Experimental Research

Vitamin D receptor gene polymorphisms and risk of breast cancer in Iranian women

Seyedeh Maryam Kazemi\textsuperscript{a,b}, Aghil Esmaielbandbodi\textsuperscript{c}, Ziba Veisi Malekshahi\textsuperscript{d}, Mohammad Shahbaz Sardood\textsuperscript{d}, Mehrdad Hashemi\textsuperscript{e}, Keivan Majidzadeh\textsuperscript{a}, Maryam Kadkhodazadeh\textsuperscript{f}, Rezvan Esmail\textsuperscript{b}, Babak Negahdari\textsuperscript{d,}\textsuperscript{*}

\textsuperscript{a} Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
\textsuperscript{b} Toxicology and Chemotherapy Unit, German Cancer Research Center (DKFZ), Heidelberg, Germany
\textsuperscript{c} Department of Medical Genetics, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
\textsuperscript{d} Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
\textsuperscript{e} Genetics Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran
\textsuperscript{f} Department of Virology, Pasteur Institute of Iran, Tehran, Iran

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\textbf{ABSTRACT}

Objectives: Vitamin D deficiency is a driving force of common cancers like breast cancer. Vitamin D receptor (VDR) can play a tumor suppressor role by helping the precise function of vitamin D in cells such as modulation of TGF-β signaling pathway. This study aimed to investigate the association of VDR gene variants and susceptibility to breast cancer in Iranian women.

Methods: Genomic DNAs were isolated from blood samples of 161 women with breast cancer and 150 healthy women. After amplification of five positions of VDR gene, the prepared amplicons were digested with TaqI, BsmI, Cdx2, and FokI restriction enzymes.

Results: Subsequently, the digested products were electrophoresed on the 1.5% agarose gel. Odds ratios (ORs) for breast cancer were calculated for genotypes and estimated haplotypes. Binary logistic regression analysis showed TaqI (rs2228570), BsmI (rs1544410), and Apal (rs7975232) polymorphisms had the significant distribution in patients than to the normal group. Analysis of linkage disequilibrium for all pairs of SNPs showed that D′-value between SNP TaqI and SNP BsmI was significantly (p ≤ 0.05). We observed that four major haplotypes of Apal, BsmI, FokI, Cdx2, and TaqI SNPs significantly were in high frequency than predicted frequency. Among these four haplotypes, CGTAT haplotype was in a higher significant association than others with breast cancer risk (p-value = 0.0001).

Conclusion: Our results showed that FokI, BsmI, and Apal of VDR polymorphisms associated with the risk of breast cancer in Iranian population.

1. Introduction

Breast cancer is the most common cancer in women, with a high number of new cases and related death in the world [1]. Breast cancer progression and outcome have vital roles in biological, social, and health systems [2]. Vitamin D (1,25-(OH)\textsubscript{2}-Vitamin D3) deficiency is a driving force that increased the risk of common cancers [3]. Vitamin D has a differentiating and antiproliferative activity on the many types of cells [4]. Previous studies showed the role of Vitamin D compounds in cancer prevention and treatment of colorectal, breast, prostate, ovarian, bladder, lung, and skin cancers and leukemia [5–7]. Furthermore, higher cancer incidence and mortality in patients of colorectal, breast, lung and prostate cancers could be associated with low levels of the most active metabolite of vitamin D (1α,25-dihydroxy vitamin D3) in plasma.

\textbf{Abbreviations:} VDR, Vitamin D receptor; BMD, Bone mineral density; LD, Linkage disequilibrium; LOH, Loss of heterozygosity; HWE, Hardy-Weinberg equilibrium; RFLP, Restriction fragment length polymorphism; PCR, Polymerase chain reaction.

\textsuperscript{*} This study was approved by the ethical committee of Tehran Breast Cancer Research Center, (Motamed Cancer Institute).

\textit{E-mail address:} b.negahdari@tums.ac.ir (B. Negahdari).

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The function of vitamin D is performed by the binding to its receptor (vitamin D receptor (VDR)) [13]. Vitamin D -VDR combination can play a tumor suppressor role in cells by modulation of the TGF-β signaling pathway [14]. Polymorphisms in the VDR gene may influence the risk of cancer occurrence and prognosis [15]. SNP polymorphisms in VDR gene, using decreasing VDR mRNA stability, can increase serum levels of vitamin D3 [16,17]. Many polymorphisms in VDR gene were studied in various types of cancers including colorectal cancer [18,19], Human Parathyroid Tumors [20], skin cancer [21,22], prostate cancer [23,24], lung cancer [25], and breast cancer [26,27]. These SNP polymorphisms including TaqI polymorphism (rs731236) in the VDR gene, substituting T for C in exon IX of VDR gene, FokI polymorphism (rs228570), changing C to T in exon II of VDR gene, BsmI polymorphism (rs7975232), changing A to G in the intron between exon VIII and IX (INTRON 8), and Apal polymorphism (rs1544410), changing A to G in the intron between exon VII and IX, and CDXII polymorphism (rs11568820), changing G to A in exon I, could be associated with prostate cancer, breast cancer, colorectal cancer, and skin cancer [28-30].

As shown in the previous study, vitamin D status can improve the prognosis of breast, colon and prostate cancer [31,32]. Also, a great number of the previous study showed that vitamin D receptor (VDR) gene polymorphisms could influence the incidence of breast cancer and the outcome of women with breast cancer [33-37]. Then in this research, we aimed to investigate the frequency of five variants of VDR polymorphisms in Iranian women with breast cancer. Also, association of these five SNPs with breast cancer risk in this population was calculated in comparison with a normal group of women. Haplotypes including haplotypes including

2. Materials and methods

2.1. Design of study

This study included 160 breast cancer women and 151 healthy women. Blood samples of these participants were provided from I(XXX) [38]. The age restriction was not involved in sample collections of the patients. Cancer patients were verified by sonography or mammography. Cases that had other types of cancer or were treated with chemotherapy or radiotherapy were excluded from the study. The controls were approved through clinical examinations and sonography or mammography and have no prior history of cancer. This study was approved by (XXX).

3. Genotyping of VDR variants

3.1. DNA extraction

Genomic DNAs were isolated using Bioneer DNA Extraction Kit (Bioneer Company, Daejeon, Republic of Korea). Briefly, 20 μl of proteinase K and 200 μl of lysis buffer were added to 200 μl whole blood and incubated 10 min at 60 °C. 100 μl phenol was added to this mixture. Then, whole lysate transferred into a DNA purification column. After centrifuging at 8000 rpm for 1 min, the column was washed two times with 500 μl wash buffers. Finally, centrifugation was performed at 8000 rpm for 1 min and DNA was eluted by 100 μl elution buffer.

3.2. Polymerase chain reaction (PCR)

Primers for amplification of the five positions in the VDR gene, that contain our studied polymorphisms, were designed by Primer3 online software. Table 1 shows the sequences of used primers in our study. PCR reactions were performed to amplify the five positions of the VDR gene in samples. The final volume of each reaction mixture was 25 μl (12.5 μl

of 2× master mix (SinaClon, Iran), 10 pmol of each primer, 2 μl of DNA sample as template, and ultrapure water).

3.3. Restriction fragment length polymorphism (RFLP)

The amplified amplicons were digested with 10 units of TaqI, Apal, BsmI, Cdx2, and FokI restriction enzymes, for 12 h. Subsequently, the digested products were electrophoresed on the 1.5% agarose gel at 90 V for 45 min. To staining, the agarose gels were mixed with DNA safe stain (Sinaclon, XXX, Cat No: EP5082).

3.4. Statistical analysis

Data were analyzed using SPSS software (version 25) and P-values < 0.05 were considered significant. Chi-square goodness-of-fit test was used to calculate Hardy–Weinberg equilibrium (HWE) for assessment the association of allelic frequencies and distribution of genotypes. Associations between VDR genotypes and breast cancer risk were analyzed by binary logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs). These analyses were done for dominant, recessive, and codominant models for five VDR SNPs. D’ values for power estimation of linkage disequilibrium and haplotype analysis were obtained by the Haploview software version 4.2 (http://www.broad.mit.edu/mpg/haplovieview).

Unique identifying number is: researchregistry7340.

The methods are presented in accordance with STROCSS 2021 guidelines [39].

4. Results

4.1. Population characteristics

To evaluate the association of VDR polymorphisms with breast cancer, we used a case-control study design. In this study, about 311 women were analyzed, 160 women in the breast cancer group (BC group), and 151 women in control groups. 28.75% of BC group (46 women) had at least a family history for breast cancer. The mean value of women’s age in this study was 61.49 ± 17.74. We genotyped five SNPs of the VDR gene in our population. For each SNP, some data for cases and controls were missed that excluded from the study.

4.2. Association between genotypes and alleles

Frequency of alleles, and distribution of genotypes of five VDR gene SNP polymorphisms in our population were inserted in Table 2. As observed, frequency of heterozygous AC genotype of Apal polymorphism was more in breast cancer cases (40.5%) than the controls (21.6%). AA genotype of BsmI polymorphism (homozygous allele 1), had a little more value of distribution in breast cancer cases (38.5%).

| SNP Polymorphism (SNP ID) | Primers |
|--------------------------|---------|
| FokI (rs228570)           | Rev: AGCTATGTAGGGCGAAATCATG  |
| Apal (rs7975232)          | Rev: CGGAATGACAGCATGCAAAAG   |
| BsmI (rs1544410)         | Rev: ATGTCCTTGGAGTGCACATTACAC |
| TaqI (rs731236)          | Rev: GGCTGCTGAAATAATTGGTGAGA |
| Cdx2 (rs11568820)        | Rev: TAAACACCGAGGAACATTCTTCTAGAG |

a SNP ID is extracted from miRBase database (http://www.mirbase.org/).

b Forward primer.
c Reverse primer.

Table 1 The sequences of used primers for PCR.
Table 2
Allelic frequency and genotype distribution for 5 variants of VDR polymorphism.

| SNP   | BC group | Control group | p-value |
|-------|----------|---------------|---------|
|       | allele/Genotype | Frequency (number) | Frequency (%) | Frequency (number) | Frequency (%) |       |
| Apal  | A        | 155            | 64       | 196            | 71           |       |
|       | C        | 87             | 36       | 82             | 29           |       |
|       | AA       | 53             | 44       | 83             | 60           | <0.0001|
|       | AC       | 49             | 40       | 30             | 22           |       |
|       | CC       | 19             | 16       | 26             | 19           |       |
| BsmI  | A        | 171            | 63       | 143            | 53           |       |
|       | G        | 99             | 37       | 125            | 47           |       |
|       | AA       | 52             | 39       | 42             | 31           | 0.53   |
|       | AC       | 49             | 40       | 30             | 22           |       |
|       | CC       | 19             | 16       | 26             | 19           |       |
| FokI  | T        | 143            | 52       | 207            | 78           |       |
|       | C        | 133            | 48       | 57             | 22           |       |
|       | CC       | 45             | 33       | 25             | 19           | <0.0001|
|       | TC       | 43             | 31       | 7              | 5            |       |
|       | TT       | 50             | 36       | 100            | 76           |       |
| Cdx2  | G        | 157            | 64       | 142            | 55           |       |
|       | A        | 87             | 36       | 118            | 45           |       |
|       | AA       | 54             | 44       | 43             | 33           | <0.0001|
|       | GA       | 49             | 40       | 56             | 43           |       |
|       | GG       | 19             | 16       | 31             | 24           |       |
| TaqI  | T        | 134            | 58       | 171            | 65           |       |
|       | C        | 96             | 42       | 93             | 35           | 0.23   |
|       | CC       | 15             | 13       | 16             | 12           |       |
|       | TC       | 66             | 57       | 61             | 46           |       |
|       | TT       | 34             | 30       | 55             | 42           |       |

BC group: breast cancer group.

Table 3
Association between SNPs of VDR variants and breast cancer in our population.

| SNP   | Inheritance state | Genotype | Frequency in control group (number/%) | Frequency in BC group (number/%) | OR, 95% CI | P-value   |
|-------|-------------------|----------|---------------------------------------|---------------------------------|------------|-----------|
| BsmI  | Recessive          | AA-AG    | 119 (88.2%)                          | 101 (75.4%)                     | 1.00       | 0.0062    |
|       |                    | GG       | 16 (11.8%)                           | 33 (24.6%)                      | 2.43 (1.26-4.67) |          |          |
|       | Dominant           | AA       | 52 (38.5%)                           | 42 (31.3%)                      | 1.00       | 0.22      |
|       |                    | AG-GG    | 83 (61.5%)                           | 92 (68.7%)                      | 1.37 (0.83-2.27) |          |          |
|       | Codominant         | AA       | 52 (38.5%)                           | 42 (31.3%)                      | 1.00       | 0.022     |
|       |                    | AG       | 67 (49.6%)                           | 59 (44%)                        | 1.09 (0.64-1.86) |          |          |
|       |                    | GG       | 16 (11.8%)                           | 33 (24.6%)                      | 2.55 (1.24-5.26) |          |          |
| Apal  | Recessive          | AA-AC    | 102 (84.3%)                          | 113 (81.3%)                     | 1.00       | 0.52      |
|       |                    | CC       | 19 (15.7%)                           | 26 (18.7%)                      | 1.24 (0.65-2.36) |          |          |
|       | Dominant           | AA       | 53 (43.8%)                           | 83 (69.7%)                      | 1.00       | 0.01      |
|       |                    | AC       | 68 (56.2%)                           | 56 (30.3%)                      | 0.53 (0.32-0.86) |          |          |
|       | Codominant         | AA       | 53 (43.8%)                           | 83 (69.7%)                      | 1.00       | 0.0038    |
|       |                    | AC       | 68 (56.2%)                           | 56 (30.3%)                      | 0.53 (0.32-0.86) |          |          |
| FokI  | Recessive          | TT-CT    | 92 (67.2%)                           | 107 (81.1%)                     | 1.00       | 0.0089    |
|       |                    | CC       | 45 (32.9%)                           | 25 (18.9%)                      | 0.48 (0.27-0.84) |          |          |
|       | Dominant           | TT       | 49 (35.8%)                           | 100 (75.8%)                     | 1.00       | <0.0001   |
|       |                    | CT-CC    | 88 (64.2%)                           | 82 (24.2%)                      | 0.18 (0.10-0.30) |          |          |
|       | Codominant         | TT       | 49 (35.8%)                           | 100 (75.8%)                     | 1.00       | <0.0001   |
|       |                    | CT       | 43 (31.4%)                           | 7 (5.3%)                        | 0.08 (0.03-0.19) |          |          |
|       |                    | CC       | 45 (32.9%)                           | 25 (18.9%)                      | 0.27 (0.15-0.49) |          |          |
| TaqI  | Recessive          | TT-TC    | 101 (87.1%)                          | 116 (87.9%)                     | 1.00       | 0.85      |
|       |                    | CC       | 15 (12.9%)                           | 16 (12.1%)                      | 0.93 (0.44-1.97) |          |          |
|       | Dominant           | TT       | 35 (30.2%)                           | 55 (41.7%)                      | 1.00       | 0.06      |
|       |                    | TC-CC    | 81 (69.8%)                           | 77 (58.3%)                      | 0.60 (0.36-1.02) |          |          |
|       | Codominant         | TT       | 35 (30.2%)                           | 55 (41.7%)                      | 1.00       | 0.16      |
|       |                    | TC       | 66 (56.9%)                           | 61 (46.2%)                      | 0.59 (0.34-1.02) |          |          |
|       |                    | CC       | 15 (12.9%)                           | 16 (12.1%)                      | 0.68 (0.30-1.54) |          |          |
| Cdx2  | Recessive          | GG-AAG   | 98 (80.3%)                           | 92 (73.6%)                      | 1.00       | 0.21      |
|       |                    | AA       | 24 (19.7%)                           | 33 (26.4%)                      | 1.46 (0.81-2.66) |          |          |
|       | Dominant           | GG       | 64 (52.5%)                           | 61 (48.8%)                      | 1.00       | 0.57      |
|       |                    | AG-AA    | 58 (47.5%)                           | 64 (51.2%)                      | 1.16 (0.70-1.91) |          |          |
|       | Codominant         | GG       | 64 (52.5%)                           | 61 (48.8%)                      | 1.00       | 0.45      |
|       |                    | AG       | 34 (27.9%)                           | 31 (24.8%)                      | 0.96 (0.53-1.74) |          |          |
|       |                    | AA       | 24 (19.7%)                           | 33 (26.4%)                      | 1.44 (0.77-2.71) |          |          |

BC group: breast cancer group, OR: odds ratio, CI: confidence interval.
than controls (31.3%). The percentage of heterozygous CT genotype of FokI polymorphism was higher in breast cancer cases (31.4%) than controls (5.3%). Furthermore, for Cdx2 polymorphism, the GG genotype (homozygous allele 1) was a common (52.5%) in breast cancer cases versus 48.8% in controls. Also, about TaqI, the heterozygous TC genotype (was more common among breast cancer cases (56.9%) than controls (46.2%) (Table 2).

The VDR Apal, BsmI, and TaqI allelic frequency and genotype distribution of the control population confirmed the Hardy-Weinberg equilibrium (HWE) (P = 0.60, P = 0.53, P = 0.23), whereas the FOKI, Cdx2 allele frequencies missed the HWE (P = 0.0001, P = 0.0001) (Table 2).

4.3. Association of VDR polymorphism with breast cancer risk

Odds ratios for breast cancer associated with each VDR polymorphism in our population are given in Table 3. Univariate logistic regression statistical analysis to survey association of BsmI (rs15444110) with breast cancer between the two study groups represented powerful significant differences under the recessive and codominant models of inheritance. In the recessive model, the GG genotype, as recessive, had little chance to be patients (OR: 2.43, 95% CI = 1.26–4.67, p < 0.01). Therefore, this genotype had a protective effect in the control group. Also in codominant model, the AA genotype was more common among breast cancer cases (38.5%) than controls (31.3%); but GG genotype had 24.6% in controls (OR = 4.67, p < 0.01) (Table 3).

In case of Apal polymorphism, in recessive state, differences in genotypes distribution were not significant between two groups (control vs patients). In dominant state, A/C, and C/C genotypes had more chance to be patient (OR = 0.53, 95% CI = 0.32–0.86, p = 0.01). In codominant model, AC genotype was somewhat more common among breast cancer cases (40.5%) than controls (21.6%) (OR = 0.39, 95% CI = 0.22–0.69, P = 0.01), leading to a protective effect against breast cancer in CC genotype were compared with AC genotype (OR = 0.87, 95% CI = 0.44–1.73, P < 0.01).

About FokI in all models, differences in frequency of genotypes were significant between two groups (control vs patients). In dominant state, C/T, and C/C genotypes had more chance to be patient (OR = 1.18 95% CI = 0.10–0.30, P = 0.001). In the codominant model, CC, and CT genotype were somewhat more common among breast cancer cases than controls (OR 0.27, 95% CI 0.15–0.49, and OR 0.08, 95% CI = 0.03–0.19 respectively, P = 0.001)). Also in the recessive model, CC genotyping was more common in breast cancer patients versus controls (OR 0.27, 95% CI = 0.15–0.49, P < 0.01).

For C.DX2 and TaqI polymorphisms in all models, differences in genotype distribution were not significant between two groups (control vs patients).

4.4. Analysis of linkage disequilibrium and haplotypes association with breast cancer

The D’ value of standardized measure of LD, and the corresponding P-values, were calculated for all pairs of SNPs on our population. As shown in Table 4, most of the SNPs were not in tight and highly significant LD with each other. Only, TaqI showed a somewhat high LD to the SNP BsmI in the population (D’ value: 0.1405). This D’ value was near to significant statistically (p = 0.0508).

Analysis of Five-markers haplotypes was performed with all five SNPs (Apal, BsmI, FokI, Cdx2, and TaqI respectively) through the VDR gene. Table 5 shows the frequencies for the estimated 5-marker haplotypes among breast cancer and healthy control women. We observed four major haplotypes (haplotypes 7, 12, 17 and 19) significantly were in high frequency than predicted frequency. As shown in Table 5, these haplotypes are AGGCC, CAGGC, CACAT, and CGTAT respectively. Haplotype 19 (CGTAT) was in a higher significant association than others with breast cancer risk because of a global P-value of 0.0001.

5. Discussion

Vitamin D receptor polymorphisms are interesting single nucleotide polymorphisms for investigating their association with multiple cancers. This study aimed to investigate the association of VDR gene variants and susceptibility to breast cancer in Iranian population. Five variants of VDR polymorphisms were selected for genotyping purposes: rs2228570 (FokI), rs15444110 (BsmI), rs7975232 (Apal), rs731236 (TaqI), and rs11568820 (Cdx2). Allelic frequency and genotype distribution and agreement with Hardy–Weinberg equilibrium for five variants were assessed in this study. Also, the association of these five variants and breast cancer in women was calculated in this study. As seen in our study, distribution of FokI, BsmI, and Apal polymorphisms had a significant difference in patients than to the normal group. Furthermore, the genotype distribution FokI was in Hardy–Weinberg equilibrium, but the genotype distribution of BsmI and Apal was not in Hardy–Weinberg equilibrium. Loss of heterozygosity (LOH) events in BsmI and Apal alleles of our population could be a hypothesis that can disrupt this equilibrium.

To identify candidate genes for breast cancer detection, many genes were studied in the previous researches [40–42]. Association of polymorphisms with breast cancer was analyzed in many previous studies [43,44]. McCullough et al. evaluated the association of VDR polymorphisms including BsmI, Apal, TaqI, and FokI with the risk of breast cancer. Their results show that had not any significant association between these SNPs and the risk of breast cancer [34]. Another study in Iran by Shahbazi et al. was shown that the BsmI polymorphism had a significant association with breast cancer risk, but FokI had not significant association in Iranian populations [36]. Our results verify that both FokI and BsmI have a significant association with risk of Breast Cancer. In Yiallourou et al. research, FokI polymorphism was associated with the risk of breast cancer staging and survival among Caucasian women [45]. This verifies our result of the FokI polymorphism association with breast cancer risk.

In Pulto et al. Study, the association of Cdx2 VDR polymorphism with the treatment efficacy of positive and negative estrogen breast cancer cell lines using vitamin D was investigated. Results showed that the cases with variant homozygote AA of Cdx2 VDR polymorphism could affect the clinical treatment of negative estrogen breast cancer cell lines [46]. This result is inconsistent with the results of our study that showed no association between genotypes of Cdx2 polymorphism and breast cancer. Perna et al. demonstrated that homozygotes for rare alleles of TaqI polymorphism had increased risk for mortality in breast cancer women. Also, FokI polymorphism was significantly not associated with breast cancer prognosis. These results in our study contradict this article. But, Cdx2 polymorphism in this article and our study both were not associated significantly with breast cancer risk [47]. Overall, a meta-analysis study in 2016 by Lu et al. showed that all FokI, BsmI, Apal, and TaqI variants of VDR polymorphisms were not associated with breast cancer risk [48]. Our results showed that FokI, BsmI, and Apal could be associated with breast cancer.

The power of linkage disequilibrium (LD) of all pairs of these variants
in the breast cancer population is calculated for the first time in this study, although there was not any powerful linkage between SNPs of the VDR gene. Also, haplotypes of these five polymorphisms of the VDR gene showed that four haplotypes associated with breast cancer risk, significantly. In 2004 Thakkinistian et al. performed a meta-analysis for surveying haplotypes and linkage disequilibrium of researches on the BsmI, Apal, and TaqI polymorphisms in bone mineral density (BMD). Their results show the existence of a strong LD between the BsmI and TaqI polymorphisms [49]. In our study, we verified this result, that there is a stronger LD between BsmI and TaqI polymorphisms than other pairs of polymorphisms.

6. Conclusions

Our results showed that some VDR polymorphisms are associated with the risk of breast cancer. Breast cancer is a complex disease, and many factors contribute to it. However, identifying the causative agents will help the early prediction and diagnosis of the disease. It is recommended to investigate other polymorphisms of the VDR gene in breast cancer, considering the role of vitamin D receptors in the regulation of cell cycle in breast cancer. We hope to find new genes and polymorphisms involved in breast cancer in the world for early diagnosis and more effective treatment.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Sources of funding

No funding was secured for this study.

Ethical approval

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Table 5

Haplotypes frequency in case and control groups, and their association with breast cancer.

| Apol | BsmI | FokI | Cdx2 | TaqI   | Total | BC group | Control group | OR (95% CI) | P-value |
|------|------|------|------|--------|-------|----------|--------------|-------------|---------|
| 1    | A    | A    | T    | G      | 0.1176 | 0.1037   | 0.1285       | 1.00        | –       |
| 2    | A    | G    | T    | A      | 0.0702 | 0       | 0.1119       | 1.37 (0.47-3.99) | 0.56     |
| 3    | A    | G    | T    | G      | 0.0679 | 0.0546   | 0.088        | 2.60 (0.62-10.94) | 0.19     |
| 4    | A    | A    | C    | G      | 0.0588 | 0.0532   | 0.0674       | 0.08 (0.01-1.04) | 0.055    |
| 5    | A    | A    | T    | G      | 0.0547 | 0       | 0.0678       | 1.03 (0.17-6.27) | 0.97     |
| 6    | C    | G    | T    | G      | 0.053  | 0.0716   | 0.0252       | 0.47 (0.13-1.75) | 0.26     |
| 7    | A    | G    | C    | G      | 0.0493 | 0.0648   | 0.05         | 0.02 (0.00-0.53) | 0.02     |
| 8    | C    | A    | T    | G      | 0.0455 | 0.0334   | 0.0525       | 1.40 (0.30-6.53) | 0.67     |
| 9    | A    | A    | T    | A      | 0.0454 | 0.0735   | 0.0213       | 3.31 (0.26-41.96) | 0.36     |
| 10   | A    | A    | C    | A      | 0.0436 | 0.0404   | 0.0252       | 0.31 (0.06-1.53) | 0.15     |
| 11   | A    | A    | T    | A      | 0.0367 | 0.0406   | 0.0438       | 0.95 (0.08-11.51) | 0.97     |
| 12   | C    | A    | C    | G      | 0.0356 | 0.0325   | 0.0412       | 0.08 (0.01-0.66) | 0.019    |
| 13   | A    | G    | T    | G      | 0.035  | 0.0508   | 0.0446       | 1.25 (0.28-5.49) | 0.77     |
| 14   | A    | G    | C    | G      | 0.032  | 0.0778   | 0.0055       | 11.80 (0.49-285.35) | 0.13     |
| 15   | C    | A    | T    | A      | 0.0306 | 0.0595   | 0.0222       | 0.54 (0.11-2.54) | 0.44     |
| 16   | C    | A    | C    | G      | 0.029  | 0.0289   | 0.0273       | 1.20 (0.17-8.68) | 0.85     |
| 17   | C    | A    | C    | A      | 0.0266 | 0       | 0.0478       | 0.02 (0.00-0.78) | 0.037    |
| 18   | A    | A    | C    | G      | 0.0258 | 0.0201   | 0.0135       | 3.62 (0.12-111.21) | 0.46     |
| 19   | C    | G    | T    | A      | 0.0239 | 0.0205   | 0.0129       | 5.64 (2.64-7.43) | <0.0001  |
| 20   | C    | G    | C    | G      | 0.01  | 0       | 0.0252       | 1.55 (0.20-11.97) | 0.67     |
| 21   | A    | G    | C    | A      | 0.0191 | 0.0281   | 0.0107       | 0.33 (0.01-11.32) | 0.54     |
| 22   | A    | G    | T    | A      | 0.0183 | 0.017   | 0.0204       | 0.76 (0.06-9.46) | 0.83     |
| 23   | C    | A    | T    | A      | 0.0182 | 0.0216   | 0.0061       | 0.22 (0.01-6.39) | 0.38     |
| 24   | C    | G    | T    | G      | 0.0153 | 0.0291   | 0.0178       | 0.09 (0.00-1.92) | 0.12     |
| Rare | *    | *    | *    | *      | 0.0281 | 0.0875   | 0.0078       | 8.39 (0.29-243.29) | 0.22     |

Rare (*): All haplotypes with estimated frequencies <0.01% in total population.

BC group: breast cancer group, OR: odds ratio, CI: confidence interval.

Consent

Not applicable.

Author contribution

Seyyedeh Maryam Kazemi, Aghil Esmaieli-bandboni and Ziba Veisi Malekshahi: conceptualized and designed the study, drafted the initial manuscript, and reviewed and revised the manuscript. Mohammad Shahbaz Sardood, Mehrdad Hashemi, Keivan Majidzadeh-A, Maryam Kadkhodazadeh: Designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. Dr. Babak Negahdari: Coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Availability of data and material

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Registration of research studies

1. Name of the registry: This study was approved by the ethical committee of Tehran Breast Cancer Research Center, (Motamed Cancer Institute).
2. Unique Identifying number or registration ID: Hyperlink to the registration (must be publicly accessible):

Guarantor

The Guarantor is the one or more people who accept full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.
Declaration of competing interest

The authors deny any conflict of interest in any terms or by any means during the study. All the fees provided by research center fund and deployed accordingly.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jamsu.2021.103150.

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