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Original article

Association of VDR *ApaI* and *TaqI* Polymorphisms with Breast Cancer Risk in Libyan Women

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Abstract
Vitamin D receptor (VDR) gene polymorphisms, particularly *ApaI* (rs7975232) and *TaqI* (rs731236), have been inconsistently associated with breast cancer (BC) risk across diverse populations. No data exist on their role in Libyan women. The objective of this study was to investigate the association between VDR *ApaI* and *TaqI* polymorphisms and BC susceptibility in a Libyan case-control cohort. Methods: This study included 56 histologically confirmed BC patients and 58 healthy controls. Genotyping was performed using PCR-Restriction Fragment Length Polymorphism (PCR-RFLP), validated by Sanger sequencing. Genetic association was tested under multiple inheritance models (codominant, dominant, recessive, over-dominant, log-additive). Linkage disequilibrium (LD) and haplotype analyses were conducted. Results: No statistically significant association was found for either polymorphism. Analysis of the *ApaI* polymorphism revealed a non-significant, protective trend for the homozygous variant (*a/a*) genotype (OR = 0.48, 95% CI: 0.15–1.54, P = 0.45, codominant model). Conversely, a non-significant trend toward higher risk was observed for the *TaqI* homozygous variant (*t/t*) genotype (OR = 2.40, 95% CI: 0.73–7.86, P = 0.28, codominant model). For both markers, the observed genotype distributions did not deviate significantly from Hardy-Weinberg equilibrium (P > 0.05). A significant moderate linkage disequilibrium was observed between the two SNPs (D' = 0.7786, P = 0). Haplotype analysis revealed no significant association with BC risk. Conclusion: This first genetic association study of VDR *ApaI* and *TaqI* in Libyan women does not support a major role for these specific polymorphisms in BC etiology. The findings provide crucial baseline genetic data for North Africa and underscore the importance of population-specific studies to clarify the heterogeneous global patterns of association of VDR variants.
Keywords. Vitamin D Receptor, *ApaI* and *TaqI* Polymorphisms, Breast Cancer, Genetic Association, Libyan Population.

Introduction

Breast cancer (BC) is still the most prevalent cancer diagnosis and a primary driver of female cancer mortality worldwide [1]. Its etiology is multifactorial, involving complex interactions between environmental exposures, reproductive factors, and genetic susceptibility [2,3]. While high-penetrance mutations in genes such as BRCA1 and BRCA2 confer significant risk, they account for only 5-10% of cases, highlighting the importance of investigating moderate-risk genetic variants in polygenic susceptibility models and driving programmed cell death [3]. The vitamin D receptor (VDR), a member of the nuclear hormone receptor superfamily, mediates the pleiotropic biological actions of 1,25-dihydroxyvitamin D3 [4,5]. In addition to its classic role in maintaining calcium homeostasis, vitamin D exerts potent anti-proliferative and pro-differentiative effects, as well as inducing programmed cell death (apoptosis) [6,7]. These anti-cancer properties are primarily mediated through genomic signalling via the VDR, which regulates the transcription of hundreds of target genes involved in cell cycle control and differentiation [8,9].

Genetic polymorphisms in the VDR gene may influence the receptor's expression, stability, or function, thereby modulating individual responses to vitamin D and potentially affecting cancer risk [10]. Two commonly studied variants, *ApaI* (rs7975232) and *TaqI* (rs731236), have been specifically implicated in modulating mRNA stability and VDR expression levels [11]. The *TaqI* polymorphism, located in exon 9, involves a T to C transition at codon 352 (ATT to ATC). Although this is a synonymous mutation that does not alter the amino acid sequence (isoleucine), it is in strong linkage disequilibrium with the 3' untranslated region (UTR) and is thought to influence mRNA longevity [11]. Similarly, the *ApaI* polymorphism (rs7975232), located in intron 8, is characterized by a G-to-T transversion. Although intronic, this variant is part of a high linkage disequilibrium block at the 3' end of the VDR gene, which may influence mRNA stability and overall gene expression through its association with other functional sites within the VDR locus [11,12]. However, epidemiological evidence linking *ApaI* (rs7975232) and *TaqI* (rs731236) variants to BC risk has been marked by significant inconsistency across different ethnic populations. Studies have reported positive associations in Egyptian, Iranian, and Chinese cohorts [13–15], while

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others from Turkey, Ethiopia, and meta-analyses of predominantly Caucasian populations have found no significant links [16–19]. This heterogeneity may be due to genetic differences between populations, variations in LD patterns, and environmental factors, including vitamin D status [20,21]. However, no genetic data are currently available on VDR polymorphisms in relation to breast cancer in Libya, a North African country with a distinct genetic background and environmental context.

In Libya, breast cancer is reported as the most common cancer among Libyan women with an incidence rate of 23.2% [22]. Moreover, it has been found that the incidence rate of 18.8 new cases per 100,000 each year, with a younger age than that of European women [23]. However, no genetic data are currently available on VDR polymorphisms in relation to breast cancer in Libya, a North African country with a distinct genetic background and environmental context. Addressing this research gap is essential to ensure that the global understanding of breast cancer genetics reflects all populations, rather than just a few well-studied regions. Therefore, this case-control study represents the first effort to evaluate the link between VDR *ApaI* and *TaqI* polymorphisms and BC susceptibility in Libyan women by utilizing rigorous genotyping and detailed genetic modelling. This study seeks to clarify how these variants influence breast cancer susceptibility, providing a more definitive understanding of genetic risk within the Libyan population.

Methods

Study Population and Ethical Approval

A total of 120 women (60 BC patients and 60 healthy controls) were recruited for this study. PCR amplification was successfully performed for 114 samples (58 BC and 56 control samples). PCR-RFLP genotyping was subsequently successful for 113 (99.1%) samples for the *ApaI* polymorphism and 109 (95.6%) samples for the *TaqI* polymorphism. Samples that failed PCR amplification or produced unscorable RFLP patterns were excluded from the corresponding genotype analyses. All participants provided written informed consent before joining the study. This study was formally approved by the Bioethics Committee at the Biotechnology Researcher Center (BEC-BTRC) (Ref No: NBC: 001. H. 26. 06).

DNA Extraction and PCR Amplification

Genomic DNA was isolated from peripheral blood samples using the Epicenter Master Pure™ Complete DNA and RNA Purification Kit. DNA concentration and purity (260/280 ratio) were verified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). A 489-bp fragment of the VDR gene, encompassing both the *ApaI* and *TaqI* loci, was amplified via polymerase chain reaction (PCR) using previously published primers (Forward: 5'-GGACAGAGCATGGACAGGGAGC-3' and Reverse: 5'-GGGCGTTAGCTTCATGCTGCAC-3') [15].

Genotyping by PCR-RFLP and Validation

To determine the genotypes, the PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) technique was used. The 489-bp PCR products were digested in two separate reactions using *ApaI* and *TaqI* restriction endonucleases (New England Biolabs, USA) following the manufacturer's recommended conditions. Genotypes were assigned based on whether the specific SNP created or removed a restriction site. For simplicity and consistency throughout the manuscript, the *ApaI* genotypes are denoted as (*A/A*, *A/a*, and *a/a*), corresponding to the actual nucleotide genotypes (*G/G*, *G/T*, and *T/T*), respectively. Likewise, the *TaqI* genotypes are denoted as (*T/T*, *T/t*, and *t/t)*, corresponding to (*T/T*, *T/C*, and *C/C*), respectively.

For the *TaqI* (rs731236) locus, the T allele (ancestral thymine) lacked the recognition site, leaving a single 489-bp fragment. In contrast, the t allele (variant cytosine) created a 5'-T^CGA-3' recognition site, cleaving the product into 297-bp and 192-bp fragments. For the *ApaI* (rs7975232) locus, the (*A*) allele (ancestral) remains undigested (489 bp), while the (*a*) allele (variant cytosine) created a 5'-GGGCC^C-3' recognition site, resulting in 273-bp and 216-bp fragments. The digested products were determined on 2% agarose gels and visualized under UV light. To ensure genotyping accuracy, a random subset of samples (n = 4), representing all genotype combinations, was validated by bidirectional Sanger sequencing (Ribosite Biotechnology, Tunisia). These results showed 100% agreement with our RFLP results.

Statistical Analysis

Allele and genotype frequencies were calculated for all participants, and a chi-square goodness-of-fit test was used to ensure the control group followed Hardy-Weinberg equilibrium (HWE). To explore the relationship between these SNPs and breast cancer risk, five different genetic models (codominant, dominant, recessive, over-dominant, and log-additive) were tested using unconditional logistic regression to generate Odds Ratios (ORs) and 95% Confidence Intervals (CIs). Linkage disequilibrium (LD) between the two SNPs was estimated using the standardized coefficient (D') and the correlation coefficient (r). Furthermore, haplotype frequencies were inferred using the Expectation-Maximization (EM) algorithm, and their association with BC risk was analysed relative to the most frequent haplotype. The overall significance of haplotype distribution was assessed using a global likelihood ratio test. All genetic analyses were performed using SNPStats [24], while descriptive statistics were conducted using IBM SPSS Statistics v26. All tests were two-sided, and a P-value < 0.05 was considered statistically significant.

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Results

PCR-RFLP analysis was successful for 109 (95.6%) samples for *TaqI* and 113 (99.1%) for *ApaI*, with failed reactions excluded. The genotype distributions for both *ApaI* (P = 0.78) and *TaqI* (P = 0.11) in the control group were in Hardy-Weinberg equilibrium, indicating no major genotyping error or population stratification.

Genotyping of VDR ApaI Polymorphism

Genotyping of the *ApaI* polymorphism was performed via PCR-RFLP analysis. As shown in (Figure 1-I), the undigested PCR product was 489 bp. The homozygous (A/A) genotype was identified by a single 489 bp fragment (absence of the restriction site), while the homozygous (*a/a*) genotype resulted in two fragments of 273 bp and 216 bp. The heterozygous (*A/a*) genotype displayed all three fragments (489, 273, and 216 bp).

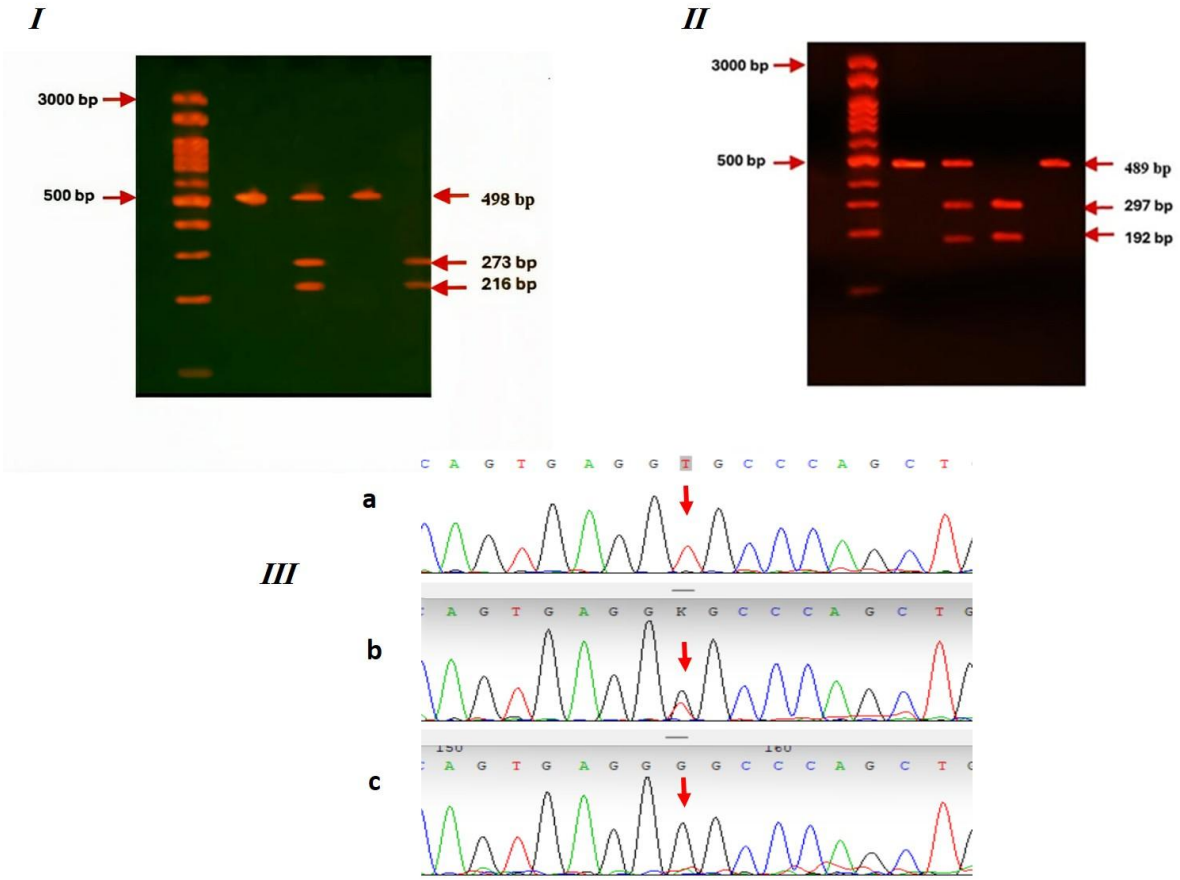

Figure: (1). (I) An agarose gel showing the three different ApaI genotypes. Lane 1, a negative control; lane 2, 3000bp DNA marker; Lane 3, undigested PCR amplicon; lane 4, heterozygous (A/a) genotype; lane 5, homozygous (A/A) genotype; lane 6, homozygous (a/a) genotype. (II). An agarose gel showing the three different TaqI genotypes. Lane 1, a negative control; lane 2, 3000bp DNA marker; lane 3, undigested PCR amplicon; lane43, heterozygous (Tt) genotype; lane 5, homozygous (T/T) genotype; lane 6, homozygous (tt) genotype. (III). Chromatogram of the genotyping of ApaI polymorphism (rs7975232, indicated by the red arrow) of the VDR gene. (a) DNA sequencing analysis representing the (A/A-T/T) genotype. (b) DNA sequencing analysis representing the (A/a-T/G) genotype. (c) DNA sequencing analysis representing (a/a-G/G) genotype

Genotyping of VDR TaqI Polymorphism

The *TaqI* polymorphism was successfully genotyped via PCR-RFLP analysis (Figure 1-II). The undigested PCR amplicon was 489 bp. The homozygous TT genotype was identified by the presence of the 489 bp fragment, indicating an absence of the restriction site. Complete digestion of the product yielded two fragments of 297 bp and 192 bp, characteristic of the homozygous tt genotype. The heterozygous Tt genotype was characterized by the presence of all three fragments (489, 297, and 192 bp).

Validation of Genotyping via Sanger Sequencing

To validate the accuracy of the PCR-RFLP genotyping, representative samples were subjected to Sanger sequencing. The resulting chromatograms (Figure 1-III) showed clean, well-separated peaks, confirming the presence of the polymorphic site. Sequence analysis confirmed the homozygous (*A/A –T/T*), the heterozygous (*A/a)* (characterized by an overlapping (*T/G)* signal), and the homozygous (*a/a – G/G*) genotypes. These sequencing results were in 100% agreement with the initial PCR-RFLP findings, confirming the reliability of the genotyping method.

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Single-SNP Association Analysis

The allele and genotype distributions of *ApaI* (rs7975232) and *TaqI* (rs731236) are summarized in (Tables 1 and 2). No statistically significant associations were observed for either SNP under any of the tested genetic models (Tables 3 and 4). For the *ApaI* polymorphism, the minor allele (*a*) was more frequent in breast cancer cases (42%) than in controls (34%) (Table 1); however, this difference was not statistically significant (P = 0.230).

Table 1. Allele frequencies of VDR ApaI and TaqI Polymorphisms

SNP	Allele	Cases (%)	Controls (%)	P-value
ApaI	*A*	58%	66%	0.230
	a	42%	34%	
TaqI	*T*	69%	60%	0.203
	t	31%	40%	

For the *ApaI* polymorphism, the heterozygous genotype (*A/a*) was the most frequently observed genotype in both breast cancer cases (48%) and controls (47%) (Table 2). The homozygous variant genotype (*a/a*) was more frequent in cases (18%) than in controls (11%), whereas the homozygous wild-type genotype (*A/A*) was more common in controls (42%) than in cases (34%). However, the overall genotype distribution did not differ significantly between cases and controls (χ^2 = 1.573, P = 0.456).

Table2. Genotype frequencies of VDR ApaI and TaqI Polymorphisms

SNP	genotype frequencies (n = 120)							
		All subjects		Cases		Controls		P-value
	Genotype	Count	Proportion	Count	Proportion	Count	Proportion	
Apa1	***A/A***	43	0.38	19	0.34	24	0.42	0.456
	A/a	54	0.48	27	0.48	27	0.47	
	a/a	16	0.14	10	0.18	6	0.11	
	NA	7	---	4	---	3	---	
TaqI	T/T	48	0.44	24	0.47	24	0.41	0.295
	T/t	44	0.4	22	0.43	22	0.38	
	t/t	17	0.16	5	0.1	12	0.21	
	NA	11	---	9	---	2	---	

Genotype-based analysis showed no statistically significant association under any genetic model (codominant, dominant, recessive, overdominant, or log-additive) (Table 3). In the codominant model, the heterozygous genotype (*A/a*) showed a non-significant trend towards a reduced risk of breast cancer compared with the wild-type genotype (OR = 0.79, 95% CI: 0.35–1.77). Likewise, the homozygous variant genotype (*a/a*) showed a non-significant trend towards a reduced risk (OR = 0.48, 95% CI: 0.15–1.54). The dominant (*A/a* + *a/a* vs. *A/A*) (OR = 0.71, 95% CI: 0.33–1.51) and recessive (*a/a* vs. *A/A* + *A/a*) (OR = 0.54, 95% CI: 0.18–1.61) models also suggested reduced odds of breast cancer, although these associations were not statistically significant. Similarly, no statistically significant associations were observed under the overdominant or log-additive models (OR = 0.97, 95% CI: 0.46–2.02; OR = 0.71, 95% CI: 0.41–1.24, respectively).

Table 3. Association test (Genetic Model) for SNP (ApaI)

SNP *ApaI* association with response status (n = 113, crude analysis)					
Model	**Genotype**	**Case**	**Control**	**OR (95% CI)**	**P-value**
Codominant	*A/A*	19 (33.9%)	24 (42.1%)	1.00	0.45
	A/a	27 (48.2%)	27 (47.4%)	0.79 (0.35-1.77)	
	a/a	10 (17.9%)	6 (10.5%)	0.48 (0.15-1.54)	
Dominant	*A/A*	19 (33.9%)	24 (42.1%)	1.00	0.37
	A/a-a/a	37 (66.1%)	33 (57.9%)	0.71 (0.33-1.51)	
Recessive	*A/A-A/a*	46 (82.1%)	51 (89.5%)	1.00	0.26
	a/a	10 (17.9%)	6 (10.5%)	0.54 (0.18-1.61)	
Overdominant	*A/A-a/a*	29 (51.8%)	30 (52.6%)	1.00	0.93
	A/a	27 (48.2%)	27 (47.4%)	0.97 (0.46-2.02)	
Log-additive	---	---	---	0.71 (0.41-1.24)	0.23

These results indicate that neither the alleles nor the genotypes of *ApaI* are significantly associated with breast cancer risk in this sample (n = 113). The results across all genetic models are visually summarized in (Table 3), which illustrates the odds ratios and 95% confidence intervals for each model, highlighting the non-significant trends.

Similarly, for the *TaqI* polymorphism (rs731236), the minor allele (t) was less frequent in breast cancer cases (31%) than in controls (40%) (Table 2), but this difference was not statistically significant (*P* = 0.203). For the *TaqI* polymorphism, the

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homozygous wild-type genotype (*T/T*) was the predominant genotype in both breast cancer cases (47%) and controls (41%), followed by the heterozygous genotype (*T/t*) (43% and 38%, respectively) (Table 2). Although the homozygous variant genotype (*t/t*) was more frequent in controls (21%) than in cases (10%), the overall genotype distribution was not significantly different between the two groups ($\chi^2 = 2.443$, $P = 0.295$).

Genotype-based analysis showed no significant association under any genetic model (codominant, dominant, recessive, overdominant, or log-additive) (Table 4). In the codominant model, patients with the heterozygous (*T/t)* genotype had the exact same odds of disease as the baseline (*T/T)* reference group (OR = 1.00, 95% CI: 0.44–2.27). Meanwhile, homozygous variant carriers (*t/t*) showed a non-significant trend toward higher risk (OR = 2.40, 95% CI: 0.73–7.86). This same increased risk trend appeared under the recessive model (*t/t* vs. *T/T* + *T/t*: OR = 2.40, 95% CI: 0.78–7.36). The dominant model (*T/t* + t/t vs. *T/T*) also showed a slight, non-significant risk elevation (OR = 1.26, 95% CI: 0.59–2.69), while the overdominant model showed no real link (OR = 0.81, 95% CI: 0.37–1.73) and the log-additive model trended slightly higher (OR = 1.38, 95% CI: 0.81–2.36).

Table 4. Association test (Genetic Model) for SNP (TaqI)

SNP *(TaqI)* association with response status (n = 109, crude analysis)					
Model	**Genotype**	**case**	**Control**	**OR (95% CI)**	**P-value**
Codominant	*T/T*	24 (47.1%)	24 (41.4%)	1.00	0.28
	T/t	22 (43.1%)	22 (37.9%)	1.00 (0.44-2.27)	
	t/t	5 (9.8%)	12 (20.7%)	2.40 (0.73-7.86)	
Dominant	*T/T*	24 (47.1%)	24 (41.4%)	1.00	0.55
	T/t-t/t	27 (52.9%)	34 (58.6%)	1.26 (0.59-2.69)	
Recessive	*T/T-T/t*	46 (90.2%)	46 (79.3%)	1.00	0.11
	t/t	5 (9.8%)	12 (20.7%)	2.40 (0.78-7.36)	
Overdominant	*T/T-t/t*	29 (56.9%)	36 (62.1%)	1.00	0.58
	T/t	22 (43.1%)	22 (37.9%)	0.81 (0.37-1.73)	
Log-additive	---	---	---	1.38 (0.81-2.36)	0.23

As observed for *ApaI*, neither the alleles nor the genotypes of TaqI are significantly associated with breast cancer risk in this sample (n = 109). These results across all genetic models are visually summarized in Table 4, which highlights these non-significant trends.

Linkage Disequilibrium and Haplotype Analysis

The Linkage Disequilibrium (LD) analysis of the *ApaI* and *TaqI* polymorphisms revealed a significant moderate association between the two loci (D' = 0.7786, *r = -0.4555*, D = -0.106 $P < 0.001$). The negative correlation coefficient (r) reflects an inverse relationship in allele pairing, indicating that the alleles are partially co-inherited. Haplotype frequencies were inferred using the EM algorithm, and four distinct haplotypes were identified (Table 5). The distribution of these haplotypes did not differ significantly between breast cancer cases and controls, as indicated by the global association test (Global $P = 0.24$). Using the most frequent haplotype, T-a (34.5%), as the reference group, no individual haplotypes showed a statistically significant association with breast cancer risk. The t-A haplotype exhibited a non-significant trend toward a risk effect (OR = 1.52, 95% CI: 0.81 - 2.87, $P = 0.20$). Conversely, the rare t-a haplotype (3.1%) showed a reduced Odds Ratio (OR = 0.26, 95% CI: 0.02 - 2.92, $P = 0.28$), though the association was not significant and the estimate was imprecise due to the low frequency of this variant in the study population.

Table 5. Haplotype Analysis of VDR ApaI and TaqI Polymorphisms

Haplotype (*TaqI-ApaI*)	**Total Frequency**	**Cases Frequency**	**Controls Frequency**	**OR (95% CI)**	***P*-value**
T-a	0.345	0.364	0.324	1.00 (Reference)	---
t-A	0.319	0.254	0.379	1.52 (0.81 - 2.87)	0.20
T-A	0.305	0.328	0.286	0.99 (0.48 - 2.03)	0.98
t-a	0.031	0.054	0.012	0.26 (0.02 - 2.92)	0.28
Global association test	—	—	—	—	0.24

Discussion

Breast cancer susceptibility is influenced by a combination of genetic, environmental, and lifestyle factors [25]. Vitamin D, in particular, is known to influence oncogenesis by regulating cellular differentiation and proliferation, and its deficiency has been linked to increased cancer progression [26,27]. This study represents the first investigation of VDR gene polymorphisms *ApaI* (rs7975232) and *TaqI* (rs731236) in relation to breast cancer risk among Libyan women. Overall, our case-control analysis found no statistically significant association between these polymorphisms and breast cancer, with all p-values exceeding 0.05. Genotype and allele distributions for *ApaI* and *TaqI* were in HWE in both cases and controls (P = 0.11–1.00), indicating genetic stability and minimal population stratification or genotyping bias. The present study found no significant association between the VDR *ApaI* polymorphism and breast cancer susceptibility in Libyan women. Although

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the minor allele (a) and the homozygous variant genotype (*a/a*) were observed more frequently among cases than controls, neither the allele frequencies (P = 0.230) nor the overall genotype distribution (P = 0.456) differed significantly between the two groups. Moreover, under multiple genetic models (codominant, dominant, recessive, overdominant, and log-additive), no statistically significant associations were detected. For the *ApaI* SNP, in the codominant model, a non-significant trend toward reduced risk was observed for the homozygous variant (*a/a*) genotype relative to the wild-type baseline (OR = 0.48, 95% CI: 0.15–1.54; P = 0.45), while all other models similarly showed no significant associations (P > 0.05). In comparison, Kazemi et al. (2022) reported a statistically significant protective effect specifically for the heterozygous (*A/a*) genotype (OR = 0.39, 95% CI: 0.22–0.69; P = 0.0038), in an Iranian population, with no significant effect observed for their (*a/a*) genotype group. Although both studies show that *ApaI* variants generally lower risk rather than increase it, they differ on which exact genotype is most affected. This variation suggests that the codominant and dominant genetic models are the most useful ways to look at this marker. These slight differences highlight how genetic patterns can change between different populations, showing why we need larger studies with diverse ethnic groups to confirm these trends.

Consistent with previous results, no significant association was observed between the VDR *TaqI* polymorphism and breast cancer risk. The (T) allele and (T/T) genotype were more frequent among cases, whereas the (t/t) genotype was more common in controls; however, neither the allele frequencies (P = 0.203) nor the overall genotype distribution (P = 0.295) showed statistically significant differences. These results indicate that the *TaqI* polymorphism does not appear to contribute substantially to breast cancer susceptibility in the studied Libyan population. In addition, under multiple genetic models, both the (*T/T*) and (*T/t*) *TaqI* genotypes were slightly more frequent in cases than in controls (47.1% vs. 41.4% for (*T/T*), and 43.1% vs. 37.9% for *T/t*), respectively. Because they increased together, the calculated odds of disease for (*T/t*) carriers were identical to the (*T/T*) reference group (OR = 1.00, 95% CI: 0.44–2.27). In contrast, the homozygous variant (*t/t*) genotype was twice as frequent in controls (20.7%) as in cases (9.8%). This structural shift confirms that analysing the data under a recessive model framework (OR = 2.40, 95% CI: 0.78–7.36; P = 0.11) provides the most accurate reflection of the *TaqI* locus trend. Genotypic analysis across all models revealed no statistically significant associations. Under the recessive model (t/t vs. *T/T* + *T/t*), a non-significant trend toward increased risk was observed (OR = 2.40, 95% CI: 0.78–7.36; P = 0.11), while the overdominant model showed no meaningful association (OR = 0.81, 95% CI: 0.37–1.73; P = 0.58). These findings suggest that the *TaqI* polymorphism is unlikely to have a substantial independent effect on breast cancer risk; however, the observed recessive trend warrants further investigation in larger, multi-center studies. Linkage disequilibrium (LD) analysis between *ApaI* and *TaqI* revealed a statistically significant, moderately strong association (D' = 0.779; r = –0.456; D = –0.106; P < 0.001), suggesting partial co-inheritance of these loci in this population. Haplotype analysis identified four distinct combinations: (*T/a*, *t/A*, *T/A*, and *t/a*). The (*T/a*) haplotype was the most common and was used as our baseline reference group. The (t/A) haplotype showed a non-significant trend toward higher risk (OR = 1.52, 95% CI: 0.81–2.87; P = 0.20), while the (*T/A*) group showed virtually no link to disease risk (OR = 0.99, 95% CI: 0.48–2.03; P = 0.98). On the other hand, the rare t-a haplotype suggested a potential protective effect (OR = 0.26, 95% CI: 0.02–2.92; P = 0.28), though its low frequency makes this estimate less precise. Overall, the global haplotype test confirmed that these distributions were not statistically different between the groups (P = 0.24).

Comparison with previous studies reveals variable results across populations. For *ApaI*, several studies have found no significant association, including a meta-analysis by Lu et al. (2016) in primarily Caucasian populations [19], an Ethiopian study [16], and a Turkish case–control study [28], consistent with our findings. Conversely, significant associations have been reported: in Iran, Matini et al. (2020) observed increased risk with the (*A/a*) genotype and a higher frequency of the a allele in patients[15]; Kazemi et al. (2022) reported increased risk under the dominant model, with a protective effect for heterozygous (*A/a*) carriers [29]; in Egypt, Abd-Elsalam et al. (2015) reported elevated risk for postmenopausal women with the (*a/a*) genotype [30]; and El-Shorbagy et al. (2017) reported increased risk associated with (*A/A*) in *ApaI* among Egyptian women [13]. Similarly, an Australian study observed a significantly increased risk of breast cancer with *ApaI* (*A/a*) and (*a/a*) genotypes [31]. In contrast, a study from China reported that individuals with the (*A/A*) genotype had a lower risk of breast cancer compared to those with the (*a/a*) genotype [14]. These differences suggest that the effect of *ApaI* on breast cancer risk may vary according to ethnicity and environmental factors. For *TaqI*, findings of no significant association in the current study align with prior studies in Turkey [17], Egypt [30], and a global meta-analysis [18]. However, other reports suggest potential risk associations: Matini et al. (2020) found higher risk among (*t*) allele carriers in Iran [15]; El-Shorbagy et al. (2017) reported increased risk associated with (*T/t*) in *TaqI* and (*A/A*) in *ApaI* among Egyptian women [13]; and Curran et al. (1999) observed population-specific allele frequency differences in Australia [31]. These contrasting findings indicate that *TaqI* polymorphism's influence on breast cancer risk may be population-dependent.

Conclusion

In conclusion, this study provides the first evidence regarding VDR *ApaI* (rs7975232) and *TaqI* (rs731236) polymorphisms in Libyan women, showing no statistically significant association with breast cancer risk. These findings highlight that genetic risk factors identified in one population cannot be universally generalized and emphasize the importance of population-specific studies in understanding the complex aetiology of breast cancer [3,21]. Although some trends in genetic models and haplotype analyses were observed, together with moderate linkage disequilibrium between the two loci, larger multi-centre studies are required to confirm potential population-specific effects.

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Conflicts of Interest
The authors declare no conflict of interest related to the publication of this work.

Ethics
This study was formally approved by the Bioethics Committee at the Biotechnology Researcher Centre (BEC-BTRC) (Ref No: NBC: 001. H. 26. 06).

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