance in BRCA1, BRCA2, and PALB2: Implications for Clinical Decision-Making in Breast Cancer

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Abstract

Background

Germline pathogenic variants in high-penetrance breast cancer susceptibility genes, including BRCA1, BRCA2, and PALB2, play a pivotal role in risk assessment, therapeutic decision-making, and preventive strategies. The reclassification of VUS through standardized frameworks and emerging evidence is essential to enhance their clinical utility.

Materials and Methods

We retrospectively analyzed 6,369 breast cancer patients who underwent 52-gene panel testing (2020–2024). VUS in BRCA1, BRCA2, and PALB2 were re-evaluated using ACMG/AMP and ClinGen criteria, based on public databases, literature, and internal data.

Results

Among 4,215 patients with VUS, 436 variants were identified in the three genes (BRCA1: 105; BRCA2: 257; PALB2: 74). Overall, 13 variants (3%) were reclassified: 3 (0.69%) as likely pathogenic and 10 (2.3%) as likely benign.

Conclusion

Although VUS reclassification rates are low, their clinical impact is substantial, influencing therapeutic decisions and reducing uncertainty. These findings support the need for ongoing variant reinterpretation in precision oncology.

Background

High-penetrance susceptibility genes such as *BRCA1*, *BRCA2*, and *PALB2* are central to the clinical and surgical management of breast cancer patients. Pathogenic/Likely Pathogenic (P/LP) variants in these genes confer a markedly increased lifetime risk of breast and ovarian cancer and are therefore critical for risk stratification, patient counseling, and individualized management. In clinical practice, the identification of such variants directly informs decisions regarding intensified surveillance strategies, the use of targeted systemic therapies such as PARP inhibitors, and the consideration of risk-reducing surgical interventions, including prophylactic mastectomy or salpingo-oophorectomy[1,2]. Moreover, the presence of pathogenic or likely pathogenic variants guides therapeutic planning and follow-up, underscoring the importance of accurate genetic testing in optimizing both oncologic outcomes and preventive strategies.

However, the increasing use of next-generation sequencing has led to a growing number of detected variants of uncertain significance (VUS), which represent a major challenge in clinical genetics. By definition, VUS lack sufficient evidence to determine their association with disease risk, thereby limiting

their clinical utility and complicating patient management[2,3]. Importantly, individuals harboring VUS are generally managed as non-carriers of pathogenic variants and are not eligible for targeted therapies or risk-reducing interventions based solely on these findings, which may lead to uncertainty in clinical decision-making[3]. In this context, the reclassification of VUS through the integration of updated population data, functional studies, standardized interpretation frameworks and constant evaluation of ACMG/AMP and Clingen specific criteria is of paramount importance. Accumulating evidence indicates that a proportion of VUS can be reclassified over time, with significant implications for treatment planning, surgical decision-making, and genetic counseling, ultimately improving the precision of patient care[1,4,5,6].

Materials and Methods

In the current study, 6369 patients with breast cancer were evaluated, after being referred to Genekor's laboratory for NGS based genetic testing in 52 genes, including *BRCA1, BRCA2*, and *PALB2*. Before undergoing molecular genetic testing, they all provided an informed consent process, granting permission for the anonymized utilization of their genetic data in scientific research and potential publication of research findings. Retrospectively, ClinGen expert panel–specified variant classification guidelines for *BRCA1, BRCA2*, and *PALB2* genes were applied to variants of uncertain significance (VUS) identified in patients, during the period from January 1, 2020, to December 31, 2024. The data were derived from VCEP documentation, unpublished internal data, publicly available databases, and international scientific literature.

Results

A total of 6369 patients diagnosed with breast cancer underwent hereditary cancer predisposition testing using a 52-gene next-generation sequencing (NGS) panel that included the high-penetrance susceptibility genes *BRCA1, BRCA2*, and *PALB2*. Among the analyzed cohort, 4215 patients were characterized as non-negative for pathogenic or likely pathogenic variants but were found to harbor at least one variant of uncertain significance (VUS). Overall, 5563 VUS were identified across the entire multigene panel, illustrating the substantial burden of uncertain findings generated by contemporary high-throughput genomic testing in hereditary cancer diagnostics. Within the subset of high-risk breast cancer susceptibility genes, 436 VUS were detected in *BRCA1, BRCA2*, and *PALB2*. Specifically, 105 VUS were identified in *BRCA1*, 257 in *BRCA2*, and 74 in *PALB2*. *BRCA2* accounted for the highest proportion of uncertain variants identified in this cohort, representing approximately 59% of all VUS observed in the three high-risk genes, while *BRCA1* and *PALB2* represented 24% and 17.0%, respectively (Fig. 1).

Retrospective reinterpretation of these variants was performed using the ACMG/AMP classification framework in combination with ClinGen Variant Curation Expert Panel (VCEP)-specified criteria for *BRCA1, BRCA2*, and *PALB2*. Reassessment incorporated updated population frequency data, publicly available databases, functional evidence, segregation data, published literature, and internal laboratory observations collected during the study period from January 1, 2020, through December 31, 2024.

Application of these reinterpretation criteria resulted in the reclassification of 13 out of 436 VUS (3.0%) identified in *BRCA1, BRCA2*, and *PALB2*. Among the reclassified variants, 3 variants (0.69% of all VUS) were upgraded to likely pathogenic (LP), whereas 10 variants (2.3%) were downgraded to likely benign (LB). The observed predominance of downgrading over upgrading is in agreement with previous studies demonstrating that the majority of reclassified VUS ultimately prove to be non-pathogenic after additional evidence accumulates. Of the three variants upgraded to likely pathogenic, two were identified in *BRCA1* and one in *BRCA2*. The proportion of upgraded variants within each gene corresponded to 1.9% of *BRCA1* VUS and 0.39% of *BRCA2* VUS. No *PALB2* variants were upgraded to likely pathogenic during the study period. Conversely, among the variants downgraded to likely benign, six were identified in *BRCA1*, three in *BRCA2*, and one in *PALB2*. Therefore, likely benign reclassifications accounted for 5.7% of *BRCA1* VUS, 1.17% of *BRCA2* VUS, and 1.35% of *PALB2* VUS (Fig. 2).

Discussion

The clinical management of breast cancer has been significantly transformed by the identification of germline variants in high-penetrance susceptibility genes such as *BRCA1, BRCA2*, and *PALB2*, which directly inform risk assessment, surveillance strategies, and therapeutic decision-making. However, the increasing use of multigene panel testing has led to a substantial rise in the detection of variants of uncertain significance (VUS), posing a persistent challenge in clinical interpretation. In the present cohort, VUS were identified in a considerable proportion of patients, with 436 variants detected specifically in the 3 high-risk genes, highlighting the burden of uncertainty associated with genetic testing in hereditary breast cancer. Although most VUS remain unresolved at the time of initial reporting, their presence complicates patient counseling and may lead to ambiguity in clinical management, as current guidelines recommend that VUS should not be used to guide medical or surgical decisions[7].

In this context, the reclassification of VUS represents a critical process for improving the clinical utility of genetic testing. In our study, 3% of VUS in *BRCA1, BRCA2*, and *PALB2* were reclassified following the application of ACMG/AMP and ClinGen gene-specific criteria. While this proportion may appear modest, it is consistent with previous reports indicating that a subset of VUS can be resolved over time as additional evidence accumulates[8]. Importantly, reclassification has direct and meaningful clinical implications. Variants reclassified as likely pathogenic may render patients eligible for targeted therapeutic strategies, such as PARP inhibitor treatment, and may influence decisions regarding risk-reducing surgical interventions and intensified screening protocols[9]. In our cohort, the identification of three variants reclassified as likely pathogenic underscores the potential for reanalysis to uncover clinically actionable findings that were previously unrecognized.

Conversely, the reclassification of VUS to likely benign has equally important implications. In this study, the majority of reclassified variants were downgraded to likely benign, thereby reducing uncertainty for affected individuals. This has significant psychosocial and clinical benefits, as patients carrying VUS often experience anxiety related to a possible hereditary cancer predisposition and may overestimate their cancer risk. The reclassification of such variants enables more accurate risk communication and

may prevent unnecessary interventions or intensified surveillance. Furthermore, inappropriate clinical management based on unresolved VUS has been reported in some settings, reinforcing the importance of continuous variant reinterpretation to avoid overtreatment[10].

The relatively low reclassification rate observed in this cohort may reflect both the inherent complexity of variant interpretation and the limited availability of functional and population-level data for rare variants. Nevertheless, the application of standardized frameworks, such as the ACMG/AMP guidelines and ClinGen expert panel specifications, provides a robust and reproducible approach to variant classification. As such, the reclassification of VUS in high-risk breast cancer susceptibility genes is essential for bridging the gap between genetic findings and clinical action.

The dynamic nature of variant interpretation highlights the importance of longitudinal genetic counseling and patient follow-up. Patients should be informed that VUS classifications may change over time as additional clinical, functional, and population-based evidence becomes available. Establishing clear recontact policies and encouraging periodic reassessment of genetic findings are therefore critical components of modern hereditary cancer care. Reclassification to likely pathogenic status may alter eligibility for targeted therapies, intensified screening, or prophylactic interventions, while downgrading to likely benign may substantially reduce psychological distress and prevent unnecessary medical procedures. In conclusion, the reclassification of VUS in high-risk breast cancer susceptibility genes is essential for bridging the gap between genetic findings and clinical action. Although only a minority of variants may be reclassified in the short term, the clinical significance of these changes is substantial, enabling personalized treatment and management for some patients while relieving others from the uncertainty and psychological burden associated with hereditary cancer risk. These findings reinforce the importance of periodic re-evaluation of genetic test results, multidisciplinary collaboration between molecular laboratories and genetic counseling services, and the integration of evolving genomic evidence into precision oncology practice.

Declarations

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Author Contribution

Conceptualization, A.M., D. B., ; methodology, A.M., D. B; software,A.M., D. B; validation, A.M., D. B; formal analysis, A.M., D. B; investi-gation, ALL AUTHORS; resources, ALL AUTHORS; data curation, A.M., D. B; writing—original draft preparation, A.M.; writing—review and editing,D.B., V.V..; visualization, A.M., D. B;

supervision, V.V.; project administration, A.M., D. B. All authors have read and agreed to the published version of the manuscript.

Data availability:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics declaration: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of EXEM (Hellenic Society of Breast Surgery), Protocol No: 00115, approval date is 6 April 2026..

Consent to participate: All examinees provided an informed consent, granting permission for the anonymized utilization of their genetic data in scientific research and potential publication of research findings.

Consent to Publish: All examinees provided an informed consent, granting permission for the anonymized utilization of their genetic data in scientific research and potential publication of research findings.

Accordance: In accordance with the Helsinki Declaration

Clinical trial number: not applicable

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Figures

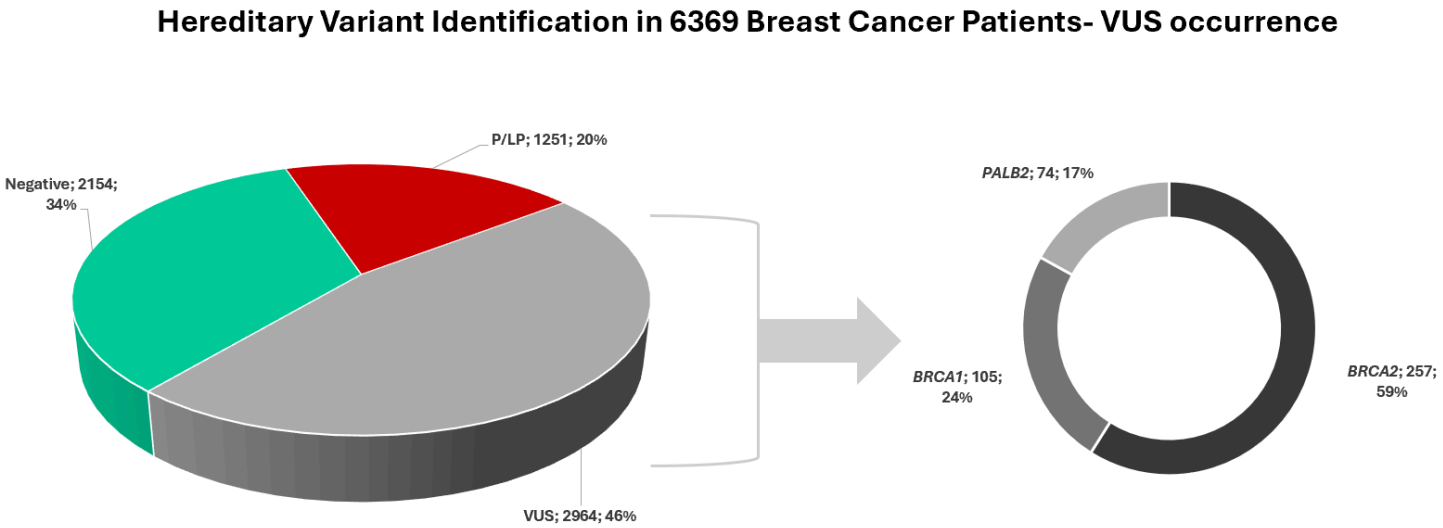


Figure 1

Identification of VUS variants in 6369 breast cancer patients. In a total of 4215 positive samples, 5563 VUS were identified in a 52-gene NGS panel analysed. 436 VUS were identified in high-risk genes *BRCA1, BRCA2* and *PALB2*.

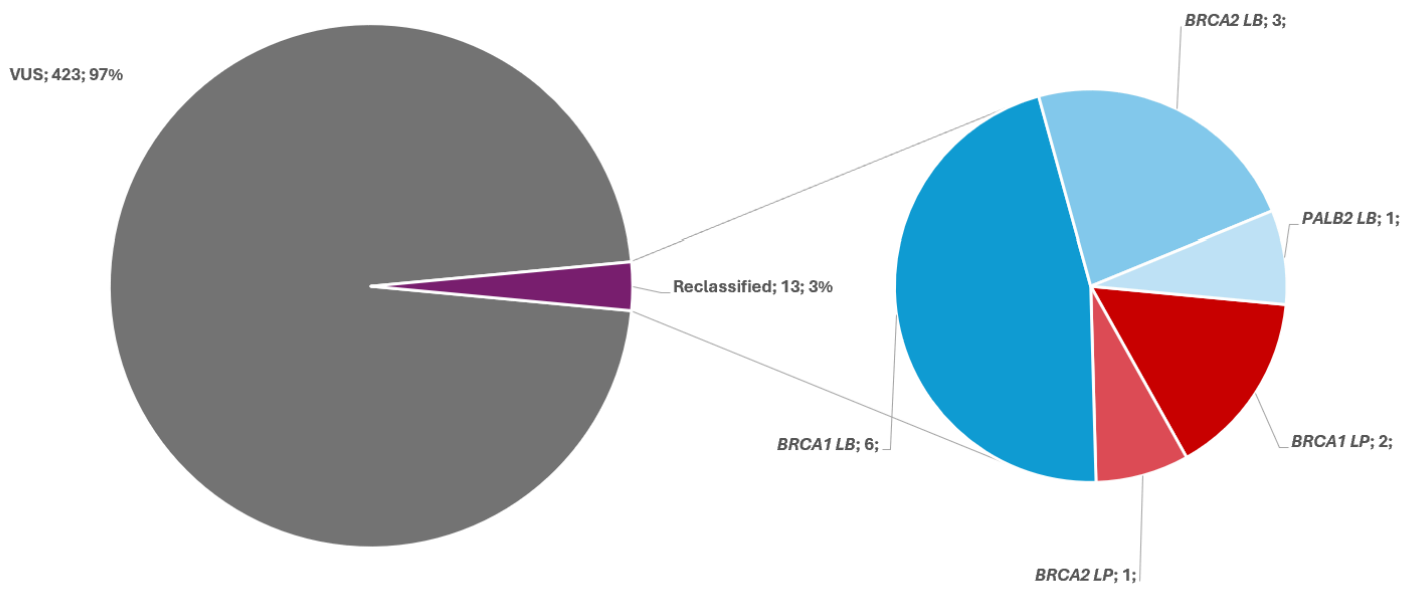


Figure 2

VUS reclassification in 3 high-risk genes. In total, 3 variants were reclassified to Likely Pathogenic (LP) in *BRCA1* and *BRCA2* genes, while 10 were reclassified to Likely Bening in *BRCA1, BRCA2* and *PALB2* genes.