RESEARCH ARTICLE

Genetic Profiling of GADD45A (1506T>C) Single Nucleotide Polymorphism rs3783469 in Breast Cancer Development

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Abstract

Objective: To assess the association between the growth arrest and deoxyribonucleic acid damage 45 alpha (1506 thymine > cytosine) polymorphism (reference single nucleotide polymorphism cluster ID 3783469) and breast cancer development in Pakistani females.

Method: The case-control study was conducted at Riphah International University, Islamabad, Pakistan, in collaboration with Pakistan Railway General Hospital, Rawalpindi, Pakistan, from November 22, 2023, to October 8, 2024, and comprised diagnosed cases of breast cancer in group 1 and healthy controls in group 2. Blood samples were collected, and allele-specific tetra amplification refractory mutation system polymerase chain reaction and gel electrophoresis were carried out to determine the association of respective genotypes and allele frequencies using specific primers. Data was analysed using SPSS 27.

Results: Of the 340 women, 170(50%) with mean age 51.06±9.74 years (range: 30-76 years) were in group 1, and 170(50%) with mean age 56.84±11.5 years (range: 26-82 years) were in group 2. The thymine-cytosine genotype was more frequent in group A compared to group B [110(64.7%) vs. 33(19.4%)], while the thymine-thymine genotype predominated in group B [137(80.6%) vs. 60(35.3%); p<0.0001]. The cytosine-cytosine genotype was absent in both the groups. Genotype distribution and allele frequencies were significantly associated with breast cancer risk (p<0.0001). The associations were significant regardless of age stratification p<0.05).

Conclusion: The incidence of breast cancer development was found to be elevated among females carrying the thymine-cytosine genotype, whereas the thymine-thymine genotype played a protective role among the healthy subjects.

Key Words: Breast cancer, GADD45A, Single nucleotide polymorphism.

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Introduction

Breast carcinoma is a significant global health concern, with the World Health Organisation (WHO) reporting approximately 2.3 million new cases and 685,000 deaths in 2023.[1] According to 2022 WHO cancer statistics for Pakistan, breast cancer accounted for an estimated 31.3% of cancer incidence and 32.4% of prevalence among females of all ages, based on Global Cancer Observatory (GLOBOCAN) databases provided by the International Agency for Research on Cancer (IARC).[2] In Pakistan, breast cancer ranked first among top-10 malignancies diagnosed at Shaukat Khanam Memorial Cancer Hospital and Research Centre (SKMCH&RC) during the year 2023.[3]

The growth arrest and deoxyribonucleic acid damage 45 alpha (GADD45A) gene, also known as deoxyribonucleic acid (DNA) damage-inducible transcript (DDIT) 1 protein,[4] is located on chromosome 1, and produces a 165-amino acid protein regulated by the tumour suppressor protein p53. It plays a crucial role in responding to DNA damage, mediating apoptosis, stress signalling, cell cycle arrest, and DNA repair in the Gap 2 to Mitosis (G2/M) phase.[5]

Research has linked GADD45A expression to environmental stressors, like radiation and starvation, emphasising its role in growth arrest and DNA repair mechanisms. Its activation can occur through p53-dependent or p53-independent pathways, triggered by ionising radiations, like X-rays, alpha (α), beta (ß) and gamma rays, or non-ionising radiations, like ultraviolet (UV) and microwaves.[6]

A study identified a GADD45A variant (1506 thymine > cytosine [T>C]) in ovarian carcinoma patients in the Chinese population, suggesting that this variant may alter transcription factor binding, influencing gene expression and tumour development. This gene's dysregulation has been implicated in cancer progression due to its roles in cell growth, apoptosis and DNA repair mechanisms.[7]

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Despite growing evidence regarding the role of GADD45A polymorphisms in carcinogenesis, data from the Pakistani population, to our knowledge, has remained limited. Considering ethnic variations in genetic susceptibility and the high burden of breast cancer in Pakistan, evaluating population-specific genetic risk factors is essential. The current study was planned to determine the association and risk of GADD45A (1506T>C) polymorphism, and to assess its distribution across genotype and age groups in Pakistani samples.

Patients and Methods

The case-control study was conducted from November 22, 2023, to October 8, 2024, at the Biochemistry Department and Multidisciplinary Laboratory of Islamic International Medical College (IIMC), Riphah International University, Islamabad, Pakistan, in collaboration with Pakistan Railway General Hospital, Rawalpindi, Pakistan. After approval from the institutional ethics review board, the sample size was calculated using Open Epi calculator,[8] for a case-control ratio of 1:1, with odds ratio (OR) 1.90, confidence interval (CI) 95%, power 80%, 53.2% exposure among controls and 68.3% exposure among the cases.[7] The sample was raised using non-probability convenience sampling technique. Those included were women diagnosed with breast cancer regardless of histological grade and type, with no precancerous conditions or any other chronic disease. Those excluded were women with genetic syndromes, endocrinological abnormalities, any other accompanying malignancy both at present and in the past. Age- and ethnicity-matched healthy controls were enrolled from the general population. The diagnosed cases of breast cancer formed group 1 and healthy controls formed group 2.

After taking written informed consent from all the subjects, 3ml peripheral blood was withdrawn, genomic DNA was extracted using 7% Chelex solution (Bio-Rad Laboratories, Inc, USA) and was stored at -80˚C for further analysis.[9] Genotyping of GADD45A (1506T>C) reference single nucleotide polymorphism (SNP) cluster ID 3783469 (rs3783469) was performed by tetra-primer amplification refractory mutation system polymerase chain reaction (Tetra-ARMS PCR) in a thermal cycler (Major Sciences, United States). Primers were designed in the light of data available online.[10] Four sets of primers were used for the amplification of T and C alleles. Two were outer forward (FO-5'-CCTGCACTGCGTGCTGGTGACGGTAAGG-3') and reverse (RO-5'- ATCTCTTCCGCTGCTGGCAGTCCCCAGC-3'). Two were inner allele-specific forward (T-allele-5'-GTGGGGGTCAGGAGGGTGGCTGCCGTT-3') and reverse (C-allele-5'-TGAAAGTCCAGCCACACTCTAGTCGGCCG-3'). PCR amplification was performed in a final volume of 25µl in a 0.2ml PCR tube containing 2µl of genomic DNA, 12.5µl 2x Thermo Scientific PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) which consisted of 0.05U/µl Taq DNA polymerase, reaction buffer, 4ml MgCl (Magnesium chloride), 4mM of each dNTP (Deoxyribonucleotide triphosphates), 0.5µl of primer mix (Macrogen, South Korea) and 10µl of PCR water (Invitrogen, Thermo Fisher Scientific, USA), in a Veriti 96-well thermal cycler (Major Sciences, USA). The amplification included initial denaturation at 95˚C (5min), 35 amplification cycles of denaturation at 95˚C (30sec), annealing at 64˚C (45sec), extension at 72˚C (30sec), a final extension at 72˚C (5min) and the cycle was terminated to hold at 4˚C (30min). Genotype and allele frequencies were visualised using electrophoretograms on 2% agarose gel using a 1x Tris/Borate/ Ethylenediaminetetraacetic acid (EDTA) (TBE) buffer and 3-5 drops of 1% ethidium bromide. It was compared to 50 base pair (bp) Gene Ruler ladder (Thermo Fisher Scientific, Waltham, MA, USA). The amplified DNA fragments were visualised as white bands against a dark field on an ultraviolet (UV) trans-illuminator (Gene Box SN.DR4V2/2987, model No. GBox F3, UK), and the images were securely stored in a gel document system (Figure 1).

Data was analysed using SPSS 27. Descriptive statistics included the calculation of frequencies and percentages. Chi-square test was employed to examine associations between variables and the genotype of interest in cases and controls through cross-tabulation. Fisher's exact test was applied where the cell count was <5. Crude odds ratio (OR) and 95% CIs were calculated using VassarStats.[11] Genotype frequencies in the control group were analysed for adherence to Hardy-Weinberg equilibrium (HWE) using the chi-square test, with deviations considered statistically significant at $p \leq 0.05$.[12] Allele frequencies were calculated ($p+q=1$) using an online statistical tool.[13]

Results

Of the 340 women, 170(50%) with mean age 51.06±9.74 years (range: 30-76 years) were in group 1, and 170(50%) with mean age 56.84±11.5 years (range: 26-82 years) were in group 2. Among the cases, three major ethnicities enrolled were Punjabis 95(55.9%), Pathans 56(32.9%) and Kashmiris 19(11.2%). The corresponding number in controls were Punjabis 118(69.4%), Pathans 34(20%) and Kashmiris 18(10.6%). Those aged ≤35 years were 14(8.2%) cases in group 1 and 15(8.8%) controls in group 2. Those aged >35 years were 156(91.8%) group 1 and 155(91.2%) group subjects. In group 1, there were 112(65.9%) sporadic cases and 58(34.1%) familial cases.

The frequency of the T allele (p) was 0.903, while the C

Table-1: Intergroup comparison of GADD45A rs3783469 genotype and allele frequencies.

Genotype (N=340)	Cases n=170 (%)	Controls n=170 (%)	OR (95%CI)	p-value
TT	60(35.3)	137(80.6)	Reference	<0.0001
TC	110(64.7)	33(19.4)	7.6(4.64-12.46)	
Allele Frequencies				
T Allele	230(67.6)	307(90.3)	4.4(2.90-6.80)	<0.0001
C Allele	110(32.4)	33(9.7)		

GADD45A: Growth arrest and deoxyribonucleic acid damage 45A, rs: reference single nucleotide polymorphism cluster ID, TT: Thymine-thymine, TC: Thymine-cytosine, OR: Odds ratio, CI: Confidence interval.

Table-2: Association of GADD45A genotype with age groups and family history in breast cancer cases and controls.

Genotype	Cases n(%)	Controls n (%)	OR (95%CI)	p-value
Age Group ≤ 35				
TT	5(2.9)	13(7.6)	Reference	0.005
TC	9(5.3)	2(1.2)	11.7(1.85-74.12)	
Age Group > 35				
TT	55(32.3)	124(72.9)	Reference	<0.0001
TC	101(59.4)	31(18.2)	7.35(4.4-12.26)	
Family History				
Genotype	**Absent n (%)**	**Present n (%)**	**OR (95%CI)**	**p- value**
TT	42(24.7)	18(10.6)	Reference	0.403
TC	70(41.2)	40(23.5)	0.75(0.38-1.47)	

GADD45A: Growth arrest and deoxyribonucleic acid damage 45A, TT: Thymine-thymine, TC: Thymine-cytosine, OR: Odds ratio, CI: Confidence interval.

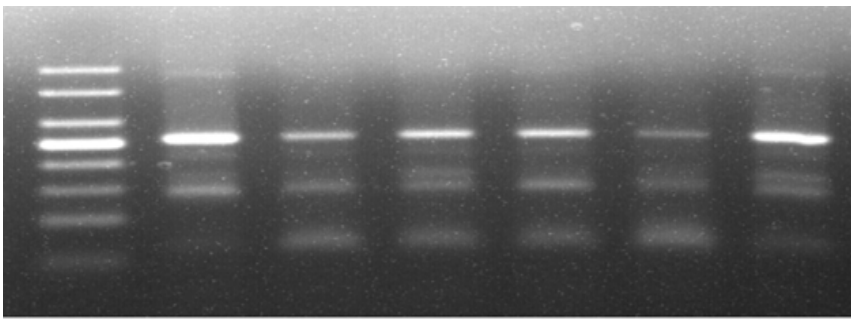

Figure: Electrophorectogram of GADD45A (1506T>C). Lane 1 represents 50 base pair (bp) ladder. Lanes 2,3,5 and 6 represent TT genotype. Lane 4 and 7 represent TC genotype. The control band is at 253bp.
GADD45A: Growth arrest and deoxyribonucleic acid damage 45A, T: Thymine, C: Cytosine.

allele (q) was 0.097. Genotype distribution in the group 2 did not deviate significantly from Hardy-Weinberg equilibrium (p=0.16). A higher prevalence of the TC genotype was observed in group 1 cases 110(64.7%), TT genotype was more common in controls 60(35.3%), and the CC genotype was not observed in either group, OR=7.6; 95%CI: 4.64-12.46; p<0.0001). The T allele was more frequent in group 2 controls, while the C allele was predominant in group 1 cases (OR=4.4; 95%CI: 2.90-6.80; p<0.0001 (Table 1, Figure). A statistically significant association was found between genotype (p<0.0001), age group ≤35 years (p=0.005) and age group >35 years (p<0.0001). No significant association was observed between genotype and family history (Table 2).

Discussion

In the current study, the median age at the time of breast cancer diagnosis was 51 years compared to 59 years in the US, 49 years in Iran and 53 years in Japan.[14] The median age of diagnosis varies globally, influenced by regional differences in risk factors, early detection, cancer screening practices, and biological factors, like genetics and hormones. Regions like East Asia, the Middle East and Africa tend to have younger median ages at diagnosis, likely due to a combination of population demographics and cancer control efforts.

The current study found a strong association of genotype with age ≤35 years and age >35 years among both the cases and controls (Table 2). In our study 91.2% breast cancer females were aged >35 years and 8.2% were aged <35 years. A study in Ukraine reported distinct features of breast cancer in younger women (≤35 years), highlighting aggressive tumour characteristics, and the importance of optimised diagnostic and personalised treatment.[15] In Pakistan, Hussain et al. reported that approximately 5% of breast cancer cases occur before age 35, and around 25% before age 50.[16] This discrepancy in younger cases may reflect population demographics, regional differences, lifestyle factors, environmental exposures and underlying genetic and hormonal influences.

The current study did not observe any statistically significant association between rs3783469 and family history (Table 2). In the current study, sporadic (65.9%) predisposition was more than familial (34.1%) breast cancer. Similarly, a Pakistani study reported that the majority of females (82.53%) had no familial breast cancer history. This may point towards the influence of recessive alleles, potentially linked to parental consanguinity.[17] In contrast, 69% of Israeli patients had a significant family history of breast cancer.[18] This may be attributed to an increased awareness towards genetic predisposition to cancer, leading to higher rates of screening programmes and genetic testing in countries with more developed healthcare systems.

In 2008, Desjardins et al. analysed the genotype distribution for rs3783469 and reported non-significant

values (p>0.556).[18] However, in the current study observed a much stronger association between GADD45A (1506T>C) polymorphism rs3783469 and breast cancer, indicating a strong association between genotype and allele frequencies of this variant and breast cancer susceptibility (Table 1).

Yuan et al. examined the polymorphism of the variant (1506T>C) in the Chinese population, in relation to ovarian cancer susceptibility and prognosis, and reported different patterns. Both the TT and TC genotypes were more frequent among the controls (OR=1.61, p>0.0021), and the CC genotype was found in ovarian carcinoma (OR=2.75, p>0.0021), with a higher prevalence in ovarian cancer cases than in the controls.[7] The current results justified the opposite pattern where the TT genotype was expressed more by the controls, the TC genotype was more frequent in the cases, and the CC genotype was entirely absent in both the groups, showing highly significant association with breast carcinoma. These differences may be attributed to genetic diversity, demographic variations across different regional populations, and also the absence of CC genotype in the study population.

Regarding allelic frequencies, the current study observed that the major allele (T) was more prevalent among the controls, whereas the minor allele (C) was more frequently observed in breast cancer cases, suggesting a possible protective role of the T allele. Yuan et al. reported an association between the T allele and increased GADD45A mRNA (Messenger ribonucleic acid) expression, as well as improved overall survival in ovarian cancer patients.[7] Although their study focussed on prognosis rather than disease susceptibility, the observed beneficial association of the T allele is in line with the potential protective effect suggested in the present study.

This further signifies that breast and ovarian cancers have same aetiological factors due to environmental and genetic risk factors.[19]

The current study has limitations. The small sample size limits the generalisability of the findings. No adjustments were made for potential confounding variables, and, therefore, the estimated associations should be interpreted as exploratory. Larger, more diverse studies, integrating genetic and clinicopathological data are needed to validate the results and enhance understanding of breast cancer mechanisms for personalised treatment strategies.

Conclusion

The GADD45A (1506T>C) polymorphism rs3783469 was significantly associated with breast cancer. The TT genotype showed a protective pattern in the control group, while the TC genotype was associated with higher odds of breast cancer. Significant associations were observed between genotype and age groups, but no significant association was found between family history and breast cancer. Understanding demographic variations is essential for addressing broader implications for women's health.

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AUTHORS' CONTRIBUTIONS:
VR: Concept, design, drafting, data acquisition, interpretation, data analysis and agreement to be accountable for all aspects of the work.
FF: Revision and final approval.
AR: Concept, design, revision and final approval.
SA: Drafting and data analysis.