Original Article

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Reclassification of *BRCA1* and *BRCA2* Variants of Unknown Significance in a Turkish Cohort; A Single-Center, Retrospective Study

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ABSTRACT

Objective: Accurate classification of *breast cancer susceptibility gene (BRCA)1/2* variants is important to delineate candidates for surgical or medical treatment. We retrospectively analyzed *BRCA1/BRCA2* sequencing data and reclassified the *BRCA1/2* variants of unknown significance (VUS) in Turkish patients with breast, ovarian, pancreatic and prostate cancers.

Materials and Methods: *BRCA1/BRCA2* sequence data of a large cohort were retrospectively analyzed. The sequencing data were reinterpreted in the context of American College of Medical Genetics guidelines, the Evidence-based Network for the Interpretation of Germline Mutant Alleles *BRCA1/2* classification rules, and current public genomic databases.

Results: Among the total of 2,713 patients, 254 (9.36%) had *BRCA1* or *BRCA2* variants. A total of 264 *BRCA1/BRCA2* variants were detected. Of these, 130 (49.2%) were pathogenic variants (PV), 24 (9%) were likely pathogenic (LP) and 110 of 264 variants (41.6%) were VUS. For the 119 *BRCA1* variants, 68% (*n* = 81) were PV, 7.5% (*n* = 9) were LP, and 24.5% (*n* = 29) were VUS. Similarly, for the 145 *BRCA2* variants, 33.7% (*n* = 49) were PV, 10.3% (*n* = 15) were LP, and 55.8% (*n* = 81) were VUS. Reanalysis of the 110 *BRCA1+BRCA2* VUS variants led to 22 (20%) being reclassified. Of these 22, 45.4% (*n* = 10) were reclassified as P/LP and 54.6% (*n* = 12) were reclassified as benign/likely benign.

Conclusion: These results show that it may be possible to reclassify VUS, in this case *BRCA1/2* VUS, in light of changing genetic data. These results demonstrate the importance of VUS reclassification of *BRCA1/2* variants in clinical management, surgical decisions, risk counseling and screening.

Keywords: *BRCA1* and *BRCA2* genes; breast cancer screening; breast conserving surgery; PARP inhibitors; sequencing

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Key Points

- *Breast cancer susceptibility gene (BRCA)1/BRCA2* sequence data was retrospectively analysed to reclassify *BRCA1/2* variants of unknown significance (VUS) in Turkish patients with breast, ovarian, and prostate cancers for improved clinical decision-making.
- Retrospective analysis of *BRCA1/2* sequencing data from 2,713 patients using American College of Medical Genetics guidelines, Evidence-based Network for the Interpretation of Germline Mutant Alleles rules, and public genomic databases.
- VUS reclassification is crucial for accurate *BRCA1/2* variant interpretation, impacting treatment, surgical planning, and genetic counseling.

Introduction

Breast cancer susceptibility gene 1 (*BRCA1*) and *breast cancer susceptibility gene 2* (*BRCA2*) are tumor suppressor genes that are involved in DNA repair, cell cycle regulation, and genome stability (1). Germline *BRCA1/2* gene mutations are associated with an increased risk of breast, ovarian, prostate, and several other cancers. *BRCA1/2* sequencing is increasingly being used to determine the therapeutic options, both preventive surgery in breast and ovarian cancers and medical treatments with poly (ADP) ribose polymerase inhibitors (PARPi) in breast, ovarian, prostate, and pancreatic cancers (1-3). Germline *BRCA1/2* variants may be classified into "pathogenic (P)", "likely pathogenic (LP)", "variant of unknown clinical significance (VUS)", "likely benign (LB)", or "benign (B)" (4, 5). Cases with P and LP variants will benefit from targeted treatment (surgery or chemotherapy) (1, 2). However, patients with B, LB, and VUS variants should have their treatment plans organized similarly to those who do not have P or LP variants. Diagnosing *BRCA1/2* PV guides the planning of effective surgery and chemotherapy, patient follow-up, and the consideration of prophylactic surgical options for asymptomatic

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individuals (2, 6). With the increased use of targeted therapy with PARPi in the treatment of *BRCA*-positive cancers, the accurate classification of *BRCA* gene variants guides treatment planning. Over the years, the improvement in next-generation sequencing (NGS) technology has led to an increase in the use of *BRCA1/2* testing and, consequently, reports of VUS variants. With the expanded use of NGS and *BRCA1/2* testing, the detection of VUS has become increasingly frequent. *BRCA1/2* VUS are a significant challenge for molecular genetic testing in specific breast, prostate, and ovarian cancers (7, 8). The rate of detection for VUS of *BRCA* was reported as 10–20% in women who were tested for *BRCA* variants (9).

Several guidelines and bioinformatic tools have been used to diminish the challenges in classifying *BRCA* variants (4, 5). Multiple guidelines and bioinformatic tools have been developed to address the challenges of variant interpretation. Among these guidelines, the American College of Medical Genetics and Genomics (ACMG) guidelines [the Association for Molecular Pathology (AMP)/ACMG 2015] are widely recognized as a reliable variant interpretation system (5). However, due to gene-specific complexities, more tailored approaches have been required to eliminate uncertainties. For this purpose, the current guideline provides detailed, *BRCA*-specific instructions to support variant curation and address discrepancies and uncertainties in variant classification. Variant classification of *BRCA1* and *BRCA2* genes depends on the current Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium classification (5).

In this retrospective study, previously reported *BRCA1* and *BRCA2* VUS were reinterpreted according to the 2015 ACMG guidelines, the ENIGMA *BRCA1/2* classification rules, and current public genomic databases. This study aimed to investigate changes in *BRCA* variant classification over time and thus highlight the importance of reanalyzing VUS variants in light of changing genetic data for clinical decision-making and patient management.

Materials and Methods

Study Population

Sequence data (January 2018 to August 2023) of a total of 2,713 patients with breast and ovarian cancer, prostate cancer, or pancreatic tumors who were referred to the "Mikrogen Genetic Diagnosis Center" for *BRCA1/BRCA2* sequencing were retrospectively analyzed (Figure 1). This study was approved by the Yüksek İhtisas University Medical School Ethical Committee (approval number: 296, date: 14.04.2025).

BRCA1/2 Sequencing

QIAamp DNA Blood Kit (QIAGEN, Aarhus, Denmark) was used to isolate DNA from blood samples. NGS of *BRCA1* and *BRCA2* genes was performed on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA, USA) using primers covering exon/exon-intron junctions in the *BRCA1/BRCA2* genes with the Qiaseq targeted DNA panel (DHS-102Z-96) according to the manufacturer's instructions. NGS achieved a minimum 20x read depth for >98% of targeted bases. The human genome Hg19 sequence was used as a reference to identify genetic variants. FASTQ, BAM, and VCF files were obtained. The bioinformatic analysis of VCF files was performed using NextGene (SoftGenetics, State College, PA, USA), Geneticist Assistant (SoftGenetics), and Franklin Genoox (Genoox, Israel). Variant annotation and filtering were conducted using a comprehensive set of public databases, including ClinVar, the Human Gene Mutation Database, and dbSNP, as well as population frequency datasets such as the Exome Aggregation Consortium, Genome Aggregation Database (gnomAD), Turkish Variome and the 1000 Genomes Project. Functional predictions were assessed using *in silico* tools, including PolyPhen-2, SIFT, MutationAssessor, and SpliceAI (Figure 2).

Variant Classification

All the *BRCA1/2* variants were reclassified in the context of the specific ACMG/AMP guideline for *BRCA1/2* variant classification (6, 7). This guideline uses ACMG/AMP variant classification criteria and contains additional specific updates for variant interpretation of *BRCA1/2* genes. Variant classification of *BRCA1/2* gene variants was made according to two main criteria. PV are weighted as very strong, strong, moderate, or supporting (PVS1, PS1–4, PM1–6, PP1–5), and benign variants are defined as standalone, strong, or supporting (BA1, BS1–4, BP1–6). The detected variants were classified as "P", "LP", "VUS", "LB", or "B" according to specific ACMG/AMP guidelines for *BRCA1/2* variant classification criteria.

Statistical Analysis

Chi-square tests were used to compare reclassification patterns between *BRCA1* and *BRCA2*. We assessed whether the overall reclassification rates and the distribution of reclassification types (P/LP vs. B/LB)

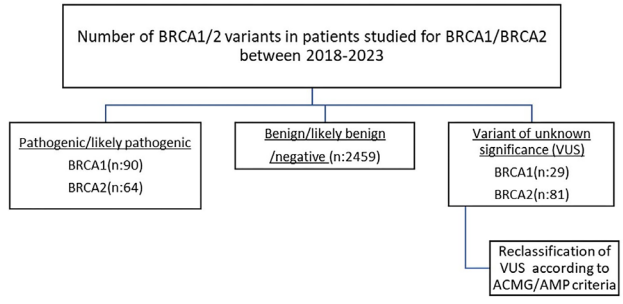

Figure 1. The number of reported patients and the VUS reclassification flow chart

VUS: Variant of unknown significance; ACMG: American College of Medical Genetics; AMP: Association for Molecular Pathology; *BRCA: Breast cancer susceptibility gene*

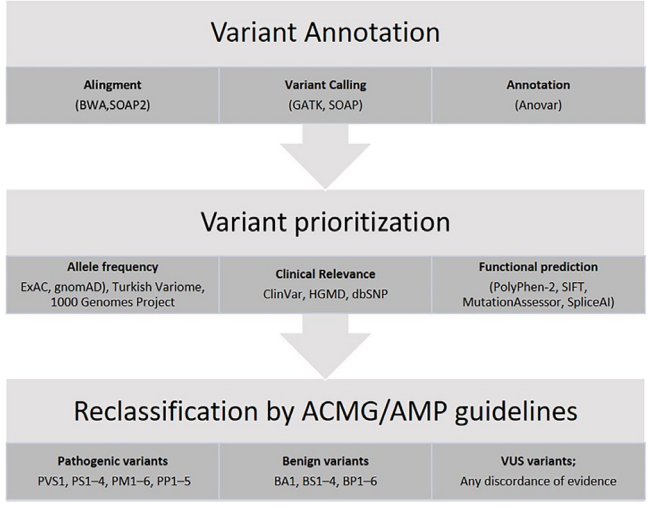

Figure 2. *BRCA1/BRCA2* variant reclassification workflow

ACMG: American College of Medical Genetics; AMP: Association for Molecular Pathology; *BRCA: Breast cancer susceptibility gene;* HGMD: Human Gene Mutation Database; ExAC: Exome Aggregation Consortium; gnomAD: Genome Aggregation Database

differed significantly between the two genes. All statistical tests were analyzed with a significance level of $p<0.05$. Analyses were conducted using SPSS for Windows, version 29.0 (IBM Corp., Armonk, NY, USA).

Results

The *BRCA1/BRCA2* sequence data of 2,713 patients were retrospectively analyzed regardless of cancer type. Among these, 254 (9.36%) harbored a total of 264 had *BRCA1* or *BRCA2* variants (Figure 1). On the initial analysis, 5.7% (154/2713) with *BRCA1* or *BRCA2* variants had P and LP variants, while 3.7% (100/2713) had VUS variants. The majority of the 2,713 patients (90.6%; *n* = 2459) had no variant or only B/LB variants.

Among the patients with *BRCA1/BRCA2* variants, 51.1% (130/254) PV, 9.4% (24/254) had LP, and 39.3% (100/254) VUS. Ten patients were carriers of multiple VUSs (two *BRCA2* VUS *n* = 6; two *BRCA1* VUS *n* = 3; and one patient had one *BRCA1* and one *BRCA2* VUS). For the 119 *BRCA1* variants, 68% (*n* = 81) were PV, 7.5% (*n* = 9) were LP, and 24.5% (*n* = 29) were VUS. Similarly, for the 145 *BRCA2* variants, 33.7% (*n* = 49) were PV, 10.3% (*n* = 15) were LP, and 55.8% (*n* = 81) were VUS. The type of variants were: 105 (39.7%) missense; 97 (36.7%) frameshift; 42 (15.9%) non-sense; 9 (3.4%) splice site; 5 (1.8%) intronic; 4 (1.5%) stop gain; and 2 (0.7%) in-frame deletions.

Reclassification of BRCA1/2 Variants

The 110 *BRCA1* and *BRCA2* VUS variants were reanalyzed, comprising 105 missense, two stop-gain, two in-frame deletions, and one intronic variant. In total, 22 (20%) were reclassified, with 40.9% (9/22) reclassified as P/LP, and 59.1% (13/22) as B/ LB. The *BRCA1* VUS rate dropped from 24.3% (29/119) to 17.6% (21/119). Among the reclassified variants, the status of 50% (4/8) of the *BRCA1* VUS variants changed to B or LB, and 50% (4/8) of them changed to P or LP. The frequencies of the specific *BRCA2* VUS changed from 56.2% (81/145) to 40.9% (59/144). The status of 64.2% (9/14) of *BRCA2* VUS variants changed to B or LB, and 35.7% (5/14) of them changed to P or LP. The VUS classification of 80% (88/110) of the *BRCA1/2* VUS did not change (Table 1).

The reclassification rate between *BRCA1* and *BRCA2* was not different (p = 0.358). Similarly, the distribution of reclassification types (P/LP vs. B/LB) did not differ significantly between the two genes (p = 0.838). We calculated a 95% Wilson score confidence interval based on 22 reclassified variants out of 110 to estimate the reclassification rate. The resulting confidence interval ranged 13.6% to 28.4%.

Discussion and Conclusion

Identifying PV in *BRCA1/2* is essential when managing breast, ovarian, and prostate cancers, particularly regarding follow-up and treatment selection (10). As *BRCA1/2* testing becomes increasingly integrated into routine clinical practice, the frequency of VUS findings has also risen. The increasing presence of VUS variants complicates patient counseling and clinical management, so genetic experts strongly recommend reclassification to resolve these uncertainties. Updates in databases, the development of bioinformatics solutions, and functional studies increase the likelihood of identifying the pathogenicity of VUS variants. The reclassification of VUS variants is important in genetic diagnosis and is recommended by genetic experts. Several previous studies have reported that the rates of *BRCA1/2* PV were about 6–15% (11, 12). Several studies have reported *BRCA1/2* variant prevalence in the Turkish population. Celik Demirbas et al. (13) found P *BRCA1* and *BRCA2* variants in 7.8% and 5.4% of 3,184 hereditary breast and ovarian cancer (HBOC) patients, respectively. Bahsi and Erdem (14) reported 9.4% P, 0.3% LP, and 6.4% VUS variants among 1,419 patients with HBOC. Boga et al. (15) found 9.9% P and 5.7% VUS rates in Turkish HBOC patients.

In our cohort, the initial detection rate of *BRCA1/2* PV was 5.4%, but after the reclassification of VUS variants, the rate of PV increased to 6%, which is similar to the results of Zang et al. (16) (6%). In the current study, the *BRCA1/2* VUS dropped from 3.6% to 2.8% after reclassification, which aligns with previous reports. Several studies have reported a range of VUS rates for *BRCA* variants (3.9–22.5%) (17-20), and our VUS rate is consistent with the previous studies. Zanti et al. (21) reported the VUS rate as 23.4% for *BRCA1* and *BRCA2* genes in a case-control evidence study from 11,227 *BRCA1* and *BRCA2* variants (2025) in 96,691 female breast cancer cases and 303,925 healthy controls. VUS rates have ranged from study to study due to the types of cancers included in the study, the size of the cohort, and the bioinformatics tools and databases used during the years the study was reported. The majority of the previous studies have been reported from breast cancer cases. Our cohort consisted mainly of breast cancer patients but also included ovarian and prostate cancer patients.

The current study's rate of VUS reclassification (20%) is very similar to previous reports [Mighton et al. (18), 14.7%; Benet-Pagès et al. (22), 20%; Innella et al. (23), (20%). Our rate of VUS reclassification is higher than the rates reported by both Mersch et al. (24) (7.7%) and Macklin et al. (25) (11.3%). Several studies reported that most of the reclassified VUSs were downgraded, which is similar to our results; however, the frequency of VUSs reclassified to P is nearly the same as the frequency of VUSs reclassified to benign in our cohort (17, 18, 22, 23). There is a lack of sufficient studies on the reclassification of *BRCA1* and *BRCA2* VUS variants in the Turkish population. Özdemir et al. (26) reclassified variants identified in 26 genes, including *BRCA1* and *BRCA2*, in a cohort of 137 cancer patients. In this study, 33.6% of the variants initially classified as VUS were downgraded, while 20.83% were upgraded. However, the results are not specific to the reclassification of *BRCA1* and *BRCA2* VUS variants; they also include the reclassification outcomes of VUS variants in the other 26 genes.

Genetic authorities recommend a periodic reassessment of VUS variants to avoid uncertainty in clinical decision-making. The periodic re-evaluation of VUSs is recommended in clinical practice; however, no specific time interval has been reported regarding how often reevaluation should be performed. The recommended time interval for periodic reassessment of VUS variants changes among several studies (23, 27). AMP guidelines suggest that reassessing VUS variants every two years may be enough to show the changes in classifications (27). Although guidelines recommend re-evaluating VUS variants every two years, this period may be shorter in cases of earlier than expected recurrence, metastasis, or aggressive tumour progression. Indeed, in our study, we identified cases whose classification changed in <3 years (Table 1). Mighton et al. (18) reported that two years is an ideal time interval for the reclassification of *BRCA1/2* VUS variants. Innella et al. (23) reported that the average time from the initial classification of VUS variants to reclassification was 49.4 months for *BRCA1/2* variants. Nevertheless, they recommended 3 years for periodic reassessment of VUS variants. The present study's mean duration between the initial VUS report and the first reclassification was 33.7 months.

Table 1. Reclassification of variants of unknown significance in *BRCA1/2* genes

Patient no	Gene	Variant/dbSNP number	Initial classification	Clinical significance (Clinvar)	Type of evidence	ACMG/AMP reclassification	Time between initial classification and first reclassification
1	*BRCA2* *NM_000059.4*	c.9857T>A rs398122624	VUS	LB	BP4, BP6	LB	13 months
2	*BRCA2*	c.632-4_632-3del rs431825341	VUS	VUS	PVS1, PM2	LP	Recent study
3	*BRCA2*	c.8249_8251delAGA rs80359703	VUS	VUS	PM1, PM2, PM4	LP	Recent study
4	*BRCA2*	c.1232T>C rs79597821	VUS	LB	BP1, BP3 BP4, PM2	LB	36 months
5	*BRCA2*	c.8452G>A rs80359094	VUS	LP	PM1, PP3, PM2, PP5	LB	38 months
6	*BRCA2*	c.10095delCinsGAATTATATCT rs276174803	VUS	LP	PVS1, PM2, BP6	LP	23 months
7	*BRCA2*	c.9934A>G rs80359254	VUS	LB	BP4, BP6, PM2	LB	48 months
8	*BRCA2*	c.516G>T rs80359790	VUS	LP	PP3, PP5, PM2	LP	38 months
9	*BRCA2*	c.8524C>T rs80359104	VUS	LP	PP3, PP5, PM2	LP	68 months
10	*BRCA2*	c.9257G>C rs574271678	VUS	LB	BS2, BP6, PM2	LB	65 months
11	*BRCA2*	c.6080G>A rs431825337	VUS	LB	BP4, BP6, PM2	LB	50 months
12	*BRCA2*	c.3318C>G rs129855035	VUS	LB	BP4, BP6, PM2	LB	28 months
13	*BRCA2*	c.1232T>C rs79597821	VUS	LB	BP6, PM2	LB	47 months
14	*BRCA2*	c.8452G>A rs80359094	VUS	LB	BP6, PM2, PM5	LB	33 months
15	*BRCA1*	c.4986+5G>A rs397509211	VUS	P	PS3, PS4, PM2	P	48 months
16	*BRCA1*	c.3082C>T rs80357049	VUS	B	BS3, BP5, BS2	B	54 months
17	*BRCA1*	c.5236C>A rs80357146	VUS	LP	PM1, PM2, PM5	LP	9 months
18	*BRCA1*	c.53T>A rs80356929	VUS	P	PS3, PS4, PM2, PM5	P	39 months
19	*BRCA1*	c.754C>T rs273902786	VUS	LB	BS3, BP6, PM2	LB	24 months
20	*BRCA1*	c.4418T>C rs374519494	VUS	LB	BP1, BP4, PM2	LB	12 months
21	*BRCA1*	c.1703C>T rs80356910	VUS	LB	BP6, PM2	LB	12 months
22	*BRCA1*	c.5321G>C rs397509246	VUS	P	PS3, PS4, PM2	P	58 months

ACMG: American College of Medical Genetics; AMP: Association for Molecular Pathology; VUS: Variant of unknown significance; P: Pathogenic; LP: Likely pathogenic; LB: Likely benign; B: Benign; PM: Pathogenic moderate evidence; BP: Benign supporting evidence; PS: Pathogenic strong evidence; BS: Benign Strong evidence

Two of the reclassified variants are still reported as VUS by ClinVar but based on updates to databases and guidelines in this study, these variants were reclassified as LP. Some potential P or PV may be classified as VUS, according to the ClinVar database. ClinVar provides a broad collection of data on genetic variants, and this data is based on reports from different laboratories and research studies, which can sometimes lead to conflicting classifications. For example, The *BRCA2* c.632-3_632-2del(rs431825341) variant was found in one female patient with breast cancer (invasive ductal carcinoma). It was previously classified as VUS in our cohort and is still reported as VUS in the ClinVar database. After reclassification, it is upgraded to LP according to *BRCA1/2*-specific ACMG/AMP criteria. The frequency of this variant is extremely low in all databases (gnomAD (Genome); 0.0007%, gnomAD (Exome); very rare, 1000 Genomes; no observation); therefore, it was assigned as PM2 according to population data. The effect of a variant on protein is defined as loss of function due to a null variant (intronic within ±2 of a splice site) in the gene *BRCA2*, and it is assigned to PVS1. The *BRCA2* c.8249_8251del(rs80359703) variant was found in one female patient with breast cancer. The frequency of this variant is also extremely low in all databases [gnomAD (Genome); 0.0007%, gnomAD (Exome); 0%, 1000 Genomes; no observation], so it was assigned as PM2 according to population data. Protein coding length changes because of an in-frame variant in gene *BRCA2,* so it was assigned as PM4.

Over time, as the data entered into databases has increased, and more information has accumulated, the VUS rate in *BRCA1* and *BRCA2* decreased from around 13% to 2%, but VUS rates for non-*BRCA* genes are still reported as higher (20–40%) (8). The current study's *BRCA1/2* VUS rate was 3.6% before reclassification, and after reclassification, the VUS rate was revised to 2.8%.

Genetic counseling of *BRCA* VUS variants is one of the critical problems in the clinical management of cancer patients. *BRCA* VUS diagnosis causes high anxiety in both cancer and non-cancer patients (7). Limited studies have reported about the impact of VUS variants in the clinical management of patients with *BRCA1/2* VUS variants (8, 28, 29). Culver et al. (28) reported similar mastectomy rates in patients with VUS variants and *BRCA*-negative patients, but *BRCA*-negative patients have higher anxiety compared to patients with VUS variants. Welsh et al. (8) reported that the patients with *BRCA1/2* VUS variants had higher prophylactic mastectomy rates compared to *BRCA*-negative and untested patients (33% *vs.* 25%). Still, rates are lower than those of patients with *BRCA* P mutations (33% *vs.* 83%). Morgan et al. (30) reported a similar rate of prophylactic mastectomy among breast cancer cases with P *BRCA1/2* and VUS variants. In Morgan's study, VUS cases without breast cancer did not choose prophylactic mastectomy. VUS results can be confusing for patients and physicians and can cause difficulties in providing accurate information to the patient. In a study conducted among breast cancer specialists, most physicians (71%) had difficulty interpreting VUS reports, and 39% stated that they did not know how to provide counseling (28). Therefore, due to the problems in counseling, interpretation of clinical effects, and planning of patient treatment in patients with VUS variants, re-evaluation of VUS variants is essential for accurate interpretation and access to clinical geneticists would also be helpful.

Study Limitations

The current study has some limitations. The result of the study presents a limited study population from one center and one ethnic group. However, the variant reclassification is made according to databases that mainly include European population data. There is no information about the presence of mutations in non-*BRCA* genes, and this is also another limitation of the study. Patients with VUS variants may have P/LP variants in non-*BRCA* genes. Based on some limitations of the current study, further studies are needed and should include a large study population and information about non-BRCA genes.

Improvements in bioinformatic tools and database updates may eventually diminish the reclassification rates. The current guidelines recommend reporting the *BRCA1/2* VUS variants; however, none recommend making clinical decisions according to VUS results. VUS reclassification will be necessary in the clinical management of the disease. Most VUS variants are downgraded after initial classification, preventing the patients from unnecessary surgery, therapy, and misdiagnosis. The upgraded VUS variants will facilitate surgical decisions, targeted therapy options, reproductive decision-making, and predictive at-risk family member screening.

In conclusion, this study demonstrates that the *BRCA1/2*-specific ACMG/AMP classification guidelines and current databases can be effectively used for VUS re-classification. VUS reclassification is important to avoid unnecessary treatment and to provide accurate risk management. This study supports the utility of *BRCA*-specific ACMG/AMP guidelines and updated genomic databases in improving the clinical utility of genetic testing.

Ethics

Ethics Committee Approval: This study was approved by the Yüksek İhtisas University Medical School Ethical Committee (approval number: 296, date: 14.04.2025).

Informed Consent: All participants provided their written informed consent before undergoing molecular analysis.

Footnotes

Authorship Contributions

Surgical and Medical Practices: L.Ö., S.A., E.Ü.; Concept: L.Ö., S.A., E.Ü.; Design: L.Ö.; Data Collection or Processing: L.Ö., S.A., E.Ü.; Analysis or Interpretation: L.Ö., S.A., E.Ü.; Literature Search: L.Ö.; Writing: L.Ö., S.A., E.Ü.

Conflict of Interest: No conflict of interest was declared by the authors.

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