Association of rs2228570 and rs11568820 polymorphisms in the *Vitamin D Receptor* gene with breast cancer risk in Vietnamese women

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Abstract

Recent research has increasingly concentrated on the potential correlation between breast cancer risk and vitamin D, particularly focusing on the vitamin D receptor (VDR) gene. The VDR gene's polymorphisms including rs2228570 (Fok1) and rs11568820 (Cdx2), have been implicated in modifying breast cancer susceptibility, though findings vary across populations. This study investigates the association of these VDR polymorphisms with breast cancer risk in Vietnamese women. A case-control study involving 100 breast cancer patients and 100 healthy controls was conducted, with genomic DNA isolated and SNP genotyping performed. The results revealed a significant protective effect of the rs2228570 (Fok1) SNP under the overdominant model (AG vs. AA + GG) with an odds ratio (OR) of 0.52, indicating statistical significance (p-value = 0.03).

In contrast, rs11568820 (Cdx2) showed no significant association with breast cancer risk across all genetic models analyzed. Although haplotype analysis did not reveal any significant associations, the AT haplotype exhibited a non-significant trend toward increased risk relative to the reference haplotype (OR = 1.56, 95% CI: 0.89–2.75, p-value = 0.1231). These findings highlight the importance of considering population-specific genetic factors in breast cancer risk assessment and underscore the need for further research to understand these complex genetic associations fully.

Keywords: Breast cancer, rs2228570 (Fok1), rs11568820 (Cdx2), Vitamin D receptor, Polymorphisms, Vietnamese women.

Introduction

Recent research has increasingly concentrated on the potential correlation between breast cancer risk and vitamin D. The interest in vitamin D and its analogs arises from multiple studies showing their ability to suppress cell growth in breast cancer cell lines, suggesting that they may have a potential function in decreasing the occurrence of breast cancer[7]. In cellular physiology, the mechanistic function of 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) has been extensively researched. The biologically active form of vitamin D binds to the vitamin D receptor (VDR), creating a complex between the ligand and receptor that functions as a transcription factor[7]. This complex functions as a transcription factor, regulating the expression of more than 60 genes involved in cell differentiation, metastasis, proliferation and apoptosis[12].

The VDR gene on chromosome 12q13 produces the VDR protein, which is crucial for the effectiveness of vitamin D. Single nucleotide polymorphisms (SNPs) in the VDR gene have been associated with the potential contribution to breast cancer development by modifying the receptor's function and structure. Nevertheless, there is still debate among populations regarding the association between these VDR SNPs and the risk of breast cancer. Studies conducted in Canada, the United States, Germany, Australia, the United Kingdom and Canada have shown varying results [1-5, 9-11]. In Canada, the Fok1 VDR SNP's minor homozygous genotype shows a lower breast cancer risk (OR = 0.71), contrasting with the United States, where it increases risk (OR = 1.34).

Similarly, in Germany, the TaqI SNP correlates with a higher risk for estrogen receptor-positive tumors but not for estrogen receptor-negative ones, showing regional variability. In Australia, significant correlations with breast cancer risk were found for the ApaI and TaqI SNPs, whereas Fok1 (rs2228570) showed no significant associations (OR = 0.99). Recent studies from the UK and Canada show strong links between other VDR SNPs (BsmI, variable-length poly(adenylate) sequence and Fok1), with family history playing a role.

Additionally, the Cdx2 VDR SNP (rs11568820) is associated with more aggressive tumor characteristics, such as estrogen receptor-negative and HER2-positive status, in breast cancer patients. This suggests that it may serve as a biomarker for predicting treatment responses in aggressive breast cancer cells. These differences show that more research is needed to fully understand how VDR SNPs, like rs2228570 (Fok1) and rs11568820 (Cdx2), affect the risk of getting breast cancer in different groups of people. Breast cancer risk in Vietnamese women and the VDR gene polymorphisms rs2228570 (Fok1) and rs11568820 (Cdx2) are the subjects of this study. The research attempts to find genetic markers that could be significant for assessing the risk of breast cancer in this population by examining the frequency of these SNPs and their potential linkage.

Material and Methods

Study sample: In this study, 100 women with a diagnosis of breast cancer were matched with an equal number of healthy persons to act as controls. Vietnamese women were recruited to donate peripheral blood samples at the Ho Chi Minh City Oncology Hospital. Diagnostic imaging techniques like sonography or mammography were used to confirm breast cancer diagnoses. Patients who had already gotten cancer treatments were not allowed to take part in the study. Individuals with negative cancer screening tests were chosen as controls. The written informed consent of all the participants was obtained. The Ho Chi Minh City Oncology Hospital Ethical Committee granted the study ethical permission (No. 177 / ĐĐĐ - CĐT).

Genomic DNA Isolation: DNA extraction was performed utilizing an optimized in-house Salting-out method [8]. To summarize, 1.000 µl of cell lysis buffer was mixed with 500 µl of whole blood and then centrifuged at a speed of 10.000 rpm for 5 minutes. After resuspending the pellet in 300 µl of nuclei lysis buffer, it was centrifuged again. The DNA was partitioned into the aqueous phase using 500 µl of chloroform and 100 µl of 5M NaCl, followed by additional centrifugation. DNA precipitation was achieved with absolute ethanol, followed by a wash with 70% ethanol. Finally, the DNA pellet was reconstituted in 50 µl of DNase-free water.

SNP genotyping: Primers for amplifying the two SNPs in the VDR gene were designed using Primer-BLAST and uMELT online software. The primer sequences utilized in this research are provided in table 1. SNP genotyping experiments were conducted using the LightCycler 96 Instrument, which includes a 96-well thermal block and the LightCycler 480 High-Resolution Melting Master. The PCR mixture included 1X Master mix from the Kit LightCycler 480 High-Resolution Melting Dye, 3 mM $MgCl_2$, 0.2 µM of each forward and reverse primer, 10 – 20 ng of genomic DNA and water suitable for PCR. The PCR thermal cycling procedure began with a 15-minute denaturation step at 95 °C. Subsequently, the reaction was subjected to 40 cycles of denaturation, annealing and elongation, with each step lasting 30 seconds at 95 °C, 65 °C and 72 °C respectively.

Default high-resolution melting (HRM) analysis followed. Each experimental run included three positive controls and one negative control, and the genotypes were validated by Sanger sequencing. Genotype identification was achieved by analyzing normalized melting curves and normalized melting peaks.

Statistical analysis: The chi-square test was employed to evaluate the Hardy-Weinberg equilibrium (HWE), facilitating the evaluation of the interconnection between genotype distributions and allelic frequencies. These assessments encompassed five distinct genetic models namely Allelic, Codominant, Overdominant, Dominant and Recessive. Linkage disequilibrium was assessed by calculating D' values and haplotype analysis was performed simultaneously.

The study investigated the association between VDR genotypes and the risk of breast cancer by using logistic regression to calculate odds ratios and their 95% confidence intervals. Statistical significance was determined with a P value threshold set below 0.05. A P-value threshold of less than 0.05 was used to ascertain statistical significance.

Results

Population characteristics: A case-control research study included 200 women, with equally distributed patients and controls. The patient and control groups had the same mean age of 51 and 50 years respectively, indicating no significant distributional difference at a P-value of 0.22. Genotyping of two SNPs within the VDR gene was conducted in the study population; however, cases and controls with incomplete data for each SNP were excluded from the analysis.

Distribution of Genotypes and Alleles: In a study involving 100 patients and 100 healthy individuals, rs2228570 (Fok1) and rs11568820 (Cdx2) were successfully genotyped, achieving a minimum call rate of 93% for both groups. The frequencies of genotypes and alleles for rs2228570 (Fok1) and rs11568820 (Cdx2) are presented in table 2. Notably, these SNPs exhibited considerable variability, with the minor allele frequencies in controls reaching 44%. Compared to the control group (28%), breast cancer patients exhibited a marginally higher prevalence of the homozygous GG genotype of rs2228570 (Fok1). In contrast, the homozygous TT genotype of rs11568820 (Cdx2) appeared more frequently in breast cancer cases (25%) compared to controls (20%).

Additionally, the heterozygous AG genotype of rs2228570 (Fok1) was found more often in the control group (55%) than in breast cancer cases (43%). Conversely, in breast cancer patients, the TC genotype of rs11568820 (Cdx2) was more prevalent, accounting for 50% of cases, compared to the control group where it accounted for 48%.

Table 1
The primer sequences utilized for PCR-HRM analysis

SNP (Polymorphism)	Primers (5'-3')
rs2228570 (Fok1)	Forward primer: TAAGGGAAGTGCTGGCCGCCAT Reverse primer: GGCACTGACTCTGGCTCTGACCG
rs11568820 (Cdx2)	Forward primer: TTTAACTGCAACCCATAATAAGAAATAAGT Reverse primer: GTAACATCTTGTAGAAAACATAGTCCTTG

The results showed that the genotype frequencies were in agreement with HWE ($P_{HWE} > 0.05$), which means that the sample was a good representation of the Vietnamese population (Table 2). The findings imply that certain genotypes of rs2228570 (Fok1) and rs11568820 (Cdx2) exhibit varying prevalence rates in breast cancer patients compared to healthy persons, indicating a potential genetic link to breast cancer susceptibility.

The association between two VDR polymorphisms and breast cancer risk: The risk of breast cancer related to each VDR polymorphism is displayed in table 3. Based on the data table on the correlation of SNPs of VDR and breast cancer risk, rs2228570 (Fok1) shows significant and noteworthy associations under the overdominant model. Specifically, the overdominant model (AG vs. AA + GG) demonstrates a protective effect with an odds ratio (OR) of 0.52, a 95% confidence interval (CI) of 0.29 to 0.94 and a p-value of 0.03, indicating statistical significance. In contrast, the allelic, codominant, dominant and recessive models do not show significant associations, with p-values of 0.60, 0.07, 0.44 and 0.08, respectively. For rs11568820 (Cdx2), none of the genetic models show significant associations with breast cancer risk. The allelic model (T vs. C) presents an OR of 1.3 (CI: 0.86–1.96, p-value: 0.21).

The codominant model (CT vs. CC and TT vs. CC) shows ORs of 1.44 (CI: 0.72–2.90, p-value: 0.43) and 1.67 (CI: 0.73–3.82, p-value: 0.43) respectively. The overdominant model (CT vs. CC + TT) has an OR of 1.14 (CI: 0.64–2.04, p-value: 0.66), while the dominant model (CT + TT vs. CC) shows an OR of 1.51 (CI: 0.78–2.92, p-value: 0.22). Finally, the recessive model (TT vs. CC + CT) has an OR of 1.32 (CI: 0.66–2.64, p-value: 0.43). Thus, rs11568820 (Cdx2) does not display significant associations with breast cancer risk in any genetic model analyzed. These result suggest that rs2228570 (Fok1) shows a significant protective effect against breast cancer in the overdominant model, while rs11568820 (Cdx2) does not exhibit any significant association with breast cancer risk in any of the analyzed genetic models.

The study population analysis revealed that the two SNPs, rs2228570 and rs11568820, do not exhibit a robust and statistically significant LD relationship, with a D′ value of 0.08324329 and a p-value of 0.1162185. This indicates that these SNPs are not closely linked and tend to be inherited independently. Consequently, each SNP can serve as an independent biomarker for assessing breast cancer risk. This independence allows for the combination of these SNPs within haplotypes to evaluate their collective impact on breast cancer susceptibility, enabling a more nuanced genetic analysis. Thus, the lack of significant LD between rs2228570 and rs11568820 underscores their potential as separate biomarkers and highlights the importance of considering their combined effects in haplotype analyses. A haplotype analysis was subsequently conducted, focusing on four-marker haplotypes formed by two SNPs (rs2228570 (Fok1) and rs11568820 (Cdx2). The distribution of haplotypes among patients and controls and their connection with breast cancer is provided in table 4.

Table 2
Frequency Distribution of Alleles and Genotypes for rs2228570 (Fok1) and rs11568820 (Cdx2)

SNP (Polymorphism)	**Genotype Frequency (n)**			**Allele Frequency (n)**		**P_{HWE}**
rs2228570 (Fok1)	**AA**	**AG**	**GG**	**A**	**G**	
Case (n = 93)	26% (24)	43% (40)	31% (29)	48% (88)	52% (98)	0,18
Control (n = 96)	17% (16)	55% (53)	28% (27)	44% (85)	56% (107)	0,30
rs11568820 (Cdx2)	**TT**	**TC**	**CC**	**T**	**C**	
Case (n = 95)	25% (24)	50% (47)	25% (24)	50% (95)	50% (95)	1,00
Control (n = 97)	20% (19)	48% (47)	32% (31)	44% (85)	56% (109)	0,84

Table 3
Associations between SNPs of VDR and breast cancer risk

SNP	**Genetic model**		**OR**	**95% CI**	**P**
rs2228570 (Fok1)	Allelic	A vs. G	1.12	0.74 – 1.68	0.60
	Codominant	AG vs. GG	0.60	0.30 – 1.19	0.07
		AA vs. GG	1.38	0.60 – 3.19	
	Overdominant	AG vs. AA + GG	0.52	0.29 – 0.94	**0.03**
	Dominant	(AA + AG) vs. GG	0.78	0.41 – 1.48	0.44
	Recessive	AA vs. (GG + AG)	1.90	0.92 – 3.90	0.08
rs11568820 (Cdx2)	Allelic	T vs. C	1.3	0.86 – 1.96	0.21
	Codominant	CT va. CC	1.44	0.72 – 2.90	0.43
		TT vs. CC	1.67	0.73 – 3.82	
	Overdominant	CT vs. (CC + TT)	1.14	0.64 – 2.04	0.66
	Dominant	(CT + TT) vs. CC	1.51	0.78 – 2.92	0.22
	Recessive	TT vs. (CC + CT)	1.32	0.66 – 2.64	0.43

Table 4
Distribution of haplotypes among patients and controls and their correlation with breast cancer

S.N.	rs2228570 (Fok1)	rs11568820 (Cdx2)	Total	BC group	Control group	OR (95% CI)	P
1	G	C	0.30	0.30	0.31	1.00	-
2	A	C	0.23	0.20	0.25	0.83 (0.43 - 1.60)	0.5763
3	A	T	0.23	0.28	0.19	1.56 (0.89 - 2.75)	0.1231
4	G	T	0.23	0.22	0.25	0.89 (0.44 - 1.80)	0.7558

The haplotype GC has a frequency of 0.30 in both the breast cancer and the control groups, making it the reference haplotype. The ORs for the haplotypes vary between 0.83 and 1.56, with all p-values exceeding the commonly accepted significance level of 0.05. One of the haplotypes, AT, has a frequency of 0.28 in the BC group and 0.19 in the control group. The OR for this haplotype is 1.56 (95% CI: 0.89 - 2.75) with a p-value of 0.1231. This suggests that the AT haplotype may be linked to a higher risk. However, the evidence is not strong enough to be called conclusive.

Discussion

Given the relevance of VDR polymorphisms as important single nucleotide variations possibly implicated in many malignancies, this study intended to correlate VDR gene variants with Vietnamese breast cancer susceptibility. Two specific VDR polymorphisms, rs2228570 (FokI) and rs11568820 (Cdx2), were selected for genotyping. Allelic frequencies, genotype distributions and Hardy-Weinberg Equilibrium conformity were evaluated, as well as their association with breast cancer susceptibility in women.

The association between VDR polymorphisms and breast cancer risk shows significant variation across different populations and this complexity is also evident in the Vietnamese population. The SNP rs2228570 (Fok1) displays a notable protective effect against breast cancer in the Vietnamese population under the overdominant model (AG vs. AA + GG) with an odds ratio (OR) of 0.52, indicating statistical significance (p-value of 0.03). This contrasts with findings from other populations where rs2228570 (Fok1) has been associated with both increased and decreased breast cancer risks.

For instance, in Canada, the minor homozygous genotype of Fok1 is associated with a lower breast cancer risk (OR = 0.71)[2] while in the United States, the same genotype is linked to an increased risk (OR = 1.34) [3]. These conflicting results underscore the role of ethnic and environmental factors in genetic associations with breast cancer risk. In contrast, rs11568820 (Cdx2) does not show any significant association with breast cancer risk in the Vietnamese population across all genetic models analyzed. This lack of association aligns with findings from studies on the German population, where no significant correlation was observed[1]. However, it contrasts with studies conducted on Canadian[2] and African-American women[13] where rs11568820 (Cdx2) has been linked to a higher likelihood of developing breast cancer.

The Vietnamese data suggest that rs11568820 (Cdx2) may not be a significant marker for breast cancer risk in this population, highlighting the importance of considering ethnic variability in genetic research. The analysis of linkage disequilibrium (LD) between rs2228570 (FokI) and rs11568820 (Cdx2) in the Vietnamese cohort revealed a lack of strong LD, consistent with findings from an Iranian study[6]. This indicates that these SNPs are inherited independently and can serve as separate biomarkers for assessing breast cancer risk.

Haplotype analysis involving rs2228570 (FokI) and rs11568820 (Cdx2) SNPs in this study on the Vietnamese population revealed no significant associations with breast cancer risk. The GC haplotype had a frequency of 0.30 in both the breast cancer and control groups, serving as the reference haplotype. The AT haplotype showed a non-significant potential increased risk compared to the GC haplotype (OR = 1.56, 95% CI: 0.89–2.75, p-value = 0.1231). Consistent with Vietnam, the United Kingdom observed notable correlations with the BsmI and poly(adenylate) sequence polymorphisms, but not with haplotypes involving FokI and Cdx2 [5]. Meanwhile, Australian studies focused on ApaI and TaqI polymorphisms, with FokI showing no significant association[4].

In contrast, studies in other populations showed varying results. In the U.S., the haplotype FtCA (FokI F, TaqI t, VDR-5132 C, Cdx2 A) was associated with a significantly higher breast cancer risk compared to the most frequent haplotype FTCG (OR = 1.43, 95% CI: 1.00–2.05)[1]. Iranian research identified the CGTAT haplotype (comprising ApaI, BsmI, FokI, Cdx2 and TaqI) as significantly associated with breast cancer risk (p-value = 0.0001)[6]. These varying results underscore the complexity of genetic influences on breast cancer risk and the importance of conducting haplotype analyses within diverse populations to identify population-specific genetic markers and develop targeted prevention and treatment strategies.

Conclusion

The study highlights significant findings regarding the association of VDR polymorphisms, specifically rs2228570 (FokI) and rs11568820 (Cdx2), with breast cancer susceptibility in the Vietnamese population. The rs2228570 (FokI) SNP showed a notable protective effect against breast cancer under the overdominant model, indicating potential genetic protection in heterozygous individuals. However,

rs11568820 (Cdx2) did not exhibit any significant association with breast cancer risk across all analyzed genetic models. This aligns with certain international findings but contrasts with others, reflecting the genetic and ethnic diversity influencing breast cancer susceptibility.

The haplotype analysis involving these SNPs also revealed no significant association with breast cancer risk in the Vietnamese population, with the AT haplotype showing a non-significant potential increased risk compared to the GC haplotype. These results are consistent with some studies in other countries, such as the UK and Australia, but differ from findings in the U.S. and Iran, where specific haplotypes were significantly associated with breast cancer risk. Overall, this study underscores the importance of considering ethnic and population-specific genetic factors in understanding breast cancer risk and highlights the need for further research to elucidate these complex genetic associations.

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