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Research Article

Role of BRCA1 Variants in mRNA Stability and miRNA Mediated Regulation of Breast Cancer: A Case Control Study

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Abstract: ***Introduction:*** **Objective:**To identify BRCA1 gene variants and predict their potential role in development of breast cancer.**Material and Methods:**This case–control study included 250 breast cancer patients and an equal number of healthy controls recruited from Department of Radiography & Cancer Screening Affiliated Hospital, Lahore, Pakistan, between December 2025 to March 2026. Demographic information was collected using structured questionnaires, while clinical data were obtained from mammography, ultrasonography, histopathology, and immunohistochemistry reports. Polymerase chain reaction (PCR) followed by Sanger sequencing was performed to detect BRCA1 gene variants. In silico analyses were conducted to assess the functional impact of identified variants, including effects on protein function, miRNA binding sites, and mRNA secondary structure and stability.**Results:**Invasive ductal carcinoma was the most frequently observed histological subtype. Advanced age [OR: 2.81; 95% CI: 1.60–4.95; p = 0.0003] and a positive family history of breast cancer [OR: 4.32; 95% CI: 1.73–10.76; p = 0.001] were identified as significant risk factors. Six BRCA1 variants were detected. Two novel missense variants (Chr17:43082553A>T and Chr17:43093710A>T) were predicted to be deleterious, potentially disrupting interactions with PALB2 and the NLS2 site of importin-α, respectively. In silico analysis also predicted the loss of the hsa-miR-1179 binding site due to the Chr17:43093220T>C variant. Additionally, four variants were predicted to alter mRNA secondary structure and stability.**Conclusion:**BRCA1 expression is expected to change in novel variations found in this study. To further clarify and validate the functional significance of the found BRCA1 genetic variation for its involvement in breast oncogenesis and for its potential therapeutic development, however, expressional-based study is needed. Further research is required to examine additional tumor suppressor genes, such as BRCA2, in cases of breast cancer. Additionally, a multi-omics approach ought to be used in order to obtain a more comprehensive molecular knowledge of breast cancer.

Keywords: Breast cancer; BRCA1; Gene variants; miRNA

INTRODUCTION

Breast cancer rates are rising. GLOBOCAN (2020) reports 685,000 fatalities and 2.3 million new cases of breast cancer. It is estimated that by 2040, there will be three million new instances of breast cancer and up to one million fatalities from the disease[1]. One in nine women in Pakistan is at risk for breast cancer, making it the country with the greatest prevalence of the disease[2].Numerous external and endogenous risk factors contribute to the complex nature of breast cancer. One of the most important genes in the oncogenesis of breast cancer is BRCA1. Tumor development is the outcome of unchecked cell proliferation and multiplication caused by BRCA1 gene mutations[3]. Single nucleotide polymorphisms and mutations play a crucial part in determining population diversity and illness vulnerability. Non-synonymous mutations were previously thought to be important, but new research indicates that synonymous mutations may play a part in the development of cancer because of their effects on mRNA stability and structure, protein expression, microRNA (miRNA) binding, and splicing[4].

miRNA controls the post-transcriptional expression of genes. Tumorigenesis results from disruptions in gene expression caused by mutations in miRNA binding sites[5]. Moreover, mRNA's stability and translational efficiency may be impacted by alterations in its secondary structure.Six Variants found in coding areas and miRNA binding sites can change the stability and structure of mRNA, which has a major impact on the development of disease[6].

Few studies have concentrated on identifying BRCA1 mutations, and private health clinics are the only places in Pakistan where BRCA1 genetic testing is available [7-8.]However, as miRNAs are essential regulators of gene expression, it is still imperative to look at how variants affect post-transcriptional regulations such miRNA binding sites. In a similar vein, variations can alter the stability of mRNA and the function of proteins[4]. Because BRCA1 is a highly penetrant gene and breast cancer is prevalent in Pakistan, the present research was created to screen individuals for BRCA1 gene variations. Furthermore, the effects of genetic alterations on miRNA binding sites as well as the stability and structure of transcripts in oncogenesis were analyzed using in-silico methods.

MATERIALS AND METHODS

This case–control study included 250 breast cancer patients and an equal number of healthy controls recruited from Department of Radiography & Cancer Screening Affiliated Hospital, Lahore, Pakistan, between December 2025 to March 2026 .The study was carried out with approval from Institutional Ethics Committee. Demographic information was collected using structured questionnaires, while clinical data were obtained from mammography, ultrasonography, histopathology, and immunohistochemistry reports. Polymerase chain reaction (PCR) followed by Sanger sequencing was performed to detect BRCA1 gene variants. In silico analyses were conducted to assess the functional impact of identified variants, including effects on protein function, miRNA binding sites, and mRNA secondary structure and stability. In contrast to the controls, which only included healthy people, all patients with breast cancer were included. Patients with malignancies other than breast cancer were not included.

DNA Extraction and BRCA1 Gene

The phenol-chloroform technique was used to extract DNA, and the isolated DNAs were kept at -20°C. The BRCA1 gene reference sequence (ENSG00000012048) was obtained, and primer creation was done using Primer 3 online program. A thermocycler was used to conduct the polymerase chain reaction (PCR) using a reaction mixture that included Dream Taq Green PCR Master Mix (Thermo Scientific). The PCR settings were identical to those reported by Shaukat M. et al.[9]. Each sample's total volume of the PCR reaction mixture was 10µL, and the template concentration varied from 50 to 100 ng. PCR amplicons were seen using an ultraviolet transilluminator after being electrophoresed for 40 minutes at 90 volts.

Mutational Screeing

Single-strand conformational polymorphism analysis was performed on the amplified PCR products in order to screen variations based on differences in electrophoretic mobility. In order to denature the amplified products for this technique, heat shock was used while 99% formamide was present. For amplified products smaller than 500 bp, a 6% gel was used for the polyacrylamide gel electrophoresis, and for those larger than 500 bp, an 8% gel. These PCR amplicons were then electrophoresed at 120 volts for 180 minutes.

Samples were sent for Sanger sequencing after banding patterns were noted. The sequencing results were analyzed through BioEdit and Mutation Taster. The BRCA1 gene reference sequence, ENSG00000012048, was used to interpret the variants. ClinVar and Variant Effect Predictor (VEP) were then used for further analysis.

In Silico-Analysis

Predict SNP was used to forecast the effect of non-synonymous genetic variants. Using miRBase (http://www.mirbase.org), the alignment of miRNA to the mutant and reference sequences was examined. Additionally, the variant's influence on the structure and stability of BRCA1 mRNA was predicted using Visual Gene Developer software. The statistical analysis was performed using SPSS 20.0 and Microsoft Excel. P values less than 0.05 were deemed statistically significant when odds ratios were computed.

RESULTS

The majority of patients and healthy people were from the region of Punjab. Three age categories were established for the participants. The risk of breast cancer was significantly correlated with age group III of ≥60 years [OR: 2.8149 (1.5995 to 4.9538) p-value = 0.0003].The majority had a good history of nursing. In a similar vein, few individuals were nulliparous and 94.4% of patients were parous.50.8% of patients were premenopausal, and 49.2% were menopausal. According to Table I, patients with a family history of breast cancer had a considerable chance of developing the disease [OR: 4.3186 (1.7336 to 10.7581) p value = 0.001].

Table-I: Demographic Features of the Patients and Healthy Individuals

Category	Variable	Cases(n=250)	Controls(n=250)	Odds Ratio	p-Values
Age (years)	28-40	55 (22.0)	90 (35.0)	0.51	0.001*
	41-60	148 (59.2)	141 (56.4)	1.12	0.526
	≥61	47 (18.8)	19 (7.6)	2.81	0.000
Residence	Islamabad	50 (20.0)	76 (30.4)	0.57	0.0077*
	Punjab	152 (60.8)	124 (49.6)	1.58	0.012*
	KPK	29 (11.6)	22 (8.8)	1.36	0.302
	Sindh	1 (0.4)	2 (0.8)	0.50	0.570
	Azad Kashmir	15 (6.0)	21 (8.4)	0.70	0.301
	Gilgit Baltistan	2 (0.8)	4 (1.6)	0.50	0.421
	Quetta	1 (0.4)	1 (0.4)	1.00	1.000
Marital Status	Married	244 (97.6)	245 (98.0)	0.83	0.761
	Unmarried	6 (2.4)	5 (2.0)	1.20	0.761
Lactation	Yes	210 (84.0)	220 (88.0)	0.72	0.199
	No	40 (16.0)	30 (12.0)	1.40	0.199
Parity	Parous	236 (94.4)	223 (89.2)	2.14	0.0267*
	Nulliparous	14 (5.6)	27 (10.8)	0.47	0.0267*
Menopause	Pre-menopause	127 (50.8)	144 (57.6)	0.76	0.127
	Menopause	123 (49.2)	106 (42.4)	1.32	0.127
Family History	Yes	24 (9.6)	6 (2.4)	4.32	0.0017*
	No	226 (90.4)	244 (97.6)	0.23	0.0017*

The prevalence of invasive ductal carcinoma was higher.ER, PR, HER2, and Ki 67% were evaluated according on IHC data. Luminal B (35.2%) was the most common molecular subtype, followed by luminal A (30.8%). According to the Nottingham grading system, patients were most frequently classified with Grade-II. According to Table II, half of the breast lesions were classified as BI-RADS V (50.4%) based on mammography and ultrasound data.

Table-II: Classification of Patients' Breast Cancer Type Based on Mammography, Histopathology, BI-RADS, and IHC (n = 250)

Clinical features	Category	n (%)
	Ductal carcinoma in situ (DCIS)	7 (2.8)
	Invasive ductal carcinoma (IDC)	230 (92.0)

Histologic Type of Breast Cancer	Invasive lobular carcinoma (ILC)	2 (0.8)
	Other rare types	11 (4.4)
Molecular Subtype (IHC)	Luminal A	77 (30.8)
	Luminal B	88 (35.2)
	HER2-enriched	27 (10.8)
	Triple-negative breast cancer	58 (23.2)
Tumor Grade	Grade I	20 (8.0)
	Grade II	195 (78.0)
	Grade III	35 (14.0)
BI-RADS Category	BI-RADS III	3 (1.2)
	BI-RADS IV	58 (23.2)
	BI-RADS V	126 (50.4)
	BI-RADS VI	63 (25.2)

10 (now known as exon 11) of the BRCA1 gene were found during Sanger sequencing research. Patients with invasive ductal carcinoma were found to have the majority of these mutations. Both afflicted and healthy people have variants Chr17:43092418A>G and Chr17:43093220T>C (Table-III).In-silico Analysis: Variants Chr17:43082553A>T and Chr17:43093710A>T were predicted to have pathogenic consequences that alter BRCA1's function (Table-III).

Table-III: Identified Variants of the BRCA1 Gene and Predicted Changes in Its Protein

r. No	Physical Location of SNP	Variant Site	Coding Consequence (VEP)	Amino Acid Change	Predicted Effect of Variant	Participants (Patients/ Controls)	SNP Status in Current Study
	Chr17: 43093710	A > T	Missense (NLS2)	Lys 607Asn	Deleterious	20	Novel
	Chr17: 43092507	G > A	Missense (RAD51 interaction)	Met 1008Ile	Neutral	60	rs1800704
	Chr17: 43092418	A > G	Missense (RAD51 interaction)	Glu 1038Gly	Neutral	15 / 10	rs16941 4
	Chr17: 43082553	A > T	Missense (PALB2 interaction)	Asn 1403Ile	Deleterious	50	Novel
	Chr17: 43093220	T > C	Synonymous (RAD50 interaction)	Leu 771Leu	Neutral	21 / 9	rs16940 6
	Chr17: 43094127	G > A	Synonymous (c-Myc binding site)	Lys 468Lys	Neutral	5 / 0	rs1555591782

A deletion of the binding site for hsa-miR-1179 was observed in variation Chr17:43093220T>C. The variants were further examined to determine whether there were any changes in miRNA binding. Variants Chr17:43092507G>A, Chr17:43094127G>A, Chr17:43092418A>G, and Chr17:43093220T>C were predicted by the in-silico study to affect the stability and structure of the mRNA. Table IV.

Table-IV: In-silico Analysis of Impact of Identified BRCA1 Mutations on mRNA Structure, Stability, and miRNA Binding

Variant	Gibbs Energy (kcal/mol)	mRNA Structure Change	Stability Effect	miRNA ID	miRNA Alignment (R)	miRNA Alignment (M)

Chr17:43093220 T>C	-48.40 (R)	Changed	Decreased	hsa-miR-1179	Aligned	Not aligned
	-50.40 (M)					
Chr17:43093710 A>T	-45.10 (R)	No	No effect	hsa-miR-4703-5p	Aligned	Aligned
	-45.10 (M)					
Chr17:43092507 G>A	-34.70 (R)	Changed	Increased	hsa-miR-6734-5p	Aligned	Aligned
	-32.60 (M)					
Chr17:43092418 A>G	-36.00 (R)	Changed	Decreased	hsa-miR-3167	Aligned	Aligned
	-43.40 (M)					
Chr17:43082553 A>T	-39.00 (R)	No	No effect	hsa-miR-4751	Aligned	Aligned
	-39.00 (M)					
Chr17:43094127 G>A	-40.80 (R)	Changed	Increased	hsa-miR-4680-5p	Aligned	Aligned
Chr17:43093220 T>C	-48.40 (R)	Changed	Decreased	hsa-miR-1179	Aligned	Not aligned
	-50.40 (M)					
Chr17:43093710 A>T	-45.10 (R)	No	No effect	hsa-miR-4703-5p	Aligned	Aligned

DISCUSSION

The screening of BRCA1 mutations, their anticipated effects on BRCA1 function, and any dysregulation of transcriptional or post-transcriptional pathways in breast cancer are all presented in this retrospective case-control research. This study indicated that demographic risk variables, such as age and family history, increased the incidence of breast cancer. Six BRCA1 variations were found through genetic testing. Two of these variations were anticipated to have negative effects, four to disrupt the stability and structure of mRNA, and one to change the miRNA binding site.

Among Asian nations, Pakistan has the highest age-standardized incidence rates of breast cancer [10-12]. The majority of the patients in this study belonged to age group II (41–60 years), while those over 60 were shown to have a high risk of developing breast cancer [OR: 2.8149 (1.5995 to 4.9538) p value = 0.0003]. The average age for breast cancer incidence in the majority of Asian nations is between 40 and 59 years old, whereas the average age in the West is between 60 and 70 years old[13]. The numbers of children a woman has and the age at which she gives birth to her first child are additional risk factors for breast cancer. Although 94.4% of participants in this research were parous, null parity raises the risk of breast cancer[14]. In a similar vein, a Pakistani research by Ahmed F. et al.[15] found that 93% of patients with breast cancer were parous. Contrary to a previous finding that breastfeeding for a year lowers the risk of breast cancer by 4.3%, almost 84% of patients were nursing their children [16].

Menopause ends a woman's reproductive cycle and typically happens between the ages of 45 and 55. However, late menopause increases a woman's risk of breast cancer since it prolongs her exposure to hormones. In this study, there were 50.8% premenopausal and 49.2% menopausal individuals. Similarly, 54.74% of premenopausal individuals had cancer, according to Ahmed F et al. (2015). Breast cancer risk was shown to be higher in patients with a family history of the disease (OR: 4.3186 [1.7336 to 10.7581] p value=0.0017). Similarly, 5% of the cases were reported to be familial by Majeed AI et al.[7] Breast cancer risk is elevated in family instances due to inherited genetic alterations. Invasive ductal carcinoma was discovered to be the most common form. Similarly, 95.6% of patients had invasive ductal carcinoma, according to Khan ME et al. (17). About 70–80% of instances of breast cancer are invasive ductal carcinoma, according to the American Cancer Society (2023). Luminal B was detected in 35.2% of the subjects in this investigation. A study conducted in Pakistan by Alam S et al.18 also reported Luminal B with greater frequency than Luminal A. Compared to Luminal A, the Luminal B sub-type is the most common type among breast cancer patients in the current study; it has a poorer prognosis and a higher risk for metastasis.

Grade-II was the diagnosis made for the majority of cases (78%).

These findings are in line with a research by Ahmed F et al. (2016), which found that 73.2% of patients with breast cancer had Grade-II. Compared to Grade-I tumors, high-grade tumors have a worse prognosis and are linked to higher mortality.In all, six genetic variations in BRCA1 were found. Nucleotide changes in the coding region affect the secondary structure of mRNA, which interferes with translation and protein production[19]. variations Chr17:43092507G>A and Chr17:43094127G>A had higher expected mRNA stability, but variations Chr17:43092418A>G and Chr17:43093220T>C had lower estimated mRNA stability when their Gibbs free energy varied. In-silico analysis was performed on all the discovered variations to check for regulatory miRNA binding. The target BRCA1 transcript of variation Chr17:43093220T>C was not bound by miRNA hsa-miR-1179, according to miRBase. In breast cancer, upregulation of miR-1179 functions as a tumor suppressor and suppresses the Notch signaling system[20]. The lack of an anti-oncogenic regulator and the potential for oncogenesis are indicated by the non-binding of hsa-miR-1179 with BRCA1 mRNA. In these variations, the functional alterations were anticipated. In the coiled-coil domain of BRCA1, which is the interaction location of partner and localizer of BRCA2 (PALB2), a new variation Chr17:43082553A>T (Asn1403Ile) was found and was expected to be harmful. A molecular adapter between the two proteins and BRCA1 is PALB2. Defective homologous recombination (HR) may result from this mutation's alteration of the BRCA1-PALB2 relationship. Tumorigenesis and genomic instability may result from such compromised HR repair [21].

Another unique variation, Chr17:43093710 A>T (Lys607Asn), was found in the nuclear localization sequence (NLS2) and was expected to have negative effects. Importin-α recognizes NSL1 and NSL2, which facilitate the movement of BRCA1 from the cytoplasm to the nucleus. A mutation in these regions can interfere with importin-α's ability to bind with BRCA1, which prevents BRCA1 from translocating into the nucleus and keeps it in the cytoplasm. As a result, BRCA1's capacity to repair DNA in the nucleus will be weakened, which may result in the buildup of unrepaired mutations and chromosomal abnormalities[22]. The study's overall conclusion demonstrates that dysregulation of both transcription and post-transcription happens as a result of BRCA1 mutations. These BRCA1 variations can alter the protein's ability to repair DNA, which can result in oncogenesis. Two new variations in the BRCA1 gene are found in this study. Furthermore, this work illustrates the disruption of miRNA binding, mRNA structure, and stability in addition to highlighting the effect of variations on protein function. These results will also aid in the development of diagnostic biomarkers and additional treatment plans to lower Pakistan's breast cancer incidence. To overcome the selection bias, however, large-scale population-based studies that enlist patients from all parts of the nation are necessary. Although these variants must be validated in a wet lab, in-silico methods offer useful insights to estimate the impact of genetic variants.

CONCLUSION

BRCA1 expression is expected to change in novel variations found in this study. To further clarify and validate the functional significance of the found BRCA1 genetic variation for its involvement in breast oncogenesis and for its potential therapeutic development, however, expressional-based study is needed. Further research is required to examine additional tumor suppressor genes, such as BRCA2, in cases of breast cancer. Additionally, a multi-omics approach ought to be used in order to obtain a more comprehensive molecular knowledge of breast cancer.

Conflict of Interest: None

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