Experimental Research

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

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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 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 *Taq*I, *Apa*I, *Bsm*I, Cdx2, and *Fok*I 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 *Fok*I (rs2228570), *Bsm*I (rs1544410), and *Apa*I (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 *Taq*I and SNP *Bsm*I was significantly ($p \leq 0.05$). We observed that four major haplotypes of *Apa*I, *Bsm*I, *Fok*I, Cdx2, and *Taq*I 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 *Fok*I, *Bsm*I, and *Apa*I 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)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

[8–12].

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 *Taq*I polymorphism (rs731236) in the VDR gene, substituting T for C in exon IX of VDR gene, *Fok*I polymorphism (rs2228570), changing C to T in exon II of VDR gene, *Apa*I polymorphism (rs7975232), changing A to C in the intron between exon VIII and IX (INTRON 8), *Bsm*I 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. Also, haplotypes frequency and association with breast cancer risk were calculated. Finally, linkage disequilibrium for each pair of SNPs was measured.

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 tests. 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 at 4 °C 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

Table 1
The sequences of used primers for PCR.

SNP Polymorphism (SNP ID[a])	Primers
*Fok*I (rs2228570)	[b] For: AGCTATGTAGGGCGAATCATG
	[c] Rev: AGGAGATGTGAAAAATGCAAGG
*Bsm*I (rs1544410)	For: CTGAAGGGAGACGTAGCAAAAG
	Rev: ATGTCCCCAAGGTCACAATAAC
*Apa*I (rs7975232)	For: CTTCTGGATCATCTTGGCATAGAG
	Rev: AAGATAGCAGAGCAGAGTTCCAAG
*Taq*I (rs731236)	For: GCAAAGATAGCAGAGCAGAGTTC
	Rev: CTTCTGGATCATCTTGGCATAGAG
Cdx2 (rs11568820)	For: GGGCTGTGAAATAAATTTGGTGGA
	Rev: TAAACACCCAGAGAACATTCTGAGAG

[a] SNP ID is extracted from miRBase database (http://www.mirbase.org/).
[b] Forward primer.
[c] Reverse primer.

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 prepared amplicons were digested with 10 units of *Taq*I, *Apa*I, *Bsm*I, Cdx2, and *Fok*I 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 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/haploview).

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 cases) 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 *Apa*I polymorphism was more in breast cancer cases (40.5%) than the controls (21.6%). AA genotype of *Bsm*I polymorphism (homozygous allele 1), had a little more value of distribution in breast cancer cases (38.5%)

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 (%)	
*Apa*I	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	
*Bsm*I	A	171	63	143	53	
	G	99	37	125	47	
	AA	52	39	42	31	0.53
	AG	67	5	59	44	
	GG	16	12	33	25	
*Fok*I	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	
*Taq*I	T	134	58	171	65	
	C	96	42	93	35	
	CC	15	13	16	12	0.23
	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
*Bsm*I	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)	
*Apa*I	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 (59.7%)	1.00	0.01
		AC-CC	68 (56.2%)	56 (40.3%)	0.53 (0.32–0.86)	
	Codominant	AA	53 (43.8%)	83 (59.7%)	1.00	0.0038
		AC	49 (40.5%)	30 (21.6%)	0.39 (0.22–0.69)	
		CC	19 (15.7%)	26 (18.7%)	0.87 (0.44–1.73)	
*Fok*I	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%)	32 (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)	
*Taq*I	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-AG	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 *Fok*I 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 *Taq*I, the heterozygous TC genotype (was more common among breast cancer cases (56.9%) than controls (46.2%) (Table 2).

The VDR *Apa*I, *Bsm*I, and Taq1 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 *Bsm*I (rs1544410) 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 a protective effect against breast cancer (11.8% in breast cancer vs 24.6% in controls) (OR = 2.55, 95% CI = 1.24–5.26, P < 0.05) (Table 3).

In case of *Apa*I 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 *Fok*I 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 = 0.18 95% CI = 0.10–0.30, p = 0.001). In the codominant model, CC, and CT genotypes 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 = 0.0001). 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 *Taq*I 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

Table 4
Linkage disequilibrium for five SNPs of VDR gene.

	*Apa*I	*Bsm*I	*Fok*I	Cdx2	*Taq*I
*Apa*I	.	0	0.0523	0.0441	0.0817
*Bsm*I	0.9999	.	0.1099	0.0237	0.1405
*Fok*I	0.2899	0.1379	.	0.1251	0.0036
Cdx 2	0.3972	0.7534	0.1466	.	0.0508
*Taq*I	0.1289	0.0508	0.9439	0.5401	.

Upper diagonal: D′-values for the SNP combinations; lowr diagonal: *P*-values for pair wise LD.

shown in Table 4, most of the SNPs were not in tight and highly significant LD with each other. Only, *Taq*I showed a somewhat high LD to the SNP *Bsm*I 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 (*Apa*I, *Bsm*I, *Fok*I, Cdx2, and *Taq*I 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 AGCGC, CACGC, 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 (*Fok*I), rs1544410 (*Bsm*I), rs7975232 (*Apa*I), rs731236 (*Taq*I), 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 *Fok*I, *Bsm*I, and *Apa*I polymorphisms had a significant difference in patients than to the normal group. Furthermore, the genotype distribution *Fok*I was in Hardy–Weinberg equilibrium, but the genotype distribution of *Bsm*I and *Apa*I was not in Hardy–Weinberg equilibrium. Loss of heterozygosity (LOH) events in *Bsm*I and *Apa*I 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 Bsm1, Apa1, Taq1, and Fok1 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 Bsm1 polymorphism had a significant association with breast cancer risk, but Fok1 had no significant association in Iranian populations [36]. Our results verify that both *Fok*I and *Bsm*I have a significant association with risk of Breast cancer. In Yiallourou et al. research, *Fok*I polymorphism was associated with the risk of breast cancer staging and survival among Caucasian women [45]. This verifies our result of the *Fok*I polymorphism association with breast cancer risk.

In Pulito 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 *Taq*I polymorphism had increased risk for mortality in breast cancer women. Also, *Fok*I 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 *Fok*I, *Bsm*I, *Apa*I, and *Taq*I variants of VDR polymorphisms were not associated with breast cancer risk [48]. Our results showed that *Fok*I, *Bsm*I, and *Apa*I could be associated with breast cancer.

The power of linkage disequilibrium (LD) of all pairs of these variants

Table 5
Haplotypes frequency in case and control groups, and their association with breast cancer.

	*Apa*I	*Bsm*I	*Fok*I	Cdx2	*Taq*I	Total	BC group	Control group	OR (95% CI)	*P*-value
1	A	A	T	G	T	0.1176	0.1037	0.1285	1.00	–
2	A	G	T	A	T	0.0702	0	0.1119	1.37 (0.47–3.99)	0.56
3	A	G	T	G	T	0.0679	0.0546	0.088	2.60 (0.62–10.94)	0.19
4	A	A	C	G	T	0.0588	0.0532	0.0674	0.08 (0.01–1.04)	0.055
5	A	A	T	G	C	0.0547	0	0.0678	1.03 (0.17–6.27)	0.97
6	C	G	T	G	T	0.053	0.0716	0.0252	0.47 (0.13–1.75)	0.26
7	A	G	C	G	C	0.0493	0.0648	0.05	0.02 (0.00–0.53)	0.02
8	C	A	T	G	C	0.0455	0.0334	0.0525	1.40 (0.30–6.53)	0.67
9	A	A	T	A	C	0.0454	0.0735	0.0213	3.31 (0.26–41.96)	0.36
10	A	A	C	A	T	0.0436	0.0404	0.0252	0.31 (0.06–1.53)	0.15
11	A	A	T	A	T	0.0367	0.0406	0.0438	0.95 (0.08–11.51)	0.97
12	C	A	C	G	C	0.0356	0.0325	0.0412	0.08 (0.01–0.66)	0.019
13	A	G	T	G	C	0.035	0.0508	0.0446	1.25 (0.28–5.49)	0.77
14	A	G	C	G	T	0.032	0.0778	0.0055	11.80 (0.49–285.35)	0.13
15	C	A	T	A	T	0.0306	0.0595	0.0222	0.54 (0.11–2.54)	0.44
16	C	A	C	G	T	0.029	0.0289	0.0273	1.20 (0.17–8.68)	0.85
17	C	A	C	A	T	0.0266	0	0.0478	0.02 (0.00–0.78)	0.037
18	A	A	C	G	C	0.0258	0.0201	0.0135	3.62 (0.12–111.21)	0.46
19	C	G	T	A	T	0.0239	0.0205	0.0129	5.64 (3.64–7.43)	<0.0001
20	C	G	C	G	T	0.02	0	0.0252	1.55 (0.20–11.97)	0.67
21	A	G	C	A	C	0.0191	0.0281	0.0107	0.33 (0.01–11.32)	0.54
22	A	G	T	A	C	0.0183	0.017	0.0204	0.76 (0.06–9.46)	0.83
23	C	A	T	A	C	0.0182	0.0216	0.0061	0.22 (0.01–6.39)	0.38
24	C	G	T	G	C	0.0153	0.0291	0.0178	0.09 (0.00–1.92)	0.12
Rare	*	*	*	*	*	0.0281			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.

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 Thakkinstian et al. performed a meta-analysis for surveying haplotypes and linkage disequilibrium of researches on the *Bsm*I, *Apa*I, and *Taq*I polymorphisms in bone mineral density (BMD). Their results show the existence of a strong LD between the *Bsm*I and *Taq*I polymorphisms [49]. In our study, we verified this result, that there is a stronger LD between *Bsm*I and *Taq*I 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

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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.

Consent

Not applicable.

Author contribution

Seyedeh 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

Acknowledgments

Appendix A. Supplementary data

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

References

[1] Z. Tao, et al., Breast cancer: epidemiology and etiology, Cell Biochem. Biophys. 72 (2) (2015) 333–338.

[2] S.B. Wheeler, K.E. Reeder-Hayes, L.A. Carey, Disparities in breast cancer treatment and outcomes: biological, social, and health system determinants and opportunities for research, Oncol. 18 (9) (2013) 986–993.

[3] M.F. Holick, Vitamin D: its role in cancer prevention and treatment, Prog. Biophys. Mol. Biol. 92 (1) (2006) 49–59.

[4] R.R. Buras, et al., Vitamin D receptors in breast cancer cells, Breast Cancer Res. Treat. 31 (2–3) (1994) 191–202.

[5] A.J. Brown, A. Dusso, E. Slatopolsky, Vitamin D, Am. J. Physiol. 277 (2) (1999) F157–F175.

[6] C.F. Garland, et al., The role of vitamin D in cancer prevention, Am. J. Publ. Health 96 (2) (2006) 252–261.

[7] E. Giovannucci, et al., Prospective study of predictors of vitamin D status and cancer incidence and mortality in men, J. Natl. Cancer Inst. 98 (7) (2006) 451–459.

[8] E. Giovannucci, et al., A prospective study of calcium intake and incident and fatal prostate cancer, Cancer Epidemiol. Biomarkers Prev. 15 (2) (2006) 203–210.

[9] A. Kilkkinen, et al., Vitamin D status and the risk of lung cancer: a cohort study in Finland, Cancer Epidemiol. Biomarkers Prev. 17 (11) (2008) 3274–3278.

[10] E. Giovannucci, Vitamin D and cancer incidence in the Harvard cohorts, Ann. Epidemiol. 19 (2) (2009) 84–88.

[11] K. Ng, et al., Circulating 25-hydroxyvitamin d levels and survival in patients with colorectal cancer, J. Clin. Oncol. 26 (18) (2008) 2984–2991.

[12] Y. Ma, D.L. Trump, C.S. Johnson, Vitamin D in combination cancer treatment, J. Cancer 1 (2010) 101.

[13] C. Spina, et al., Vitamin D and cancer, Anticancer Res. 26 (4A) (2006) 2515–2524.

[14] Y.J. Jiang, et al., 1α, 25 (OH) 2-dihydroxyvitamin D3/VDR protects the skin from UVB-induced tumor formation by interacting with the β-catenin pathway, J. Steroid Biochem. Mol. Biol. 136 (2013) 229–232.

[15] K. Köstner, et al., The relevance of vitamin D receptor (VDR) gene polymorphisms for cancer: a review of the literature, Anticancer Res. 29 (9) (2009) 3511–3536.

[16] A.-C. Lundin, et al., Association of breast cancer progression with a vitamin D receptor gene polymorphism, Cancer Res. 59 (10) (1999) 2332–2334.

[17] J.A. Randerson-Moor, et al., Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case–control comparisons and a meta-analysis of published VDR data, Eur. J. Cancer 45 (18) (2009) 3271–3281.

[18] M.L. Slattery, et al., CDX2 VDR polymorphism and colorectal cancer, Cancer Epidemiology and Prevention Biomarkers 16 (12) (2007) 2752–2755.

[19] M.L. Slattery, et al., Dietary calcium, vitamin D, VDR genotypes and colorectal cancer, Int. J. Cancer 111 (5) (2004) 750–756.

[20] T. Carling, et al., Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors, J. Clin. Endocrinol. Metab. 83 (7) (1998) 2255–2259.

[21] N. Denzer, T. Vogt, J. Reichrath, Vitamin D receptor (VDR) polymorphisms and skin cancer: a systematic review, Derm. Endocrinol. 3 (3) (2011) 205–210.

[22] J. Han, G.A. Colditz, D.J. Hunter, Polymorphisms in the MTHFR and VDR genes and skin cancer risk, Carcinogenesis 28 (2) (2007) 390–397.

[23] S.A. Ingles, et al., Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor, J. Natl. Cancer Inst. 89 (2) (1997) 166–170.

[24] E.M. John, et al., Sun exposure, vitamin D receptor gene polymorphisms, and risk of advanced prostate cancer, Cancer Res. 65 (12) (2005) 5470–5479.

[25] R.S. Heist, et al., Circulating 25-hydroxyvitamin D, VDR polymorphisms, and survival in advanced non–small-cell lung cancer, J. Clin. Oncol. 26 (34) (2008) 5596.

[26] D. Lu, L. Jing, S. Zhang, Vitamin D receptor polymorphism and breast cancer risk: a meta-analysis, Medicine 95 (18) (2016).

[27] J. Shi, et al., Genetic variation in vitamin D-related genes and risk of breast cancer among women of European and East Asian descent, Tumor Biol. 37 (5) (2016) 6379–6387.

[28] F. Shaikh, S. Baig, Q.D. Jamal, VDR gene polymorphisms contribute to breast cancer, Asian Pac. J. Cancer Prev. APJCP 17 (2) (2016) 479–483.

[29] V. Rai, et al., Vitamin D receptor polymorphism and cancer: an update, Anticancer Res. 37 (8) (2017) 3991–4003.

[30] S. Gandini, et al., Vitamin D receptor polymorphisms and cancer, in: Sunlight, Vitamin D and Skin Cancer, Springer, 2014, pp. 69–105.

[31] A.C. Porojnicu, et al., Seasonal and geographical variations in lung cancer prognosis in Norway: does vitamin D from the sun play a role? Lung Cancer 55 (3) (2007) 263–270.

[32] T.E. Robsahm, et al., Vitamin D 3 from sunlight may improve the prognosis of breast-, colon-and prostate cancer (Norway), Cancer Causes Control 15 (2) (2004) 149–158.

[33] M. Ruggiero, et al., Vitamin D receptor gene polymorphism is associated with metastatic breast cancer, Oncol. Res. 10 (1) (1998) 43–46.

[34] M.L. McCullough, et al., Vitamin D pathway gene polymorphisms, diet, and risk of postmenopausal breast cancer: a nested case-control study, Breast Cancer Res. 9 (1) (2007) R9.

[35] J.D. McKay, et al., Vitamin D receptor polymorphisms and breast cancer risk: results from the national cancer Institute breast and prostate cancer cohort consortium, Cancer Epidemiology and Prevention Biomarkers 18 (1) (2009) 297–305.

[36] S. Shahbazi, et al., BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer, Med. Oncol. 30 (1) (2013) 393.

[37] S. Abbas, et al., Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk, Breast Cancer Res. 10 (2) (2008) R31.

[38] K. Majidzadeh-a, et al., Iranian Breast Cancer Bio-Bank: the activity and challenging issues, Cell Tissue Bank. 14 (1) (2013) 11–20.

[39] R. Agha, et al., STROCSS 2021: Strengthening the Reporting of Cohort, Cross-Sectional and Case-Control Studies in Surgery, 2021, p. 103026.

[40] Z. Yue, et al., Identification of breast cancer candidate genes using gene co-expression and protein-protein interaction information, Oncotarget 7 (24) (2016) 36092.

[41] J. Lilyquist, et al., Common Genetic Variation and Breast Cancer Risk—Past, Present, and Future, AACR, 2018.

[42] M. Kittaneh, A.J. Montero, S. Glück, Molecular profiling for breast cancer: a comprehensive review, Biomarkers Cancer 5 (2013) S9455. BIC.

[43] A.-J. Huang, et al., Polymorphism rs4919510: C> G in mature sequence of human microRNA-608 contributes to the risk of HER2-positive breast cancer but not other subtypes, PLoS One 7 (5) (2012) e35252.

[44] N. Mavaddat, et al., Genetic susceptibility to breast cancer, Molecular oncology 4 (3) (2010) 174–191.

[45] A.I. Yiallourou, et al., Association of FokI and PvuII polymorphisms with breast cancer staging and survival among Caucasian women: a prospective study, J buon 19 (3) (2014) 633–642.

[46] C. Pulito, et al., Cdx2 polymorphism affects the activities of vitamin D receptor in human breast cancer cell lines and human breast carcinomas, PLoS One 10 (4) (2015) e0124894.

[47] L. Perna, et al., Vitamin D receptor genotype rs731236 (Taq1) and breast cancer prognosis, Cancer Epidemiol. Biomarkers Prev. 22 (3) (2013) 437–442.

[48] D. Lu, L. Jing, S. Zhang, Vitamin D receptor polymorphism and breast cancer risk: a meta-analysis, Medicine (Baltim.) 95 (18) (2016) e3535.

[49] A. Thakkinstian, C. D'Este, J. Attia, Haplotype analysis of VDR gene polymorphisms: a meta-analysis, Osteoporos. Int. 15 (9) (2004) 729–734.