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RESEARCH ARTICLE
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GENETIC VULNERABILITY CAUSE PROPENSITY OF BREAST CANCER

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Objective: The moderate-risk genes (ATM, BARD1, and CHEK2) involve in breast cancer in the Pakistani population.

Abstract:
Breast cancer is a global disease affecting women of different ancestry, with Pakistan contributing to 15% of all cancer deaths. Risk factors include high energy intake, body lethargy, BMI, obesity, type II diabetes, dyslipidemia, high blood pressure, visceral obesity, and blood cholesterol imbalance. Genetic makeup plays a significant role, with mutations in genes like BRCA1, BRCA2, TP53, CHEK2, and RAD51C accounting for 20% of hereditary breast cancer in Pakistan. Moderate-risk genes, such as homozygous ataxia-telangiectasia (ATM), increase the risk of developing breast cancer. Around 20% of inherited breast cancer in Pakistan is caused by variations in high and moderate genetic risk genes. Therapeutic use for these mutations is yet to be established.

Methods:A case-control study at Jinnah Post Graduate Medical Center in Karachi, Pakistan, involved 200 participants aged 15-60 who had undergone medical and surgical intervention, chemotherapy, and radiotherapy. The study focused on ATM, CHEK2, and BARD1 gene variants on NCBI-Clinvar, focusing on hotpots, pathogenic variants, and those associated with breast cancers. Data was analyzed using SPSS, Excel, and chi square and Fisher exact tests for risk factors. The study found significant relationships with study characteristics using logistic and linear regression analysis.

Results: Breast cancer is a growing global disease influenced by genetic, environmental, personal, and lifestyle factors. Moderate-risk genes contribute to 10% of hereditary risk, with over 70% of patients having overexpressed estrogen receptors (ERs) leading to "ER-positive" tumors. Mutations in high and moderate susceptibility genes account for 20%-40% of lifetime breast cancer risk. Genetic testing and prophylactic bilateral mastectomy are effective risk reduction strategies. Mutations in BRCA1/2 gene involvement accelerate cancer growth, increasing ovarian, prostate, pancreatic, and breast cancer risks. A study focusing on triple negative patients and reference gene sequences of ATM, CHEK2, and BARD1 found that a panel of variants is needed for the population

to detect cancer. This knowledge could lead to targeted therapies, tailored approaches to breast cancer treatment, and improved genetic counseling for individuals with mutations in these genes. Obesity is a significant factor in breast cancer cases, as found in Stacy Simon's 2022 study.Breast cancer risk is non-age-dependent, affecting all age groups. American-Indian women have the least risk at 7%, while white, black, Asian/Pacific Islander, and Hispanic women have similar risk.

Conclusion: Risk factors include high energy intake, body lethargy, high BMI, obesity, type II diabetes, high blood pressure, and blood cholesterol imbalance. Mutations in the ATM gene increase the risk of breast cancer, while mutations in the CHEK2 gene decrease it. Around 20% of hereditary breast cancer is accounted for by variants in high and moderate susceptibility genes. Understanding the correlation between these genes iscrucial for refining risk assessment, enabling targeted genetic counseling, and advancing personalized cancer prevention and treatment.

Keywords: breast cancer, ATM, CHEK2, BARD1, Genetic variants, Vulnerability, onset of cancer,

Introduction:

Breast cancer is a globally feared disease with increasing risk among women of different genealogy, causing psychological effects and a high incidence rate.Breast cancer incidence varies globally due to various factors including population-specific reproductive patterns, genetic vulnerability, hormones, dietary habits, radiation exposure, and mammographic screening schemes are determinants of epidemiology of breast cancer(Vadnais C et al. 2012).Pakistan is a major contributor to breast cancer, with 634,000 women dying in 2019 (WHO report, 2019), accounting for 15% of all cancer deaths among women, and 1-in-13 females having a risk of developing it by age 74.The rate of occurrence is more or less similar to those cases reported in the West (Fox et al., 2008).Breast cancer causes an average of 969 deaths in females annually, while males experience 6 deaths annually. Females experience two deaths per five incidences (The National Cancer Registry Ireland; http://www.ncri.ie). Breast cancer affects 38% of female malignancies in Pakistan. Certain risk factors are involved in BCa but the biologist could not explicate the exact reason why a woman develops breast cancer(Iqbal et al., 2015). Suggested Risk factors include high energy intake, body lethargy, BMI, obesity, type II diabetes, dyslipidemia, high blood pressure, visceral obesity, and blood cholesterol imbalance. Genetic makeup also plays a significant role in causing breast cancer, with high-risk genes suppressing tumor development(Camet al., 2010).Mutations in genes like BRCA1, BRCA2, TP53, CHEK2, and RAD51C cause breast cancer, accounting for 20% of hereditary breast cancer in Pakistan. Moderate-risk genes increase the risk by two to fourfold in 10% of BCa in the general population. Investigations into vulnerable genes like ATM, CHEK2, and BARD1 will provide additional insights into the Pakistani population(Masoodet al., 2015).The BRIP1 gene, involved in breast cancer susceptibility, has been extensively studied in British familial breast cancer through mutational screening and sequencing.The discovery of BRIP1 and PA1B2, which are associated with the breast cancer proteins BRCA1 and BRCA2, is crucial for understanding moderate-risk breast cancer susceptibility genes.

Although, 20%-40% of lifetime risk of breast cancers are the mutations in moderate risk genes include homozygous ataxia-telangiectasia (ATM) while tumor suppressor genes like CHEK2, and BRCA1 and BRCA2 modifier genes BRIP1 and PA1B2 confer somatic mutations. Genome-wide association studies reveal low-risk common alleles in the Pakistan population, but their clinical implications are not fully investigated. This study helps examine moderate risk genes and discusses current studies and future research potentials(Hollestelle et al., 2010).Data on low- or moderate-risk variants' contribution to breast cancer is limited, and their effects on BCa are unpredictable. The first breast cancer susceptibility gene, BRCA1, was identified in 1994 but its location on chromosome 17q21 had already been determined in 1990and regulates various physiological processes, including DNA repair and cell-cyclecheckpoint regulation.After one year the BRCA2 gene's location on chromosome 13q12-13 was determined, the BRCA2 gene was found in 1995.It encodes a protein with 27 exons. It has a PA1B2 binding domain, eight BRC repeats, and two N1S.

BRCA2 regulates RAD51's DNA binding and localization. DSS1, an acidic protein, stabilizes BRCA2 and is an evolutionary conserved protein. The ATM gene, responsible for ataxia telangiectasia, is located on chromosome X's long arm.The ATM gene, a crucial protein for regulating bodily functions and maintaining cell genetic stability, is present in 1% of Americans. Mutations in this gene, caused by gene deletions or family members with positive mutations, increase the risk of developing breast cancerFabbroet al., (2004).A study found that women with the pathogenic ATM mutation are more likely to develop breast cancer, similar to germ-line BRCA 2 mutations. The CHEK2 gene, located at 22q12.1, functions as a tumor suppressor. However, this mutation results in a short and non-functional CHEK2 protein, leading to cell survival or apoptosis. The BARD1 gene encodes a protein that binds to BRCA1's N-terminal region.The CHEK2 protein, a crucial component of DNA repair, is disrupted or damaged, collaborating with TP53 and other proteins.

A mutation at 1100de1C causes a short, non-functional CHEK2 protein, leading to cell survival or apoptosis. CHEK2 and BRCA1 interact, with inherited mutations in breast cancer cases(Aquino et al., 2020).The RING motif, a cysteine-rich region in proteins controlling cell development, is crucial for tumour suppression. Mutations in BRCA1, a protein with three tandem ankyrin repeats, can cause breast or ovarian cancer. Intergenic transcript variants in this gene, including high and moderate susceptibility genes, like CHEK2, ATM, BARD1, BRIP1, and PA1B2 account for 20% of hereditary breast cancer (Suszynska et al., 2019).Previous studies show BARD1 mutations are relatively rare in BRCA patients, but their role in cancer predisposition research is not yet solidly supported. This study investigates moderate-risk genes ATM, CHEK2, and BARD1 and their correlation with BRCA1/2 in the Pakistani population.Mutational screening and sequencing of the BRIP1 gene in British familial breast cancer have identified BRIP1 and PA1B2 as moderate-risk susceptibility genes. These genes are linked to breast cancer proteins BRCA1 and BRCA2, contributing to 20%-40% of lifetime breast cancer risk. Clinical implications of these mutations are not fully investigated(Hollestelle et al., 2010).The effects of moderate genes in breast cancer are uncertain. Cell cycle checkpoint kinase 2 (CHEK2), a G2 checkpoint serine/threonine kinase, is involved in breast cancer. Ataxia-telangiectasia-mutated (ATM) mutations phosphorylate CHEK2, affecting other cell-cycle proteins like TP53, CDC25C, CDC25A, and BRCA1.These genes are heavily involved in cancer in breast.Pakistan, a developing country with rural populations, faces challenges in early detection and prevention. Given that it accounts for 40% of all female cancer cases in Pakistan, this disease is a major public health concern (Bray et al., 2018).Around 20% of inherited breast cancer in Pakistan is caused by variations in high and moderate genetic risk genesPrakash et al., 2015). Following gene involve in high and modearate Genetic vulnerability of breast cancer, are summarized in table -1. ATM gene mutations are associated with an increased risk of breast cancer.Research indicates that Asian populations, including Chinese and Japanese, have higher ATM mutation prevalence and CHEK2 mutation prevalence. Some studies showed have a higher prevalence of CHEK2 mutations in Ashkenazi Jews and individuals from Eastern European countries.BARD1 variants may increase susceptibility to breast cancer risk in these populations, providing insights into specific genetic factors contributing to Asian individuals' including China, Korea, and Japan.Moderate-risk breast cancer susceptibility genes, including CHEK2, ATM, BRIP1, PA1B2, and NBS, have been discovered, resulting in two- to four-fold higher chances of developing the disease. These genes often have a small number of mutations, increasing the risk of 20%-40% lifetime cancer. Therapeutic use for these mutations is yet to be established.

Methodology: The study was a case-control, and non-probability purposive sampling was done followed by subject's selection as per predetermined criteria.This study was carried out within one (l) year after the approval of the synopsis and obtaining ethical clearance at the Department of Physiology (BMSI), Department of Radiology and Department of Surgery, Jinnah Post Graduate medical center (JPMC), Karachi, Pakistan in collaboration with the Department of Physiology, University of Karachi, and Karachi, Pakistan.Ethical Consideration was obtained fromthe Institutional Research Board (IRB) of the Jinnah Post Graduate Medical Centre in Karachi,[REF NO.F.2-81/2020-GENL/42311 /JPMC]. The Helsinki Declaration was adhered to when conducting

the study. Both in English and the native tongue, written consent was obtained. The consent form was filled out, signed or thumbprint, later coded and secured.The sample size was calculated using an online sample size calculator, Open epi version 3.01 (http://openepi.com/Menu/OE Menu.htm) for case-controls, after inserting 9.6% proportion of late menopause among cases, (Kelsey et al., Methods in observational Epidemiology 2nd Edition), at 5% margin of error and 95% confidence interval. The required sample size for our study was 200 with 100 participants in each group. Two groups of subjects were formed: the Cases Group, contained 100 known instances of breast cancer in females, and the Control Group, contained 100 healthy people. Cases and controls were recruited using a straightforward random sampling procedure. All Naïve known cases of Breast Ca (Females), between 15-60 years of age, prior to medical and surgical intervention, chemotherapy & radiotherapy and given consent were included in study. Radiological assessment, detailed Hx were taken to make diagnosis**.** People under the age of 15 and above 60 years, as well as those who refused to give their consent, were not included in the study. Due to the unique and complex pathophysiology of medical and surgical co-morbidities were also excluded. Patients and controls were selected using a straightforward random sampling procedure.The participants underwent a questionnaire and anthropometric evaluation. Detailed information about the patient's medical history, symptoms, presenting complaints, ethnicity, family history of breast cancer, family history of residence, diagnosis, co-morbidities, and detailed dietary information were documented**.** Through venipuncture, 10 milliliters of blood were drawn from the study participants. Two 5ml tubes, one in an anti-coagulant tube and the other in a gel tube, were used to collect the material. Qiagen's QIAamp DNA mini kit (Cat. no. 51304) was used to extract DNA from blood as per the manufacturer's instructions.

Genetic analysis

For analysis of our targeted genes, we thoroughly searched for ATM, CHEK2 and BARD1 gene variants on online NCBI-Clinvar, we focused our search on gene hotpots, variants reported by many submitter previously, pathogenic/ likely pathogenic variants and those having tendency for familiar breast cancers. We analyzed from our search that for ATM, CHEK2 and BARD1, exon 8, exon 3 and exon 4 respectively were found to have many reported mutations, nearly around 18 variants in exon 8 (ATM), 24 variants in exon 3 (CHEK2) and 53 variants in exon 4 (BARD1) were found

Homo sapiens ATM serine/threonine kinase (ATM), Ref Seq Gene (LRG_135)

Chromosome #11

NCBI Reference Sequence: NG_009830.1

Size 153268 bp

Total Exons 63

Targeted exon: 8 (18 variants)

PCR Amplification

Primer Sequence for ATM Exon 8

ATML0 F	TTCTAACGCTGATGCAGCTTGAC	NG_009830.1: 29031-29053
ATML0 R	CTGATAGTTTGAAAGGAAT	NG_009830.1: 29423-29441

Product size 4ll bp

MASTER MIX CALCULATION (LX)

2X Master mix	L2.5ul
PRIMER F L0 pmol/ul	Lul
PRIMER R L0 pmol/ul	Lul
DNA 25ng/ul	4ul
WATER	6.5ul

PCR Program

95°C	3 Minutes
95°C	30 Seconds
54°C	60 Seconds
72°C	60 Seconds
72°C	60 Seconds

Ref: Sandoval, N. et all., 1999.

Homo sapiens checkpoint kinase 2 (CHEK2), Ref Seq Gene (LRG_302)
Chromosome #22
NCBI Reference Sequence: NG_008150.2
Size 61124 bp
Total 15 Exons
Targeted exon: 3 (24 variants)
Targeted exon: 3 (24 variants)

PCR Amplification
Primer Sequence for CHEK2 EX02 2&3
Forward: TGCCTTCTTAGGCTATTTTCCTAC NG_008150.2 : 21397-21420
Reverse: AACCATATTCTGTAAGGACAGGAC NG_008150.2 : 21955-21978
Product size 582 bp
MASTER MIX CLACLUTATION (LX)

2X Master mix	L2.5ul
PRIMER F L0 pmol/ul	Lul
PRIMER R L0 pmol/ul	Lul
DNA 25ng/ul	4ul
WATER	6.5ul

PCR Program
95℃ 3 Minutes
95℃ 30 Seconds
56℃ 60 Seconds
72℃ 60 Seconds
72℃ 600 Seconds

Ref: Novak, D.J., 2008.

Homo sapiens BRCA1 associated RING domain l (BARD1), RefSeqGene (LRG_297)
Chromosome # 2
NCBI Reference Sequence: NG_012047.3
Gene Size: 91067 bp
Total Exons: 11
Targeted exon: 4 (53 variants)
Targeted exon ll (53 variants)

PCR Amplification

Primer Sequence for BARD1Gene, exon ll
B42F CAGATGTTTCTGAGAGGGCT NG_012047.3: 33404-33423
B45R CTCCACTGGTGCTCAGAATG NG_012047.3: 33855-33874
Product size 47l bp

MASTER MIX CLACLUTATION (LX)

2X Master mix	L2.5ul
PRIMER F L0 pmol/ul	Lul
PRIMER R L0 pmol/ul	Lul
DNA 25ng/ul	4ul
WATER	6.5ul

PCR Program
95℃ 3 Minutes
95℃ 30 Seconds
55℃ 60 Seconds
72℃ 60 Seconds
72℃ 600 Seconds

Ref: Thai, T.H., et al., 1998.

DNA Sequencing analysis
The PCR products were then purified by ExoSap-IT PCR Product Cleanup kit according to the protocol (catalog no. 78200, Thermo Fisher Scientific). The sequence analysis (R 2 = 0.90) was performed by using a Big Dye Terminator v.3.l Sequencing Kit (catalog no. 4337456, Thermo Fisher Scientific) on SeqStudio Genetic Analyzer (Thermo Fisher Scientific) through CNCD Karachi.Variant analysis was carried out on Bioedit software. Reference gene sequence was

imported from NCBI. Gene sequence files were imported and alignment analysis was carried out.

Statistical Analysis

Both SPSS and Excel formats were used to log and compute the data. To stratify the data based on risk factors and ascertain how these modifiers affected the outcomes, the chi square analysis and fisher exact test were applied as needed. One-way analysis of variance (ANOVA) was applied to examine mean differences between quantitative variables, and in independent sample t-test were applied. Logistic regression was used to assess the result's risk factors. To ascertain genotype and allele frequency, the sample size that produced Hardy-Weinberg equilibrium was assessed. The p-value for each variant was calculated using the standard chi-squared statistics with one degree of freedom. Using logistic and linear regression analysis, significant and considerable relationships with study characteristics were discovered. The cutoff for a significant P value was 0.05. The IBM Statistical Packages for Social Sciences (IBM SPSS version 26; IBM Corp Inc, Armonk, NY) were used for the statistical study.

Results:

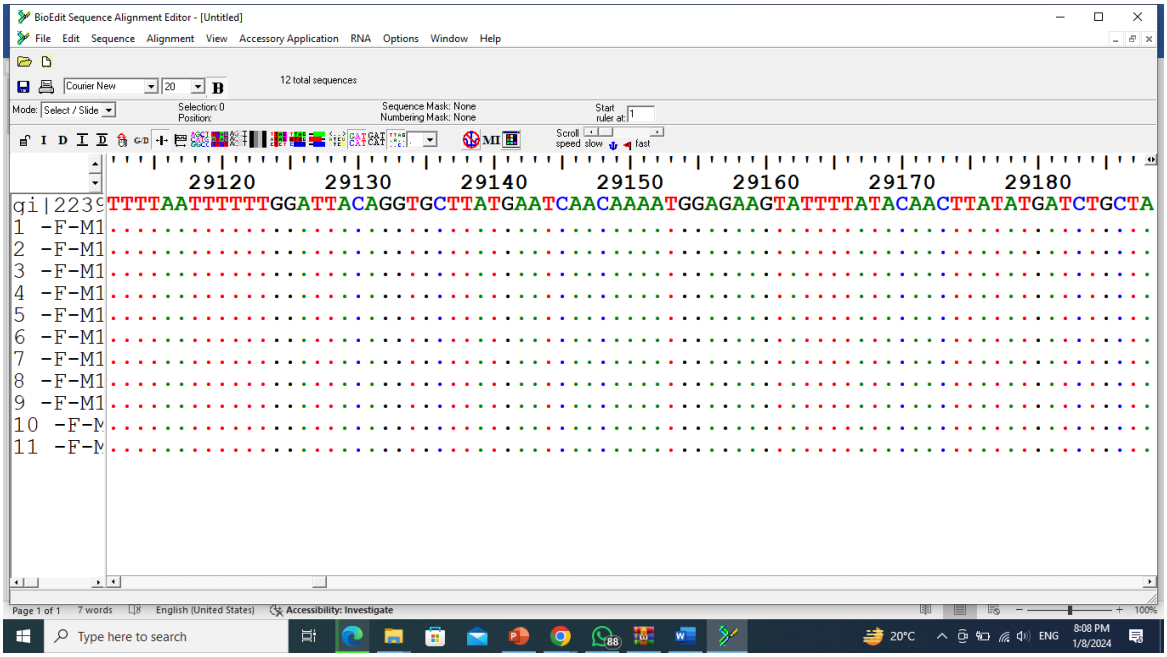
This case control study accomplished in the department of Physiology BMSI, JPMC, Department of Radiology, Department of Surgery, Jinnah Post Graduate medical center (JPMC), Karachi, Pakistan in collaboration with the Department of Physiology, University of Karachi, Karachi, Pakistan.200 individuals were interviewed and collected samples after applying inclusion and exclusion criteria and divided into two groups: i) Group A (cases) diagnosed naive breast cancer patients, Group B (controls)all healthy individuals falling in age limit, without any existing co morbidity All cases were selected from Surgery and Radiology departments, JPMC, Karachi, while the healthy individual's, age and weight matched, selected from friends, and family patient attendants. Data were collected on predefined Performa. Further it was analyzed and processed by using IBM SPSS version26.

SEQUENCING RESULTS

29133..29296 /gene="ATM"EXON 8

NUCLEOTIDE SEQUENCE VIEW

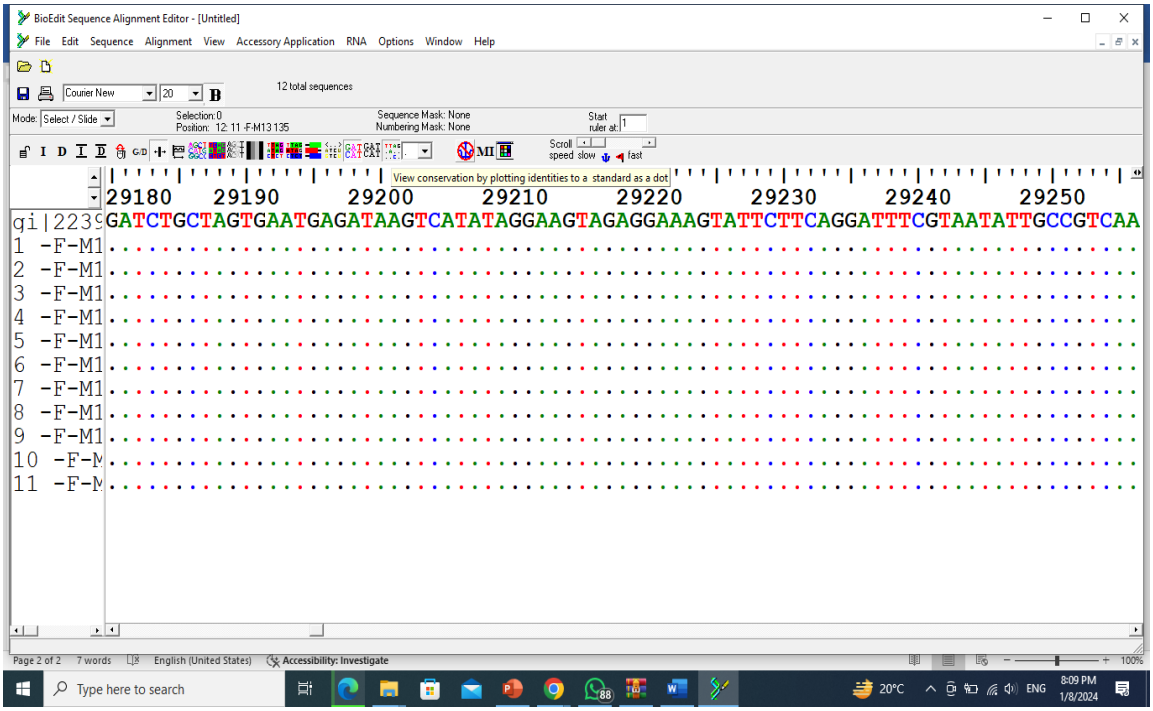
HIDE SIMILARITIES, HIGHLIGHT DIFFERENCE VIEW



29133..29296 /gene="ATM"EXON 8

NUCLEOTIDE SEQUENCE VIEW

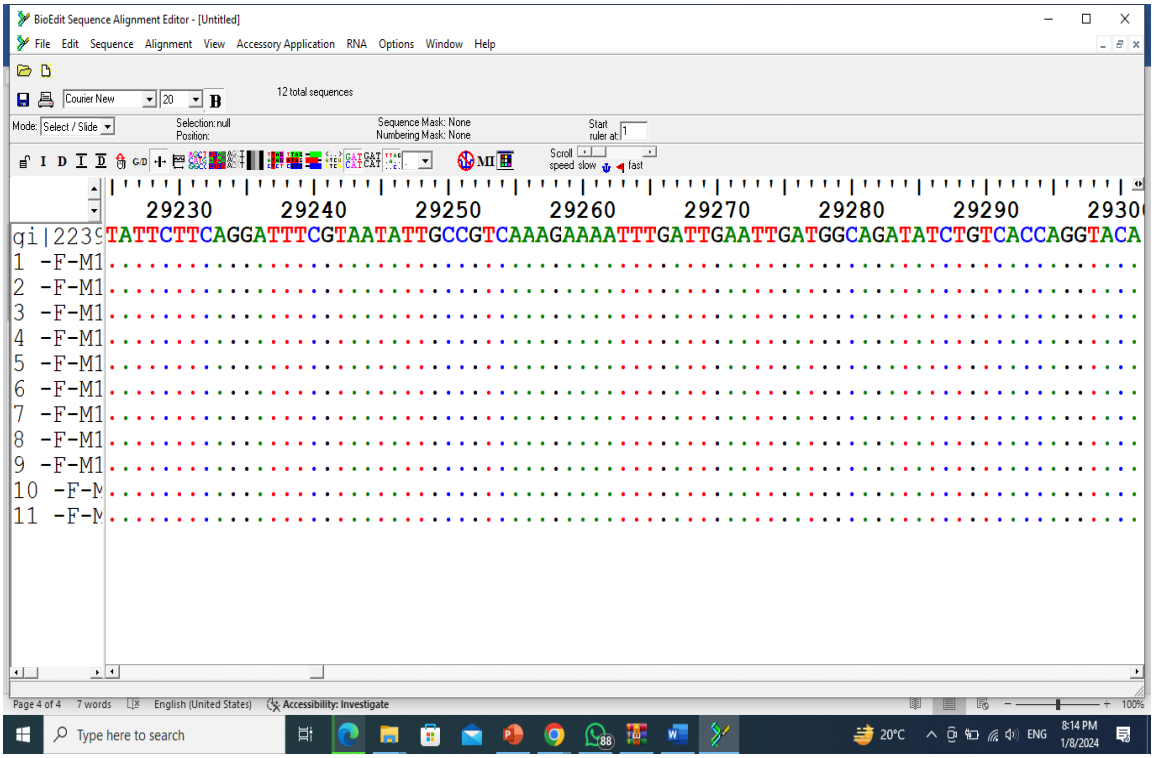
HIDE SIMILARITIES, HIGHLIGHT DIFFERENCE VIEW



29133..29296 /gene="ATM"EXON 8

NUCLEOTIDE SEQUENCE VIEW

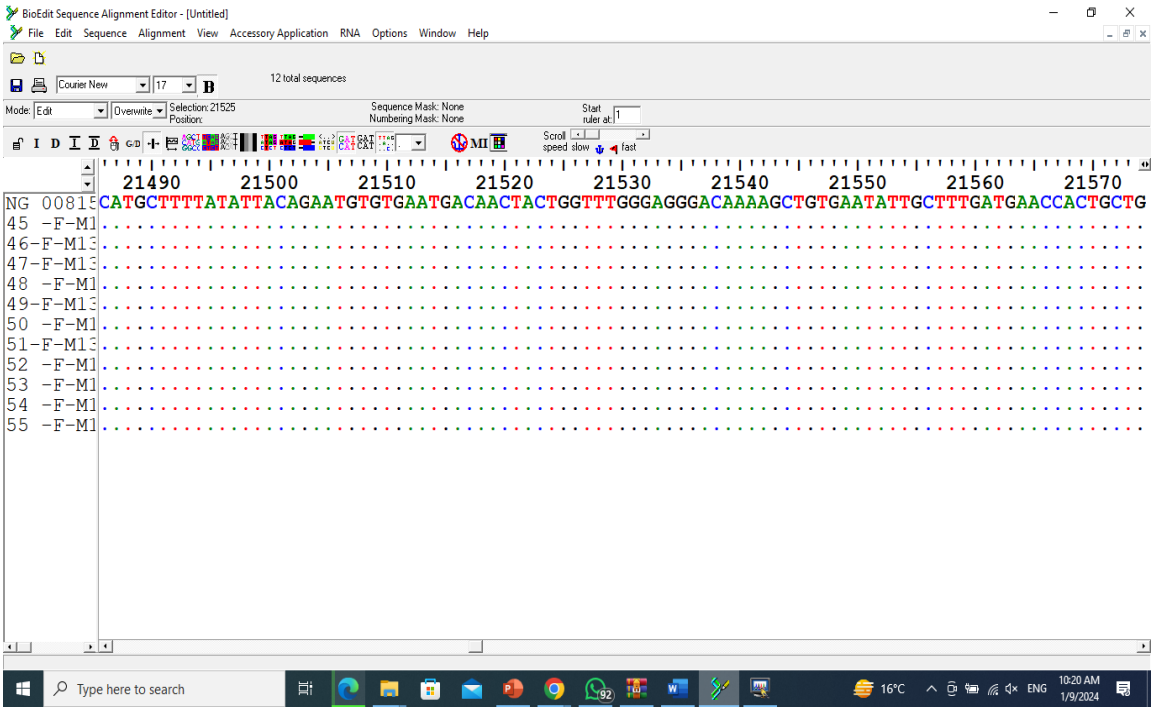
HIDE SIMILARITIES, HIGHLIGHT DIFFERENCE VIEW



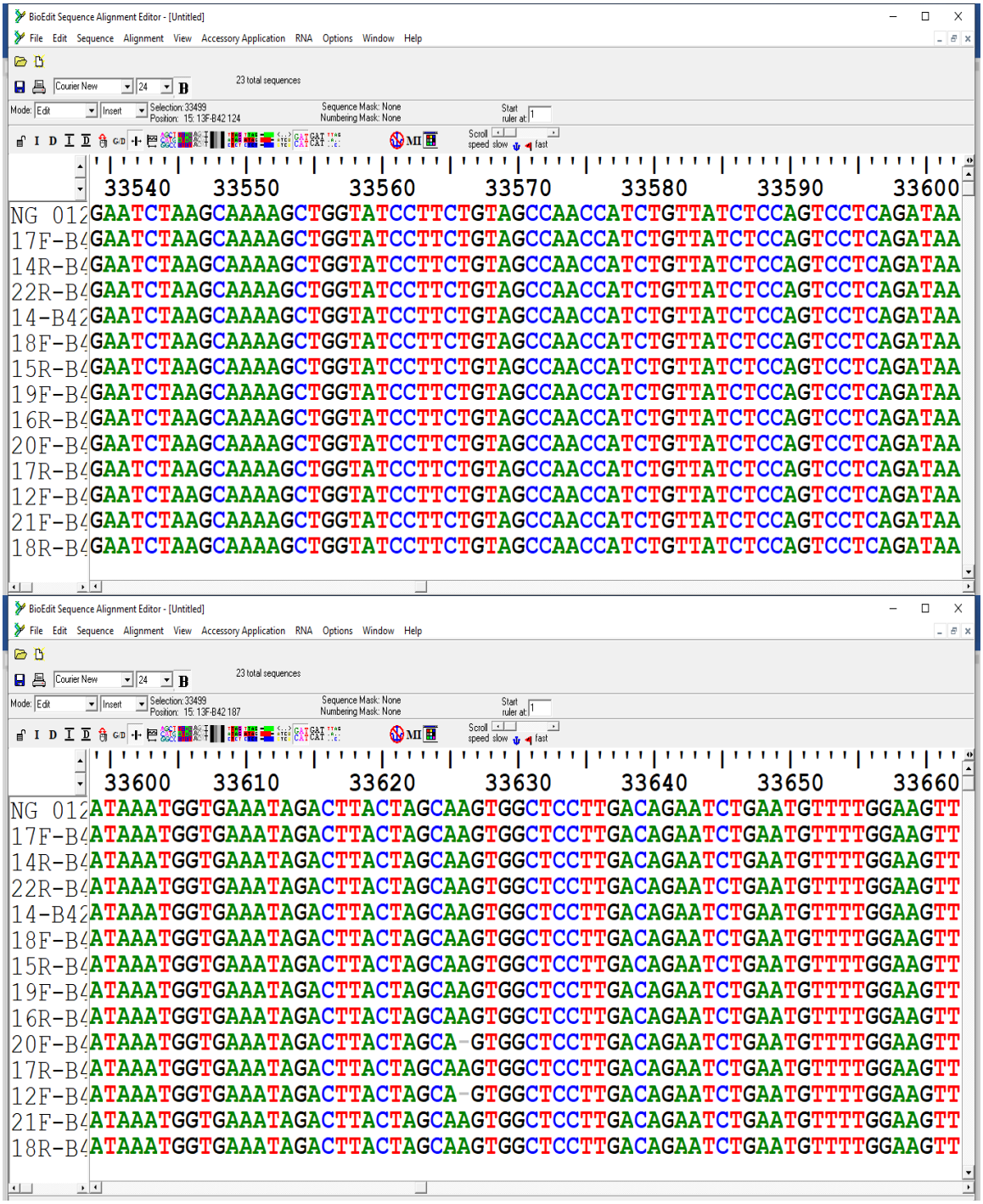
21500..21624 /gene="CHEK2" EXON 3

NUCLEOTIDE SEQUENCE VIEW

HIDE SIMILARITIES, HIGHLIGHT DIFFERENCE VIEW



SEQUENCE RESULTS- BARD1 GENE


BioEdit Sequence Alignment Editor - [Untitled]
File Edit Sequence Alignment View Accessory Application RNA Options Window Help
23 total sequences
33540 33550 33560 33570 33580 33590 33600
NG_012
17F-B4
14R-B4
22R-B4
14-B42
18F-B4
15R-B4
19F-B4
16R-B4
20F-B4
17R-B4
12F-B4
21F-B4
18R-B4
GAATCTAAGCAAAAGCTGGTATCCTTCTGTAGCCAACCATCTGTTATCTCCAGTCCTCAGATAA
BioEdit Sequence Alignment Editor - [Untitled]
File Edit Sequence Alignment View Accessory Application RNA Options Window Help
23 total sequences
33600 33610 33620 33630 33640 33650 33660
NG_012
17F-B4
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18F-B4
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20F-B4
17R-B4
12F-B4
21F-B4
18R-B4
ATAAATGGTGAAATAGACTTACTAGCAAGTGGCTCCTTGACAGAATCTGAATGTTTTGGAAGTT

Discussion

Breast cancer is a growing global disease influenced by genetic, environmental, personal, and lifestyle factors. In Pakistan, 38% of females suffer from the disease, with risk factors including high energy intake, body lethargy, BMI, obesity, type II diabetes, high blood pressure, and cholesterol imbalances. Genetic makeup plays a significant role in breast cancer, with moderate-risk

genes contributing to 10% of hereditary risk.Over 70% of breast cancer patients have overexpressed ERs, leading to "ER-positive" tumors. Tumor development occurs from gene cycle disruption, apoptosis, and DNA repair pathways. Studies show triple negative patients are linked to BRCA1/2, but not BRCA l or 2. The ATM gene, present in l% of Americans, increases the risk of breast cancer, while CHEK2 gene mutations can decrease it.The RING motif in proteins is crucial for cancer development, but a mutation in the CHEK2 protein has been linked to breast cancer. Mutations in BRCA1 cause the BARD1/BRCA1 connection to break down, suggesting a stable complex may be crucial for tumor suppression. Mutations in high and moderate susceptibility genes account for 20%-40% of lifetime breast cancer risk.Moderate genes, including ATM mutations and CHEK2 mutations, are associated with a 20%-40% lifetime risk of breast cancer in Pakistan, accounting for 40% of inherited cases. Healthcare infrastructure for early detection and prevention is lacking, accounting for 40% of inherited cases.Breast cancer is a complex disease with unique pathological characteristics, with BRCA1 and BRCA2 carriers having unique characteristics. The discovery of genetic variants and their impact on risk assessment is crucial. Family risk factors increase as affected relatives increase, and mutations in BRCA1 and BRCA2 increase the risk of breast and ovarian cancer. Mutation carriers with a strong family history are at higher risk.Breast cancer risk is influenced by genes like BRCA1, P53, CDHl, PTEN, and lKBl, with modest genetic influence from other genes.

Breast cancer susceptibility genes l and 2 increase risk, while BRCA1 and BRCA2 are associated with aggressive cancers. BRCA1/2 gene involvement accelerates cancer growth, increasing ovarian, prostate, pancreatic, and breast cancer risks. Genetic testing and prophylactic bilateral mastectomy are effective risk reduction strategies.Research on the relationship between ATM, CHEK2, and BARD1 genes and BRCA l/2 genes associated with breast cancer is crucial. Mutations in these genes may increasesusceptibility to these diseases. BARD1, a protein interacting with BRCA1, may modify BRCA1/2-associated cancer risk.The study targets genes ATM, CHEK2, and BARD1 for their potential role in breast cancer susceptibility. These genes are moderate risk genes, and understanding their interactions with high-risk genes like BRCA1 and BRCA2 is crucial for understanding hereditary breast cancer. This knowledge could lead to more effective risk assessment and management strategies.The study focused on triple negative patients and reference gene sequences of ATM, CHEK2, and BARD1. It searched for variants on NCBI-Clinvar, focusing on hotpots, pathogenic variants, and those with familiar breast cancers. Exons 8 (ATM), 3 (CHEK2), and 4 (BARD1) had numerous reported mutations.The study found that a panel of variants, excluding those mentioned in the study, is needed for the population to detect cancer. This panel would increase sample size and include multiple zones, considering the significant role of environment, diet, and genetic makeup.Research on the genetic correlations between genes like ATM, CHEK2, and BARD1 is crucial for improving risk assessment, genetic counseling, and personalized cancer prevention and treatment. This knowledge could lead to targeted therapies, tailoredapproaches to breast cancer treatment, and improved genetic counseling for individuals with mutations in these genes.Stacy Simon's 2022 study found that obese women are more likely to develop breast cancer. Our study supports this, finding that increased BMI is a significant factor in breast cancer cases. The risk of developing breast cancer is not age-dependent, with disease progression observed in all age groups, indicating that the disease can develop at any stage of life.A 2017 study found that white, black, Asian/Pacific Islander, and Hispanic women have a similar breast cancer risk, with American-Indian women at the least risk at 7%. Despite analyzing different patient groups, no particular group showed strong significance in breast cancer occurrence.

Conclusion

Breast cancer is a growing global disease influenced by genetic, epigenetic, environmental, and lifestyle factors. This study aimed to identify genetic variations in tumor suppressor genes associated with breast cancer in Pakistan. Risk factors include high energy intake, body lethargy, high BMI, obesity, type II diabetes, high blood pressure, and blood cholesterol imbalance.Our study reveals that many triple negative breast cancer patients are not linked to BRCA1/2, suggesting a

potential genetic cause in Asian populations. Mutations in the ATM gene increase the risk of breast cancer, while mutations in the CHEK2 gene decrease it. Mutations in BRCA1 may be the cause of breast or ovarian cancer. Around 20% of hereditary breast cancer is accounted for by variants in high and moderate susceptibility genes.Understanding the correlation between ATM, CHEK2, and BARD1 genes and BRCA l/2, two moderate risk genes associated with breast cancer, is crucial for refining risk assessment, enabling targeted genetic counseling, and advancing personalized cancer prevention and treatment. This research also helped to identify implications for genetic counseling, enabling more accurate risk prediction and informed decision-making for individuals with mutations in these genes. Our study found multiple ethnic groups in our subjects, from single zone, as every ethnic group has different genetic make up. Hence large and multi center study is a need to help the pateints.Researchers analyzed genetic factors in ATM, CHEK2, and BARD1 genes, focusing on hotpots, pathogenic variants, and familiar breast cancers, after selecting the exons with more reported mutations in every gene were selected to run for sequencing, but despite analyzing nearly 100 variants from each genes, we could not find any promising results, which is mainly because of two reasons, firstly our study had multiple ethnic groups, from single zone, as every ethnic group has different genetic make up. Therefore we could not generalized our findings on single ethnic group, Hence large and multi center study is a need to help the pateints. Secondly due to financial constraints, full genome sequencing could not be done on entire sample size, Hence full gene sequencing may reveal contributions of these genes in our population.

Recommendation: Breast cancer incidences varies with triple negative status. Keeping this in mind, more treatment approaches and more research is needed to understand what drives TNBC's growth and metastasis and to find better therapies for a good prognosis. Further gene markers identification should be made reachable and affordable for the targeted population for early detection of mutated genes, which can lead to breast cancer development.

Data Availability statement

All data generated or analyzed during the study are included in the manuscript.

Ethics approval and consent to participate. Approved by the department concerned. (IRB/JPMC-8-21/R091)

Consent for publication: Approved

Funding: Not applicable

Conflict of interest: The authors declared the absence of a conflict of interest.

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Table 2: Summary Table for ATM Gene: ClinVar, Search Results Targeted exon: 8 (18 variants)

Rs Number / dbSNP	Protein level	RNA level	DNA level	Variant Classification	variant Condition	Pr y rep in
rs1591510553	(p.Trp308Ter)	NM_000051.4(ATM):c.923G>A	NG_009830.1:g.29154G>A	**P	*F.Ca	2
rs1591510597	(p.Ser310fs)	NM_000051.4(ATM):c.929del	NG_009830.1:g.29160del	**P	*F.Ca	3
rs768024233	(p.Leu315fs)	NM_000051.4(ATM):c.943_944del	NG_009830.1:g.29174_29175del	**P	*F.Ca	3
rs2135265770	(p.Tyr316fs)	NM_000051.4(ATM):c.945dup	NG_009830.1:g.29176dup	**P	*F.Ca	3
rs1555068404	(p.Glu322fs)	NM_000051.4(ATM):c.964_968del	NG_009830.1:g.29195_29199del	**P	*F.Ca	2
rs587781511	(p.I1e323Val)	NM_000051.4(ATM):c.967A>G	NG_009830.1:g.29198A>G	**P	*F.Ca	7
rs2135266573	(p.Ser324fs)	NM_000051.4(ATM):c.972_979del	NG_009830.1:g.29203_29210del	**P	*F.Ca	2
rs876658832	(p.Ile326fs)	NM_000051.4(ATM):c.977_978del	NG_009830.1:g.29206TA[l]	**P	*F.Ca	3
rs1060501684	(p.Lys33lfs)	NM_000051.4(ATM):c.992del	NG_009830.1:g.29223del	**P	*F.Ca	2
	(p.Ser334Ter)	NM_000051.4(ATM):c.1001C>G	NG_009830.1:g.29232C>G	**P	*F.Ca	3
rs1555068471	(p.G1y335Ter)	NM_000051.4(ATM):c.1003G>T	NG_009830.1:g.29234G>T	**P	*F.Ca	3
rs1469427343	(p.I1e339fs)	NM_000051.4(ATM):c.1017del	NG_009830.1:g.29248del	**P	*F.Ca	15
rs587780612	(p.Glu343fs)	NM_000051.4(ATM):c.1027_1030del	NG_009830.1:g.29258_29261del	**P	*F.Ca	5
rs1565375817	(p.Met349fs)	NM_000051.4(ATM):c.1045_1052del	NG_009830.1:g.29276_29283del	**P	*F.Ca	5
rs587781984	(p.Ile352fs)	NM_000051.4(ATM):c.1053dup	NG_009830.1:g.29284dup	**P	*F.Ca	2
rs1555068609	(p.Cys353fs)	NM_000051.4(ATM):c.1058_1059del	NG_009830.1:g.29289_29290del	**P	*F.Ca	10
rs1555068615	(p.Gln355Ter)	NM_000051.4(ATM):c.1063C>T	NG_009830.1:g.29294C>T	**P	*F.Ca	2
rs201089102		NM_000051.4(ATM):c.1065+1G>T	NG_009830.1:g.29297G>T	**P	*F.Ca	2

**P (pathogenic) and *F.Ca (Familiar Cancer)

Table 3: Summary Table for CHEK2 Gene: ClinVar, Search Results Targeted exon 3 (24 variants)

Rs Number	Protein level	RNA level	DNA level	Variant classification	variant Condition	Prev repo clin
rs2053967484	(p.Glu107_Cys108insTer)	NM_007194.4(CHEK2):c.32l_324del	NG_008150.2:g.21501_21504del	**P	*F.Ca	2
	(p.Tyr113Ter)	NM_007194.4(CHEK2):c.339C>A	NG_008150.2:g.21519C>A	**P	*F.Ca	3
rs905674348	(p.Tyr113Ter)	NM_007194.4(CHEK2):c.339C>G	NG_008150.2:g.21519C>G	**P	*F.Ca	6
	(p.Trp114Ter)	NM_007194.4(CHEK2):c.34lG>A	NG_008150.2:g.2152lG>A	**P	*F.Ca	3
rs756949505	(p.Trp114Ter)	NM_007194.4(CHEK2):c.342G>A	NG_008150.2:g.21522G>A	**P	*F.Ca	6
rs756949505	(p.Trp114Ter)	NM_007194.4(CHEK2):c.342G>A	NG_008150.2:g.21522G>A	**P	*F.Ca	6
rs2146071079	(p.Arg117fs)	NM_007194.4(CHEK2):c.349del	NG_008150.2:g.21529del	**P	*F.Ca	2
rs28909982	(p.Arg117Gly)	NM_007194.4(CHEK2):c.349A>G	NG_008150.2:g.21529A>G	**P	*F.Ca	40
	(p.Asp118fs)	NM_007194.4(CHEK2):c.353del	NG_008150.2:g.21533del	**P	*F.Ca	2
rs1601825829	(p.Cys121fs)	NM_007194.4(CHEK2):c.360del	NG_008150.2:g.21540del	**P	*F.Ca	2
rs786203355	(p.Glu122fs)	NM_007194.4(CHEK2):c.364del	NG_008150.2:g.21544del	**P	*F.Ca	2
rs1555927302	(p.Glu122fs)	NM_007194.4(CHEK2):c.366del	NG_008150.2:g.21546del	**P	*F.Ca	4
rs876661050	(p.Phe125fs)	NM_007194.4(CHEK2):c.372del	NG_008150.2:g.21552del	**P	*F.Ca	4
rs1275395933	(p.Lys131Ter)	NM_007194.4(CHEK2):c.39lA>T	NG_008150.2:g.2157lA>T	**P	*F.Ca	3
rs1569158640	(p.Thr133_Asp134insTer)	NM_007194.4(CHEK2):c.400_40ldel	NG_008150.2:g.21580_2158ldel	**P	*F.Ca	2
rs730881699	(p.Lys135fs)	NM_007194.4(CHEK2):c.405del	NG_008150.2:g.21585del	**P	*F.Ca	5
rs911826883	(p.Tyr136Ter)	NM_007194.4(CHEK2):c.408C>G	NG_008150.2:g.21588C>G	**P	*F.Ca	2

Rs Number	Protein level	RNA level	DNA level	Variant classification	variant Condition	Prev repo clin
rs730881701	(p.Arg137Ter)	NM_007194.4(CHEK2):c.409C>T	NG_008150.2:g.21589C>T	**P	*F.Ca	17
rs200917541	(p.Tyr139Ter)	NM_007194.4(CHEK2):c.417C>A	NG_008150.2:g.21597C>A	**P	*F.Ca	9
	(p.His143fs)	NM_007194.4(CHEK2):c.427_429delinsA	NG_008150.2:g.21607_21609delinsA	**P	*F.Ca	9
rs1555927148	(p.Arg145fs)	NM_007194.4(CHEK2):c.432dup	NG_008150.2:g.21612dup	**P	*F.Ca	2
rs1555927148	(p.Arg145fs)	NM_007194.4(CHEK2):c.432del	NG_008150.2:g.21612del	**P	*F.Ca	2
rs1555927137	(p.Arg145fs)	NM_007194.4(CHEK2):c.433del	NG_008150.2:g.21613del	**P	*F.Ca	5
rs1064793780		NM_007194.4(CHEK2):c.444+1del	NG_008150.2:g.21625del	**P	*F.Ca	2
rs121908698		NM_007194.4(CHEK2):c.444+1G>T	NG_008150.2:g.21625G>T	**P	*F.Ca	8
rs560596101		NM_007194.4(CHEK2):c.444+2T>C	NG_008150.2:g.21626T>C	**P	*F.Ca	2

****P (pathogenic) and *F.Ca (Familiar Cancer).**

Table 4:Summary Table for BARD1Gene: ClinVar, Search Results Targeted exon ll (53 variants)

Rs Number	Protein level	RNA level	DNA level	Variant classification	Variant condition	Pre y r in c
rs758972589	(p.Arg112Ter)	NM_000465.4(BARD1):c.334C>T	NG_012047.3:g.22385C>T	**P	*F.Ca	9
	(p.Ser121Ter)	NM_000465.4(BARD1):c.362C>G	NG_012047.3:g.22413C>G	**P	*F.Ca	3
rs1420243208	(p.Lys139fs)	NM_000465.4(BARD1):c.415_416del	NG_012047.3:g.33253_33254del	**P	*F.Ca	4
rs587781806	(p.Trp146Ter)	NM_000465.4(BARD1):c.438G>A	NG_012047.3:g.33276G>A	**P	*F.Ca	4
rs155362268l	(p.Ser148Ter)	NM_000465.4(BARD1):c.441dup	NG_012047.3:g.33279dup	**P	*F.Ca	4
rs7308814ll	(p.Arg150Ter)	NM_000465.4(BARD1):c.448C>T	NG_012047.3:g.33286C>T	**P	*F.Ca	ll
rs1553622669	(p.Arg155Ter)	NM_000465.4(BARD1):c.463A>T	NG_012047.3:g.3330lA>T	**P	*F.Ca	3
rs750350372	(p.Gln164Ter)	NM_000465.4(BARD1):c.490C>T	NG_012047.3:g.33328C>T	**P	*F.Ca	2
rs786202500	(p.Gln166Ter)	NM_000465.4(BARD1):c.496C>T	NG_012047.3:g.33334C>T	**P	*F.Ca	4
	(p.Lys17lfs)	NM_000465.4(BARD1):c.512_513del	NG_012047.3:g.33350_3335ldel	**P	*F.Ca	2
	(p.Asp172fs)	NM_000465.4(BARD1):c.513del	NG_012047.3:g.3335ldel	**P	*F.Ca	2
rs746325928	(p.Asp172fs)	NM_000465.4(BARD1):c.513dup	NG_012047.3:g.3335ldup	**P	*F.Ca	3
rs1574821129	(p.Ala173fs)	NM_000465.4(BARD1):c.517dup	NG_012047.3:g.33355dup	**P	*F.Ca	3
rs1574821103	(p.Ser174fs)	NM_000465.4(BARD1):c.518dup	NG_012047.3:g.33356dup	**P	*F.Ca	2
rs776851287	(p.Gln176Ter)	NM_000465.4(BARD1):c.526C>T	NG_012047.3:g.33364C>T	**P	*F.Ca	2
rs779427628	(p.Ser179_Tyr180insTer)	NM_000465.4(BARD1):c.539_540del	NG_012047.3:g.33375AT[l]	**P	*F.Ca	7
	(p.Arg194fs)	NM_000465.4(BARD1):c.580_58ldel	NG_012047.3:g.33416AG[l]	**P	*F.Ca	3
rs876660761	(p.Ala198fs)	NM_000465.4(BARD1):c.592del	NG_012047.3:g.33430del	**P	*F.Ca	2
rs1574820590	(p.Ala200fs)	NM_000465.4(BARD1):c.596_597dup	NG_012047.3:g.33434_33435dup	**P	*F.Ca	2
rs730881415	(p.Gly203Ter)	NM_000465.4(BARD1):c.607G>T	NG_012047.3:g.33445G>T	**P	*F.Ca	
rs1559425604	(p.Lys205fs)	NM_000465.4(BARD1):c.614del	NG_012047.3:g.33452del	**P	*F.Ca	
rs1559425604	(p.Gln206fs)	NM_000465.4(BARD1):c.614dup	NG_012047.3:g.33452dup	**P	*F.Ca	5
rs587780033	(p.Lys209fs)	NM_000465.4(BARD1):c.623dup	NG_012047.3:g.3346ldup	**P	*F.Ca	8
rs1695009134	(p.Lys208fs)	NM_000465.4(BARD1):c.623_624insT	NG_012047.3:g.3346l_33462insT	**P	*F.Ca	3
rs1553622558	(p.Lys209fs)	NM_000465.4(BARD1):c.624dup	NG_012047.3:g.33462dup	**P	*F.Ca	5
rs864622223	(p.Lys209fs)	NM_000465.4(BARD1):c.627_628del	NG_012047.3:g.33465_33466del	**P	*F.Ca	7

Rs Number	Protein level	RNA level	DNA level	Variant classification	Variant condition	Pre y r in c
rs762171436	(p.Leu211Ter)	NM_000465.4(BARD1):c.632T>G	NG_012047.3:g.33470T>G	**P	*F.Ca	4
	(p.Thr210_Leu2llinsTer)	NM_000465.4(BARD1):c.632del	NG_012047.3:g.33470del	**P	*F.Ca	2
rs762171436	(p.Leu211Ter)	NM_000465.4(BARD1):c.632T>A	NG_012047.3:g.33470T>A	**P	*F.Ca	5
rs999444545	(p.Leu211fs)	NM_000465.4(BARD1):c.632dup	NG_012047.3:g.33470dup	**P	*F.Ca	2
rs1553622542	(p.Gln216fs)	NM_000465.4(BARD1):c.646del	NG_012047.3:g.33484del	**P	*F.Ca	4
rs1553622534	(p.Trp218Ter)	NM_000465.4(BARD1):c.653G>A	NG_012047.3:g.3349lG>A	**P	*F.Ca	4
rs1553622530	(p.Trp218Ter)	NM_000465.4(BARD1):c.654G>A	NG_012047.3:g.33492G>A	**P	*F.Ca	2
rs587781671	(p.Asn219fs)	NM_000465.4(BARD1):c.657_660del	NG_012047.3:g.33495_33498del	**P	*F.Ca	2
rs1064793049	(p.Glu225Ter)	NM_000465.4(BARD1):c.673G>T	NG_012047.3:g.335llG>T	**P	*F.Ca	3
rs905491643	(p.Glu234Ter)	NM_000465.4(BARD1):c.700G>T	NG_012047.3:g.33538G>T	**P	*F.Ca	2
rs587780035	(p.Gln237Ter)	NM_000465.4(BARD1):c.709C>T	NG_012047.3:g.33547C>T	**P	*F.Ca	3
rs210611087l	(p.Leu239fs)	NM_000465.4(BARD1):c.716del	NG_012047.3:g.33554del	**P	*F.Ca	3
rs587781430	(p.Gln245Ter)	NM_000465.4(BARD1):c.733C>T	NG_012047.3:g.33571C>T	**P	*F.Ca	5
rs2106110712	(p.Ser247fs)	NM_000465.4(BARD1):c.740del	NG_012047.3:g.33578del	**P	*F.Ca	2
rs1559425098	(p.Ile249fs)	NM_000465.4(BARD1):c.74l_750del	NG_012047.3:g.33579_33588del	**P	*F.Ca	2
rs1553622471	(p.Pro252fs)	NM_000465.4(BARD1):c.755del	NG_012047.3:g.33593del	**P	*F.Ca	2
rs1060501288	(p.Glu270Ter)	NM_000465.4(BARD1):c.808G>T	NG_012047.3:g.33646G>T	**P	*F.Ca	3
	(p.Glu270fs)	NM_000465.4(BARD1):c.810del	NG_012047.3:g.33648del	**P	*F.Ca	2
rs765629130	(p.Leu275Ter)	NM_000465.4(BARD1):c.824T>A	NG_012047.3:g.33662T>A	**P	*F.Ca	2
	(p.Leu275fs)	NM_000465.4(BARD1):c.824dup	NG_012047.3:g.33662dup	**P	*F.Ca	2
rs876659752	(p.Leu280fs)	NM_000465.4(BARD1):c.838_839del	NG_012047.3:g.33676_33677del	**P	*F.Ca	5
rs2106110129	(p.Gln285_Ile286insTer)	NM_000465.4(BARD1):c.856del	NG_012047.3:g.33694del	**P	*F.Ca	2
rs1553622397	(p.Glu287fs)	NM_000465.4(BARD1):c.858dup	NG_012047.3:g.33696dup	**P	*F.Ca	4
rs786201868	(p.Glu287fs)	NM_000465.4(BARD1):c.860_86ldel	NG_012047.3:g.33696AG[l]	**P	*F.Ca	4
rs1553622378	(p.Lys292Ter)	NM_000465.4(BARD1):c.873dup	NG_012047.3:g.337lldup	**P	*F.Ca	4
rs1574818696	(p.Va1298fs)	NM_000465.4(BARD1):c.89l_895del	NG_012047.3:g.33729_33733del	**P	*F.Ca	2
rs2106109750	(p.Thr309fs)	NM_000465.4(BARD1):c.925dup	NG_012047.3:g.33763dup	**P	*F.Ca	2

**P (pathogenic) and *F.Ca (Familiar Cancer).

Table 1.1: List of encoded proteins and functions of High and moderate Genetic vulnerable genes of breast cancer

Gene Name	Name of Encoded Protein	Function
ATM	ATM	DDR Signaling
BARD1	BARD1	HDR
BRCA1	BRCA1	HDR
BRCA2	BRCA2	HDR
BRIP1	BRIP1/FANCJ	HDR
CDH1	E-Cadherin	Cell-Cell Adhesion
CHEK2	CHK2	DDR Signaling
MRE11A	MRE11A/MRE11	HDR & DDR Signaling
MUTYH	MYH	BER
NBN	Nibrin/NBS1	HDR & DDR Signaling
NF1	Neurofibromin	Cytoplasmic Signaling
PALB2	PALB2	HDR
PTEN	PTEN	Cytoplasmic Signaling
RAD50	RAD50	HDR & DDR Signaling
RAD51C	RAD51C	HDR
RAD51D	RAD51D	HDR
TP53	TP53/p53	Transcription Factor