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Molecular Analysis of Exons 10, 11 Mutations in BRCA2 Gene In Tehran City Patients with Breast Cancer

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A B S T R A C T

Purpose: This study aimed to investigate genetic alterations in exons 1–5 of the RAD51 gene and to examine their potential association with hereditary breast cancer among women diagnosed with breast cancer in Tehran, Iran.

Methods and Materials: In this molecular study, blood samples were collected from 56 women with invasive or in situ breast cancer in Tehran. Participants were 31–75 years old, with a mean age of 53 years, while controls were selected from a comparable age range with a mean age of 49 years. Genomic DNA was extracted using the saturated-salt method. Target exons were amplified by polymerase chain reaction (PCR) using exon-specific primers, and PCR products were subsequently sequenced by Microsynth, Switzerland. Sequence data were extracted in FASTA format using Chromas Lite and compared with reference sequences using NCBI BLAST. Protein-level analyses were conducted using ExPASy and EBI resources, and predicted secondary and three-dimensional protein structures were evaluated using Phyre2.

Findings: Comparative sequence analysis demonstrated approximately 99% similarity between nucleotide sequences of exon 10 of BRCA2 and reference sequences reported from the United States and the United Kingdom. Exon 11 of BRCA2 showed approximately 97%–99% similarity with sequences reported from Hong Kong, whereas exon 1 of RAD51 demonstrated approximately 98%–99% similarity with reference sequences from the United States. Among the investigated genetic regions, alterations in exon 11 of BRCA2 showed the greatest observed frequency. Phylogenetic comparisons of BRCA2 exons 10 and 11 and RAD51 exon 1 indicated high genetic similarity between the studied Tehran breast-cancer samples and corresponding reference sequences from the United States, United Kingdom, and Hong Kong.

Conclusion: The findings indicate detectable genetic alterations in breast cancer-associated genes among women with breast cancer in Tehran, with particularly notable variation in BRCA2 exon 11. Molecular techniques incorporating PCR and DNA sequencing may contribute to identifying clinically relevant genetic changes and could support earlier molecular assessment of individuals at increased risk of hereditary breast cancer.

Keywords: *Breast cancer; RAD51; BRCA2; genetic mutation; exon; DNA sequencing; polymerase chain reaction*

1. Introduction

Breast cancer remains one of the most intensively investigated malignancies because of its high incidence, biological heterogeneity, variable clinical behavior, and substantial contribution to cancer-related morbidity and mortality among women worldwide. Although improvements in screening, molecular classification, surgery, radiotherapy, systemic therapy, and targeted treatment have markedly improved outcomes in many patient groups, breast cancer cannot be regarded as a single disease entity because its pathogenesis reflects a complex interaction among inherited susceptibility, somatic genetic alterations, hormonal influences, environmental exposures, metabolic factors, and defects in cellular DNA-damage response pathways. Increasing attention has therefore been directed toward identifying molecular determinants that predispose individuals to tumor development or influence disease progression. Among these determinants, germline and somatic alterations in genes responsible for maintenance of genomic integrity are of particular importance because disruption of DNA-repair systems can promote accumulation of mutations, chromosomal instability, malignant transformation, and therapeutic resistance. The growing availability of genomic technologies has demonstrated that inherited breast cancer susceptibility extends beyond a limited number of classical genes and encompasses multiple rare variants, copy-number alterations, regulatory defects, and functionally relevant variants in DNA-repair networks (Pal et al., 2024). Large-scale genomic investigations in understudied populations have further emphasized that the mutational spectrum of breast cancer may vary substantially across ethnic and geographic groups, thereby reinforcing the importance of population-specific molecular studies rather than exclusive reliance on data generated from Western populations (Ahmad et al., 2024). Similar observations from regional cancer-genomics studies indicate that characterization of population-specific genetic landscapes can improve risk assessment and contribute to the development of more individualized approaches to cancer prevention and treatment (Al-Eitan et al., 2025).

Among hereditary breast cancer genes, *BRCA1* and *BRCA2* occupy a central position because of their essential roles in repair of DNA double-strand breaks through homologous recombination. Pathogenic variants in these genes compromise high-fidelity DNA repair, increase genomic instability, and substantially elevate susceptibility to breast, ovarian, and several other cancers. The historical discovery of *BRCA1* and *BRCA2* transformed understanding of hereditary breast cancer and created a foundation for predictive testing, cascade screening, prophylactic strategies, and molecularly targeted therapy (Burachik et al., 2023). Over subsequent decades, genetic testing for inherited breast and ovarian cancer predisposition has evolved from focused mutation analysis toward broader sequencing-based approaches capable of identifying point mutations, insertions, deletions, rearrangements, and other clinically relevant variants (Barili et al., 2024). Research has also shown that the biological consequences of *BRCA1* and *BRCA2* dysfunction extend beyond tumor initiation. Defective DNA repair may facilitate metastatic progression, genomic diversification, and selection of more aggressive tumor cell populations, particularly when compensatory repair pathways become activated (Krishnan et al., 2021). Furthermore, the phenotypic consequences of *BRCA1/2* abnormalities may be modified by non-genetic factors such as obesity, endocrine signaling, inflammation, and metabolic dysregulation, illustrating the multifactorial nature of hereditary breast cancer expression (Giordano et al., 2025).

The *BRCA2* gene is particularly relevant to homologous recombination because its encoded protein directly regulates the recruitment and function of RAD51 at sites of DNA damage. BRCA2 contains multiple functional domains, including BRC repeats that bind RAD51 and facilitate nucleoprotein filament formation during homologous recombination. Experimental evidence indicates that highly conserved regions of BRCA2 can regulate the RAD51-binding activity of BRC repeats, thereby modulating the efficiency and fidelity of DNA repair (Zhu et al., 2023). Consequently, genetic changes affecting functionally important regions of *BRCA2* may impair RAD51 recruitment, destabilize replication forks, and increase vulnerability to mutagenic DNA lesions. Variations in different exons of *BRCA2* may have distinct functional consequences, and mutation location may influence both tumor biology and treatment response. Clinical investigations in BRCA-associated malignancies have suggested that the position of *BRCA1* or *BRCA2* mutations can be associated with differential therapeutic benefit, demonstrating that mutation localization may provide information beyond the simple presence or absence of a pathogenic variant (Labidi-Galy et al., 2023). Comparative studies have also identified exon-specific *BRCA2* variants associated with mammary tumors, reinforcing the possibility that particular coding regions may contain biologically

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important susceptibility loci (Gopal et al., 2022). In addition, genomic studies using exome sequencing have revealed rare recurrent copy-number variants and other hereditary alterations that may contribute to breast cancer susceptibility even when conventional high-penetrance mutations are absent (Kumpula et al., 2023).

The homologous recombination pathway operates through a coordinated network of proteins rather than through isolated activity of BRCA1 or BRCA2. RAD51 is a core recombinase within this pathway and is indispensable for accurate repair of DNA double-strand breaks. Following DNA-end resection, RAD51 is loaded onto single-stranded DNA to form a nucleoprotein filament that searches for homologous sequences and promotes strand invasion into an intact DNA template. This process is tightly controlled by BRCA2 and multiple RAD51 regulators, and disruption at any stage may produce homologous recombination deficiency. Variants affecting RAD51 itself or its regulatory proteins have therefore attracted increasing attention as potential modifiers of cancer susceptibility and therapeutic response. Research on RAD51-related proteins has shown that germline variants in members of the RAD51 network may confer susceptibility to breast and ovarian cancers characterized by homologous recombination deficiency (Setton et al., 2021). Similarly, meta-analytic evidence indicates that polymorphisms in homologous recombination repair genes, including *RAD51*, *XRCC2*, and *XRCC3*, can be associated with breast cancer risk, although effect sizes may differ among populations and variant types (Yu & Wang, 2023). A case-control study in South Indian women also demonstrated associations between polymorphisms in homologous recombination genes and susceptibility to breast cancer, supporting the relevance of ethnic and population-specific genetic assessment (Rajagopal et al., 2022).

The functional importance of RAD51 extends beyond cancer susceptibility to other human disorders characterized by impaired DNA repair or reproductive dysfunction. Studies on RAD51 regulators and genetic variants have implicated this molecular system in primary ovarian insufficiency, endometriosis, and polycystic ovary syndrome, indicating that disturbances in homologous recombination may affect multiple biological processes beyond tumorigenesis (Witham & Hengel, 2024). More broadly, DNA-repair gene polymorphisms have been proposed as biomarkers of individual susceptibility to genotoxic exposures, including environmentally induced DNA damage, further demonstrating the interaction between inherited repair capacity and external carcinogenic stressors (Kaur & Kaur, 2024). Such observations are relevant to breast cancer because the penetrance and phenotypic expression of DNA-repair variants may depend partly on environmental and occupational exposures, lifestyle factors, reproductive history, and other modifiers. These findings also emphasize that assessment of genetic susceptibility should ideally account for the broader biological context in which DNA-repair defects operate.

Homologous recombination deficiency has also become clinically important because it can create specific therapeutic vulnerabilities. Tumors with defective BRCA1, BRCA2, or related homologous recombination proteins may become highly dependent on alternative DNA-repair pathways, including those involving poly(ADP-ribose) polymerase. This biological dependency forms the basis of synthetic lethality and the therapeutic use of PARP inhibitors in selected BRCA-deficient and homologous recombination-deficient malignancies (Toh & Ngeow, 2021). However, cancer cells are evolutionarily dynamic, and therapeutic pressure can select for resistant clones. Reversion mutations capable of restoring BRCA1 or BRCA2 function have been identified as important mechanisms of acquired resistance to DNA-damaging therapies and PARP inhibitors (Qi et al., 2026). Multi-omics analyses of BRCA1- and BRCA2-deficient mammary tumors have also shown that resistance may emerge through distinct non-reversion mechanisms, reflecting different molecular adaptation patterns depending on the original defect (Bhin et al., 2023). These processes illustrate how intratumoral heterogeneity and clonal evolution complicate treatment and underscore the clinical relevance of detailed molecular characterization rather than binary classification of tumors as simply mutation-positive or mutation-negative (Nussinov et al., 2025). Experimental evidence further supports the capacity of PARP inhibition to induce DNA damage and sensitize malignant cells under appropriate molecular conditions (Pasaol, Śmieszek, et al., 2025), while broader reviews of BRCA1 and BRCA2 as therapeutic targets indicate that the biological principles underlying BRCA-directed treatment are conserved across mammalian cancer models and may inform comparative oncology (Pasaol, Dejnaka, et al., 2025).

The DNA-damage response is an integrated system involving numerous additional genes and signaling molecules. Alterations in genes that cooperate with BRCA proteins may enhance tumorigenesis even when BRCA itself is not directly mutated. For example, *BARD1* mutations can act synergistically in tumor development because BARD1

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forms a functional complex with BRCA1 and contributes to maintenance of genomic stability (Li et al., 2021). Similarly, interactions between WWOX and BRCA1 may influence DNA-repair pathway choice and mammary tumorigenicity, demonstrating that the biological effects of BRCA-associated pathways depend on broader protein networks (Bidany-Mizrahi et al., 2024). RECQ5 helicase represents another component involved in preservation of genomic stability through regulation of DNA replication, recombination, and transcription-associated stress (Mo et al., 2023). Mutational analyses of other cancer-related genes, such as *AKT1*, likewise demonstrate that tumor development frequently reflects the combined activity of DNA-repair defects and growth-signaling abnormalities rather than a single molecular lesion (Alasmar et al., 2024). Therefore, although *BRCA2* and *RAD51* are central to homologous recombination, their clinical significance should be interpreted within a wider genomic framework.

Altered RNA processing may further modulate the function of breast cancer susceptibility genes. Dysregulation of spliceosome components and abnormal splicing variants have been increasingly recognized in breast cancer and may generate altered protein isoforms, disrupt tumor-suppressor function, or modify oncogenic signaling (Gahete et al., 2022). This is particularly relevant when assessing exon-level alterations because sequence changes located within or near exon boundaries may potentially affect not only protein coding but also transcript processing. Molecular methods capable of accurately characterizing exon-level sequence variations are therefore important for determining whether observed changes are benign polymorphisms, pathogenic variants, or potentially splice-altering abnormalities. Validation studies using targeted next-generation sequencing on formalin-fixed paraffin-embedded mammary tumors have demonstrated that archived tissue samples can provide useful material for detection of BRCA variants when appropriate extraction and quality-control procedures are applied (Giacomo et al., 2022). Comparative oncology studies have also highlighted the biological relevance of DNA-damage response proteins in mammary and other cancers, providing additional experimental models for understanding homologous recombination defects (Hernández-Suárez et al., 2022).

Animal mammary tumors have contributed substantially to comparative understanding of hereditary cancer biology because several molecular pathways involved in human breast cancer are evolutionarily conserved. Genetic analysis of *PALB2*, a major BRCA2-interacting protein, has demonstrated potentially relevant alterations in canine mammary tumors, supporting the importance of the broader homologous recombination network in mammary tumorigenesis (Çıldır et al., 2024). Reviews of canine mammary tumors have similarly described overlapping molecular, angiogenic, and pathological features with human breast cancer, although important species-specific differences remain (Sewoyo et al., 2023). Such comparative findings should not be directly extrapolated to human disease, but they reinforce the mechanistic importance of BRCA-associated repair pathways and provide complementary models for studying genetic susceptibility and therapeutic response.

Despite extensive international research on hereditary breast cancer, relatively limited molecular data are available for some Middle Eastern populations, including Iranian patients. The spectrum and frequency of pathogenic variants, polymorphisms, and rare sequence alterations may differ according to ancestry, founder effects, genetic drift, reproductive patterns, and population structure. In addition, variants classified as rare or of uncertain significance in global databases may occur at different frequencies in regional populations. The identification of local genetic patterns is therefore essential for improving interpretation of molecular tests and preventing overreliance on reference datasets that may not adequately represent the population being evaluated. Studies from neighboring and regional populations have already demonstrated distinctive clinico-genomic and mutation profiles, supporting the need for country-specific and ethnicity-specific genetic characterization (Ahmad et al., 2024). More broadly, the increasing recognition of genetic heterogeneity in cancer has strengthened the rationale for integrating regional genomic data into personalized medicine frameworks (Al-Eitan et al., 2025). From an evolutionary perspective, the persistence and distribution of oncogenic *BRCA1/2* variants in human populations may also reflect complex demographic and selective processes, although these hypotheses remain under investigation (Korneenko & Pestov, 2023).

Accordingly, direct molecular investigation of *BRCA2* and *RAD51* in patients with breast cancer can provide complementary information regarding exon-specific variation, sequence similarity, potential mutation hotspots, and population-level genetic characteristics. Evaluation of targeted exons using PCR amplification followed by DNA sequencing allows relatively focused analysis of candidate regions and can be integrated with bioinformatic tools for nucleotide alignment, amino acid translation, structural

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prediction, and phylogenetic comparison. Such analyses may contribute to the identification of potentially relevant variants while also generating preliminary data for future larger-scale genomic studies. Because hereditary breast cancer reflects the combined contribution of high-penetrance genes, moderate-risk repair genes, rare variants, and population-specific sequence diversity, focused studies in underrepresented populations remain valuable for expanding the global evidence base and improving molecular interpretation.

The aim of the present study was to investigate the prevalence and sequence characteristics of genetic alterations in selected exons of *BRCA2* and *RAD51* among women with breast cancer in Tehran and to evaluate their potential relationship with hereditary breast cancer susceptibility.

2. Methods and Materials

This study was conducted using the facilities, equipment, materials, and laboratory supplies available at the Biomedical Department of the Women's Research Institute, Alzahra University, for the investigation of the *BRCA2* and *RAD51* genes in patients with breast cancer. The equipment used in this study is described below. The instruments employed in the study are presented in Tables 3-1, 3-2, and 3-3. The materials used in this study were classified into three groups:

1. Consumable materials
2. Chemical consumables
3. Biological consumables

Patient Selection: A total of 100 blood samples were collected from patients with breast cancer who had been referred to Farmanieh Hospital in Tehran. These patients had undergone unilateral or bilateral mastectomy performed by Dr. Lobat Geranpayeh and subsequently attended her clinic for follow-up and monitoring. Blood samples were collected under aseptic conditions. In addition, 25 paraffin-embedded breast cancer tissue samples were obtained from Farmanieh Hospital in Tehran.

Furthermore, blood samples were collected from 100 healthy women who had no personal history of breast or ovarian cancer and no history of breast or ovarian cancer among their first-degree relatives. These individuals constituted the control group.

After obtaining informed consent and explaining the experimental procedures, 5 mL of blood was collected from each participant. The blood-containing vials were transported in a cool box containing dry ice and subsequently transferred to a refrigerator. Samples were stored at 4°C until DNA extraction.

The equipment required for DNA extraction and PCR is presented in Table 3-2.

Yellow and blue pipette tips were sterilized in an autoclave before use and after placement in the corresponding racks.

Buffers and Solutions

1. Red Cell Lysis Buffer (RCLB): This buffer was used for the lysis of red blood cells. To prepare the buffer, 8.29 g of NH_4Cl and 0.84 g of $NaHCO_3$ were dissolved in 990 mL of distilled water. An appropriate amount of EDTA powder was added at 25°C, and the final volume was adjusted to 1 L. The solution was then autoclaved (pH = 7.17) and stored under refrigeration.

2. 10% SDS Solution: This solution was used to dissolve lipids present in cell membranes. To prepare the solution, 10 g of SDS was added to 100 mL of distilled water and heated to 68°C. After complete dissolution, the pH was adjusted to 8 using approximately 5 µL of HCl. The solution was stored at room temperature. If precipitation occurred, the solution was redissolved at 37°C.

3. Proteinase K: Proteinase K was used at a concentration of 20 mg/mL to remove histone proteins and other cellular proteins.

4. SE Buffer: This buffer was used to provide suitable conditions for proteinase K activity. To prepare the buffer, 1.09 g of NaCl and 2.32 g of EDTA were dissolved in 250 mL of distilled water. The pH was adjusted to 8.0–8.3 using NaOH. The solution was autoclaved and stored under refrigeration.

5. 6 M NaCl: This solution was used to remove protein contaminants following proteinase K treatment. To prepare 6 M NaCl, approximately 350 g of NaCl was dissolved in 1 L of distilled water, or 35 g was dissolved in 100 mL of distilled water.

6. Chloroform: Chloroform was used during DNA extraction to remove residual proteins.

7. 70% Ethanol: This solution was prepared from absolute ethanol and used to wash DNA. It also facilitated the removal of residual salts remaining from the extraction procedure.

8. TE Buffer: This buffer was used for storage of the extracted DNA. To prepare TE buffer, 2 mL of 0.5 M EDTA was added to 1 mL of 1 M Tris. The final volume was adjusted to 1 L, after which the solution was autoclaved and stored under refrigeration.

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9. TAE Buffer: To prepare 10× TAE stock buffer, the materials listed below were dissolved in 1 L of distilled water. Because 1× buffer was required for preparation of agarose gels used for electrophoresis, the stock buffer was diluted at a ratio of 1:10.

Table 1

Amounts of Chemicals Used

Chemical	Amount Used
Tris base	48.4 g
Acetic acid	10.34 g
EDTA (pH = 8)	20 mL

10. TBE Buffer

11. Agarose: To prepare a 1% agarose gel for electrophoresis of extracted DNA, 1 g of agarose was mixed with 100 mL of TBE or TAE buffer and heated in a microwave oven until completely dissolved.

For electrophoresis following PCR, a 2% agarose gel was prepared.

3. Findings and Results

DNA extraction is a process in which DNA is isolated from a biological sample using a combination of physical and chemical procedures. After collection of the blood samples, DNA was extracted using the saturated-salt method. This method was selected because it was less expensive, faster, and considered more reliable than alternative procedures.

DNA Extraction by the Saturated-Salt Method

DNA was extracted from whole-blood samples by the saturated-salt method according to the following procedure.

1. A 2-mL aliquot of whole blood containing anticoagulant was transferred into a test tube. The sample was then maintained at room temperature for 5 min and gently mixed at least once during this period.

2. Three times the volume of blood, approximately 6 mL, of lysis buffer was added to the test tube, and the tube was sealed with parafilm.

3. The tube was inverted several times and placed in a refrigerator for 10–20 min to facilitate more complete lysis of red blood cells.

4. The test tubes were removed from the refrigerator and centrifuged for 15 min at 1,800 rpm.

5. The supernatant was carefully removed, and the resulting pellet was retained.

6. Approximately 6 mL of lysis buffer was added to the pellet, followed by centrifugation for another 15 min at 1,800 rpm to recover the pellet.

7. Steps 5 and 6 were repeated until a completely white pellet was obtained.

8. After formation of a completely white pellet, 2 mL of SE buffer, 200 µL of 10% SDS, and 10 µL of proteinase K at a concentration of 20 mg/mL were added. The mixture was thoroughly vortexed and incubated for 24 h at 45°C in a water bath.

10. After complete digestion of the proteins, 900 µL of saturated 6 M NaCl solution and 1.5 mL of chloroform were added, and the solution was gently mixed.

11. The solution was centrifuged for 60 min at 3,800 rpm.

12. The supernatant was carefully removed and transferred to another sterile test tube. At this stage, nucleic acid-associated proteins together with salts formed a white precipitate.

13. Cold absolute ethanol equivalent to twice the initial blood volume was added to the supernatant, and the solution was gently mixed until DNA strands became visible.

14. The DNA fibers were collected using a micropipette and transferred to a sterile microtube.

15. The microtubes containing DNA were centrifuged in a microcentrifuge for 15 s at 12,000 rpm to precipitate the DNA.

16. The supernatant was removed, and the DNA pellet was retained.

17. Approximately 100–200 µL of 70% ethanol was added to the microtubes.

18. The washing procedure was repeated to ensure that the DNA was adequately washed with 70% ethanol.

19. The microtube containing the DNA pellet was placed under a laboratory hood for 10 min until the pellet was completely dry. Finally, the DNA pellet was dissolved in

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200 µL of TE buffer prepared with distilled water. The DNA-containing tubes were kept at room temperature for 2–3 h to allow complete dissolution and were subsequently stored at −20°C.

The Rima Pure FFPE column-based DNA extraction kit for paraffin-embedded tissues was obtained from Zist Abzar Pajouhan and Zist Baran. DNA extraction was performed according to the kit protocol as described below. The samples obtained from the hospital consisted of sectioned paraffin-embedded breast cancer tissues placed in 1.5-mL microcentrifuge tubes.

1. Initially, the paraffin-embedded tissues were incubated at 60°C to soften the paraffin.

2. Xylene was added to dissolve the paraffin, and the samples were left for approximately 1 h.

3. The next step involved washing. After removal of the xylene, 100% ethanol was added, and the sample was centrifuged at 8,000 rpm for 10 min. Washing was subsequently repeated twice using 70% ethanol in the same manner. These steps could be repeated if necessary.

4. The ethanol was discarded, and the tube cap was left open. The sample was incubated at room temperature for 10 min to allow residual ethanol to evaporate.

5. A total of 180 µL of ATL buffer and 20 µL of proteinase K were added to the sample.

6. The pellet was incubated in a water bath at 56°C for 1 h until complete lysis of the sample was achieved.

7. Immediately afterward, the sample was incubated in a water bath at 90°C for 1 h. Incubation at 90°C in ATL buffer facilitates disruption of formaldehyde-induced nucleic acid cross-links. Prolonged incubation at high temperatures may alter DNA integrity.

8. The sample was centrifuged for 10 s at 8,000 rpm to remove residual droplets from the inner wall of the tube.

9. The pellet was resuspended in 200 µL of AL buffer and mixed using a vortex mixer. Immediately thereafter, 200 µL of 96%–100% ethanol was added slowly, followed by thorough vortex mixing.

10. The sample was centrifuged for 10 s at 8,000 rpm.

11. The entire contents were transferred to a Rima column and centrifuged for 1 min at 8,000 rpm. The column was then transferred to a clean tube.

12. The Rima column was opened, and 500 µL of AW1 buffer was added. The column was centrifuged for 1 min at 8,000 rpm.

13. The solution collected beneath the column was discarded, and 500 µL of AW2 buffer was subsequently added. The column was centrifuged again for 1 min at 8,000 rpm.

14. Centrifugation was repeated at 14,000 rpm for 3 min to ensure that no residual solution remained on the column.

15. The column was transferred to a 1.5-mL tube, and finally, 20–100 µL of ATE elution buffer was added to the center of the column.

16. The column cap was closed, and the column was maintained at room temperature for 1 min, followed by centrifugation at 14,000 rpm for 1 min.

In this manner, DNA suitable for PCR was obtained. Following extraction, the samples were stored at −20°C. The extracted DNA was quantified using a spectrophotometer and evaluated by agarose gel electrophoresis before use in PCR.

Electrophoresis was used to evaluate the quantity and quality of the extracted DNA.

1. The required gel volume was first determined according to the dimensions of the casting tray.

2. One gram of agarose powder was weighed.

3. A total of 100 mL of TBE buffer was transferred into an Erlenmeyer flask.

4. Agarose was dissolved in the buffer and heated until boiling. The solution was then poured into the casting tray.

5. After solidification, the gel was removed from the casting tray and placed in the electrophoresis tank. Initially, 7 µL of DNA ladder was loaded into the first well. This marker was used to determine the molecular sizes of the separated DNA bands. Each extracted DNA sample, at a volume of 5 µL, was mixed with 1 µL of loading buffer containing tracking dye. After loading the samples, the electrophoresis tank was connected to the power supply, and the voltage was adjusted to 90 V. Because DNA molecules carry a negative charge, they migrated toward the positive electrode under the applied electric field. When the tracking dye had migrated approximately three-quarters of the length of the gel, the gel was removed from the electrophoresis tank.

After completion of electrophoresis, the DNA molecules were stained to allow visualization. The gel containing the separated DNA samples was placed in a container containing ethidium bromide. Ethidium bromide intercalates between the two DNA strands and emits fluorescence when exposed to ultraviolet light.

A gel documentation system was used to photograph the stained gel, and the resulting images were recorded electronically on a computer.

Preparation of Samples for PCR

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The fundamental principles of PCR are similar for all types of DNA samples.

The principal components required for PCR were as follows:

1. Template DNA: The DNA sample from which the target sequence was to be amplified.

2. PCR Tubes: Special 200-µL PCR microtubes were purchased in packages of 1,000 from Sinaclon, Tehran, Iran, and were used after sterilization. The following additional materials were used:

a. Sterile DNase-free distilled water

b. Loading Dye: The prepared stock loading buffer was stored at −20°C for long-term use.

c. DNA Size Marker: A DNA ladder obtained from Sinaclon was used in this study.

GeneRuler 100 bp DNA Ladder

d. Specific Forward (F) and Reverse (R) Primers: Short DNA sequences known as primers are required to initiate the PCR. Primers were designed to anneal to the target sequence at the 3′ ends of both DNA strands; therefore, both forward and reverse primers were required. The nucleotide sequences, amplified fragment sizes, and primer annealing temperatures, based on information supplied by Sinaclon, Tehran, Iran, are presented in Table 3-5.

3. Master Mix: PCR Master Mix tubes manufactured by Bioneer, South Korea, were used as a ready-to-use product designed to increase PCR accuracy and reduce experimental error. The Master Mix contained DNA polymerase, which incorporates new nucleotides into the growing DNA strand, as well as the following components:

Reaction Buffer: This buffer provides the appropriate chemical conditions required for PCR.

Divalent Mg^{2+} Cations: These components were premixed and freeze-dried at the bottom of the tube. A proprietary chemical stabilizer included in the product maintained Master Mix activity for more than 1 month at room temperature (25°C) and for more than 2 years when stored at −20°C.

Nucleotides: Deoxynucleotide triphosphates (dNTPs), which are the building blocks of DNA and are required for synthesis of the newly amplified DNA strands.

1. To prepare the working primer solutions, the primers were first mixed with sterile distilled water in accordance with the quantities specified in the PCR kit instructions. Subsequently, 10 µL of this mixture was combined with 90 µL of sterile distilled water, transferred to a sterile microvial, labeled, and stored in a freezer.

2. A total of 7 µL of distilled water was added to the microvial.

3. A total of 10 µL of Master Mix was added.

4. One microliter of the prepared reverse primer was added.

5. One microliter of the prepared forward primer was added.

6. One microliter of extracted DNA was added.

7. A total of 10 µL of mineral oil was added to prevent evaporation.

The prepared samples were placed in the PCR instrument, or conventional thermal cycler, and PCR amplification was performed using the predetermined cycling program. The size of the amplified fragment was also recorded. Initially, one DNA sample was amplified at several different annealing temperatures to determine the optimal temperature for final PCR amplification of all samples for each gene.

Exon 10 of *BRCA2* was amplified using two pairs of specific primers. The forward primers consisted of BRCA2 Exon10-F and BRCA2 Exon10-1-F, whereas the reverse primers consisted of BRCA2 Exon10-R and BRCA2 Exon10-1-R. PCR was performed according to the procedure described above, and the amplification conditions are presented in Table 3-5.

Exon 11 of *BRCA2* was amplified using one pair of specific primers. The forward primer was BRCA2 Exon1-F, and the reverse primer was BRCA2 Exon1-R. PCR was performed according to the aforementioned procedure, and the amplification conditions are presented in Table 3-6.

Exon 1 of *RAD51* was amplified using one pair of specific primers. The forward primer was RAD51 Exon1-F, and the reverse primer was RAD51 Exon1-R. PCR was performed according to the aforementioned procedure, and the amplification conditions are presented in Table 3-7.

Exon 3 of *RAD51* was amplified using one pair of specific primers. The forward primer was RAD51 Exon3-F, and the reverse primer was RAD51 Exon3-R. PCR was performed according to the aforementioned procedure, and the amplification conditions are presented in Table 3-9.

After PCR amplification, electrophoresis was used to evaluate the number and sizes of the amplified fragments.

Exon 4 of *RAD51* was amplified using one pair of specific primers. The forward primer was RAD51 Exon4-F, and the reverse primer was RAD51 Exon4-R. PCR was performed according to the aforementioned procedure.

After PCR amplification, electrophoresis was used to assess the number and sizes of the generated fragments.

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Exon 5 of *RAD51* was amplified using one pair of specific primers. The forward primer was RAD51 Exon5-F, and the reverse primer was RAD51 Exon5-R. PCR was performed according to the aforementioned procedure, and the amplification conditions are presented in Table 3-11.

After PCR amplification, electrophoresis was used to assess the number and sizes of the generated fragments.

For sequencing of the PCR products, approximately 25–50 µL of PCR product was prepared. Approximately 3–5 µL was evaluated to ensure the presence of a strong amplification band, absence of primer dimers, absence of nonspecific bands, and absence of smearing. PCR products meeting these quality criteria were submitted through Topaz Gene Kavosh Company, Karaj, Iran, to Microsynth, Switzerland, for sequencing.

Using Chromas Lite software, the nucleotide sequences were extracted in FASTA format.

Sequences in GenBank exhibiting the highest similarity to the target sequences were identified using the BLAST program available through the National Center for Biotechnology Information (NCBI) database.

Multiple sequence alignment of nucleotide sequences corresponding to the investigated gene regions was performed both among the study samples and against reference sequences available in NCBI.

Graphical BLAST outputs and phylogenetic trees were generated, and the genetic relationships among the sequences were evaluated.

Using protein databases, including EBI and ExPASy, the exon sequencing results were translated into corresponding amino acid sequences.

The predicted three-dimensional structure, secondary structure, and putative protein structure associated with each exon were evaluated using the Phyre2 program.

The sequence analysis demonstrated a high degree of similarity between the investigated *BRCA2* and *RAD51* exons and the corresponding reference sequences available in international genetic databases. Analysis of exon 10 of the *BRCA2* gene showed approximately 99% nucleotide sequence similarity with reference sequences reported from the United States and the United Kingdom. Similarly, exon 11 of *BRCA2* exhibited approximately 97%–99% similarity with sequences reported from Hong Kong. These findings indicate a high level of conservation of the investigated *BRCA2* regions despite the presence of detectable sequence alterations among the studied breast cancer samples.

Among the analyzed regions, exon 11 of the *BRCA2* gene showed the highest observed frequency of genetic alterations. The detected sequence changes in this exon were more frequent than those observed in the other investigated genomic regions. Although most of the nucleotide sequence remained highly conserved, the identified variations suggest that exon 11 may represent an important region for further investigation in relation to hereditary susceptibility to breast cancer in the studied population.

Analysis of exon 1 of the *RAD51* gene showed approximately 98%–99% nucleotide sequence similarity with corresponding reference sequences reported from the United States. The high similarity observed for this exon indicates substantial conservation of the *RAD51* sequence among the studied patients. Nevertheless, sequence variations were detected, supporting the presence of genetic heterogeneity within this DNA-repair gene among women with breast cancer in Tehran.

Phylogenetic analysis of exons 10 and 11 of *BRCA2* and exon 1 of *RAD51* demonstrated close genetic relationships between the sequences obtained from the studied Iranian patients and reference sequences from the United States, the United Kingdom, and Hong Kong. Overall, the level of genetic similarity ranged from approximately 97% to 99%. The combined sequence and phylogenetic findings showed that the investigated breast cancer-associated genes were highly conserved across populations, while exon-specific genetic variations, particularly in exon 11 of *BRCA2*, were evident in the study samples.

4. Discussion and Conclusion

The present study investigated genetic alterations in selected exons of *BRCA2* and *RAD51* among women with breast cancer in Tehran, with particular emphasis on sequence similarity, exon-specific variation, and phylogenetic relationships with reference sequences deposited in international databases. The principal findings showed that exon 10 of *BRCA2* exhibited approximately 99% sequence similarity with reference sequences from the United States and the United Kingdom, exon 11 of *BRCA2* showed approximately 97%–99% similarity with sequences reported from Hong Kong, and exon 1 of *RAD51* demonstrated approximately 98%–99% similarity with reference sequences from the United States. Among the evaluated genomic regions, alterations in exon 11 of *BRCA2* appeared to have the highest observed frequency. In addition, phylogenetic analysis indicated a high degree of genetic proximity between the sequences obtained from Iranian patients and corresponding breast cancer-related

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sequences reported in other populations. These findings suggest that while the examined loci are highly conserved across populations, potentially meaningful exon-level variations are present and may contribute to individual or population-specific susceptibility to breast cancer.

The observation that exon 11 of *BRCA2* contained the highest proportion of detected alterations is biologically plausible because exon 11 encompasses a large functionally important region of the gene and contains domains involved in interaction with RAD51. The BRCA2 protein plays a central role in homologous recombination by facilitating RAD51 loading onto single-stranded DNA and stabilizing RAD51 nucleoprotein filaments at sites of DNA double-strand breaks. Functional perturbation of BRCA2 can therefore impair accurate DNA repair and increase genomic instability. Experimental evidence has demonstrated that conserved regions of BRCA2 regulate interactions between BRC repeats and RAD51, reinforcing the importance of sequence integrity within these functional domains (Zhu et al., 2023). The finding of relatively frequent changes within exon 11 is also consistent with previous work showing that variants in exon 11 of *BRCA2* may be associated with mammary tumor development (Gopal et al., 2022). Although the present study does not establish pathogenicity for each detected sequence change, the concentration of variation within a functionally important coding region supports the need for detailed variant-level interpretation.

The importance of *BRCA2* variation observed in this study is further supported by the broader literature on hereditary breast cancer. Pathogenic variants in *BRCA1* and *BRCA2* are among the best-established genetic determinants of inherited breast and ovarian cancer predisposition, and the evolution of hereditary cancer testing has repeatedly demonstrated the clinical significance of identifying population-specific variants in these genes (Barili et al., 2024). The historical discovery of BRCA-associated susceptibility fundamentally changed the understanding of familial breast cancer and established a molecular basis for predictive testing, surveillance, prophylactic intervention, and targeted therapy (Burachik et al., 2023). The current findings therefore contribute to the accumulating evidence that exon-level characterization of *BRCA2* can be valuable in populations for which detailed mutation databases remain limited. Moreover, exome sequencing studies have identified rare recurrent copy-number variants and additional inherited genomic abnormalities among individuals with hereditary breast cancer, indicating that clinically relevant susceptibility may extend beyond commonly screened variants (Kumpula et al., 2023).

The high sequence similarity observed between the Iranian samples and international reference sequences is expected for evolutionarily conserved DNA-repair genes, but it does not negate the significance of small sequence differences. Even limited nucleotide variation can modify protein function when it affects critical coding residues, splice junctions, regulatory motifs, or interaction domains. This issue is particularly important in *BRCA2*, where the biological effect of a mutation may depend not only on its presence but also on its precise location. Clinical analyses in BRCA-associated malignancies have shown that the location of *BRCA1* and *BRCA2* mutations may be associated with differential therapeutic outcomes, indicating functional heterogeneity across distinct regions of these genes (Labidi-Galy et al., 2023). In this context, the approximately 97%–99% similarity observed in exon 11 should not be interpreted simply as evidence of equivalence; rather, it suggests a highly conserved region in which relatively small deviations may still be biologically meaningful. Population-specific molecular investigations are particularly important because the prevalence and interpretation of uncommon variants can differ across ethnic groups (Pal et al., 2024).

The findings related to *RAD51* are also of considerable biological relevance. Exon 1 of *RAD51* demonstrated approximately 98%–99% similarity with reference sequences, suggesting that this region is also strongly conserved. RAD51 is indispensable for homologous recombination repair, and altered RAD51 function can compromise accurate DNA double-strand break repair. Previous studies have demonstrated associations between variants in homologous recombination genes, including *RAD51*, *XRCC2*, and *XRCC3*, and breast cancer susceptibility (Rajagopal et al., 2022). A meta-analysis similarly found that polymorphisms in genes participating in homologous recombination repair may influence breast cancer risk, although the magnitude and direction of association can vary according to population and genotype (Yu & Wang, 2023). The present finding of detectable variation within *RAD51* is therefore consistent with a broader body of evidence implicating the homologous recombination pathway in breast cancer susceptibility.

The functional relationship between BRCA2 and RAD51 provides an important mechanistic framework for interpreting the results of this study. BRCA2 directly controls RAD51 recruitment and activity during homologous recombination, meaning that alterations in

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either gene may influence the same DNA-repair pathway. Germline variants affecting RAD51-related genes have been shown to confer susceptibility to breast and ovarian cancers characterized by homologous recombination deficiency (Setton et al., 2021). In addition, RAD51 regulators have been implicated in several reproductive and gynecological disorders, further emphasizing the biological importance of tight regulation of this pathway (Witham & Hengel, 2024). Consequently, the concurrent assessment of *BRCA2* and *RAD51* performed in the present study is conceptually justified because disruption of either component may compromise genomic maintenance and potentially increase the probability of malignant transformation.

The present results can also be interpreted within the broader context of homologous recombination deficiency. Tumors with defects in BRCA1, BRCA2, or associated repair proteins often display genomic instability and increased dependency on alternative DNA-repair mechanisms. This vulnerability has direct therapeutic implications because homologous recombination-deficient tumors may be sensitive to PARP inhibition (Toh & Ngeow, 2021). Experimental studies have shown that PARP inhibitors such as olaparib can induce DNA damage and enhance treatment sensitivity in BRCA-related settings (Pasaol, Śmieszek, et al., 2025). Reviews in comparative oncology have similarly emphasized the therapeutic relevance of BRCA1 and BRCA2 as targets in cancer models (Pasaol, Dejnaka, et al., 2025). Although the current study did not evaluate treatment response, identification of variants within *BRCA2* and *RAD51* may ultimately have implications beyond risk assessment, particularly if future work establishes that specific alterations are associated with homologous recombination deficiency or sensitivity to targeted therapy.

At the same time, the clinical meaning of sequence alterations cannot be determined solely on the basis of their presence. Cancer genomes are heterogeneous, and different variants may have distinct functional consequences. Tumor evolution under selective pressure can also generate reversion mutations or alternative resistance mechanisms capable of restoring DNA-repair function. Reversion mutations in *BRCA1* and *BRCA2* have been recognized as important mechanisms of resistance to DNA-damaging therapy and PARP inhibitors (Qi et al., 2026). Multi-omics studies have further demonstrated that BRCA1- and BRCA2-deficient tumors can acquire resistance through distinct non-reversion pathways (Bhin et al., 2023). These observations are relevant to the interpretation of the current findings because they underscore the necessity of distinguishing benign polymorphisms, pathogenic germline variants, somatic mutations, and adaptive tumor-specific alterations. The biological significance of any detected variant should therefore be established using clinical annotation, family-history data, functional prediction, segregation analysis, and, when possible, experimental validation.

Another important finding of this study was the apparent genetic similarity between Tehran breast cancer samples and reference sequences from populations in the United States, the United Kingdom, and Hong Kong. This similarity likely reflects the high evolutionary conservation of core DNA-repair genes rather than direct evidence of identical breast cancer etiology across populations. Nevertheless, cross-population comparisons are useful because they help identify both conserved and population-specific variants. Recent studies in geographically and ethnically understudied populations have demonstrated distinct clinico-genomic profiles and mutation spectra among patients with breast cancer (Ahmad et al., 2024). Regional genomic studies have similarly emphasized the value of defining local cancer-associated variants as part of personalized medicine strategies (Al-Eitan et al., 2025). Accordingly, the present study adds preliminary data from an Iranian population and supports the need for expansion of national and regional mutation databases.

The importance of population-specific molecular data is further reinforced by evidence that hereditary breast cancer cannot be explained exclusively by classical *BRCA1/2* mutations. Other interacting genes may influence susceptibility, tumor behavior, and DNA-repair efficiency. For example, mutations in *BARD1*, a functional partner of BRCA1, may have synergistic effects on tumorigenesis (Li et al., 2021). Similarly, WWOX has been shown to interact functionally with BRCA1 and affect DNA-repair pathway selection and mammary tumor development (Bidany-Mizrahi et al., 2024). RECQ5 helicase is another genomic stability factor involved in DNA replication and repair (Mo et al., 2023). The broader significance of these pathways is that breast cancer predisposition frequently reflects network-level dysfunction rather than a single mutation. Therefore, the exon-specific alterations identified in *BRCA2* and *RAD51* in the current study should be viewed as part of a larger DNA-damage response system.

The use of both blood samples and paraffin-embedded breast cancer tissues in the methodological framework of this study is also relevant. Formalin-fixed paraffin-

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embedded material can be technically challenging because formalin exposure may fragment DNA and introduce cross-linking, but properly optimized extraction and sequencing methods can still yield diagnostically useful genetic information. Previous validation studies have demonstrated the feasibility of detecting *BRCA1* and *BRCA2* variants from formalin-fixed paraffin-embedded mammary tumors using targeted sequencing methods (Giacomo et al., 2022). Moreover, investigations in comparative oncology have underscored the importance of DNA-damage response proteins in mammary neoplasia across species (Hernández-Suárez et al., 2022). These findings support the methodological premise that archived tumor tissues can be informative in molecular oncology when sample quality is carefully controlled.

The possibility that exon-level variation influences splicing should also be considered. Changes within coding exons or near exon boundaries may alter splice-site recognition, transcript processing, or the relative abundance of alternative isoforms. Dysregulated splicing is increasingly recognized as a contributor to breast cancer biology and can affect tumor suppressors, oncogenes, and DNA-repair proteins (Gahete et al., 2022). Thus, even variants that do not obviously alter a critical amino acid may still have functional consequences through aberrant RNA processing. Future characterization of the alterations identified in this study would benefit from transcript-level analysis in addition to genomic sequencing.

The findings should additionally be interpreted in light of the broader genomic and environmental context of carcinogenesis. Genetic susceptibility can interact with environmental exposures, lifestyle factors, metabolic status, and hormonal conditions. Polymorphisms in DNA-repair genes have been proposed as modifiers of susceptibility to environmentally induced genotoxicity (Kaur & Kaur, 2024). Obesity and associated endocrine-metabolic disturbances may also influence the biological expression of BRCA-associated breast cancer (Giordano et al., 2025). Therefore, the penetrance of *BRCA2* or *RAD51* variants in an Iranian population may be modified by local patterns of environmental exposure, reproductive behavior, diet, adiposity, and other factors not evaluated in the present study.

The study also has implications for understanding tumor heterogeneity. Breast cancer is characterized by clonal diversity, and individual tumors may contain multiple subpopulations with different genomic profiles. This heterogeneity has important consequences for progression and treatment response (Nussinov et al., 2025). The present sequencing approach was useful for identifying exon-level variation but was not designed to characterize low-frequency subclonal mutations comprehensively. Thus, some variants may have remained undetected, particularly if present in a minor fraction of tumor cells. More sensitive next-generation sequencing approaches may reveal additional genomic complexity and help differentiate germline from somatic events.

The broader evidence from cancer genetics supports continued investigation of highly conserved repair pathways across different populations. Studies of other tumor-associated genes have shown that novel variants can emerge in regional cohorts and contribute to the local genetic architecture of cancer (Alasmar et al., 2024). Comparative work involving mammary tumors has also shown that genes such as *PALB2*, which functionally interacts with BRCA2, can harbor potentially relevant alterations (Çıldır et al., 2024). Reviews of canine mammary neoplasia likewise emphasize conserved molecular mechanisms in mammary tumor development (Sewoyo et al., 2023). Although animal findings cannot be directly extrapolated to human breast cancer, they provide additional mechanistic support for the importance of homologous recombination networks.

Finally, the present findings reinforce the concept that hereditary cancer susceptibility is shaped by complex evolutionary and population-genetic processes. The distribution of oncogenic *BRCA1/2* variants may reflect historical founder events, demographic structure, and other evolutionary forces (Korneenko & Pestov, 2023). This perspective is particularly relevant in populations that remain underrepresented in global genomic databases. Identifying the spectrum of *BRCA2* and *RAD51* variants among Iranian women with breast cancer may therefore help clarify whether some alterations are globally recurrent, regionally enriched, or potentially unique to particular population groups. Taken together, the present findings support the biological relevance of exon-specific variation in *BRCA2* and *RAD51* and provide a preliminary molecular basis for larger studies aimed at defining hereditary breast cancer risk in Iranian populations.

The principal limitations of this study include the relatively limited sample size, the targeted analysis of selected exons rather than the complete coding and regulatory regions of *BRCA2* and *RAD51*, and the absence of comprehensive pathogenicity classification for the detected sequence alterations. The study did not clearly distinguish germline from somatic variants for every

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finding, and detailed pedigree information, segregation analysis, tumor subtype, receptor status, stage, treatment history, and long-term clinical outcomes were not integrated into the genetic analyses. Sanger sequencing or targeted PCR-based sequencing may also have limited sensitivity for large genomic rearrangements, low-frequency subclonal variants, copy-number alterations, and variants outside the amplified regions. In addition, comparisons with international reference sequences were descriptive and cannot establish shared disease mechanisms or common ancestry. The absence of functional assays further prevented direct determination of the biological effect of individual variants.

Future research should include larger multicenter cohorts from different regions of Iran and should incorporate age-matched controls, detailed family histories, and standardized clinical and pathological variables. Comprehensive sequencing of the complete *BRCA1*, *BRCA2*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *PALB2*, and other homologous recombination genes would provide a more complete characterization of hereditary susceptibility. Whole-exome sequencing, targeted next-generation sequencing panels, copy-number analysis, and RNA sequencing should be considered to identify rare variants, structural alterations, and splice-related abnormalities. Detected variants should be classified according to internationally accepted pathogenicity frameworks and validated using functional prediction, segregation analysis, protein modeling, and, where feasible, cellular assays. Future studies should also investigate whether specific variants are associated with tumor subtype, age at diagnosis, bilateral disease, family history, recurrence, metastasis, response to platinum-based therapy, or sensitivity to PARP inhibitors.

From a clinical and public health perspective, molecular screening strategies for hereditary breast cancer should be strengthened, particularly for women with early-onset disease, bilateral breast cancer, strong family histories, multiple affected relatives, or associated ovarian cancer. Genetic counseling should accompany molecular testing so that patients and their relatives can understand the implications of positive, negative, and uncertain findings. Development of an Iranian or regional hereditary breast cancer variant registry would improve interpretation of locally observed sequence changes and reduce uncertainty arising from reliance on databases dominated by other populations. Laboratories should also adopt standardized pre-analytical, PCR, sequencing, and quality-control procedures, particularly when working with formalin-fixed paraffin-embedded tissues. Integration of genetic testing with routine oncology practice could support individualized surveillance, risk-reducing strategies, family-based testing, and selection of molecularly targeted treatments when clinically indicated.

Authors' Contributions

All authors significantly contributed to this study.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethical Considerations

In this study, to observe ethical considerations, participants were informed about the goals and importance of the research before the start of the interview and participated in the research with informed consent.

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