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Association between VDR Gene Polymorphisms (*rs 1544410*, *rs 7975232*, *rs 2228570*, *rs 731236* and *rs 11568820*) and Susceptibility to Breast Cancer in a Sample of Southeastern Iranian Population

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Abstract

Background: Vitamin D receptor (VDR) is a key nuclear receptor that is associated with the risk and progression of breast cancer (BC).
Objectives: The present study investigated the *Fok1*, *Bsm1*, *Taq1* and *Cdx2* polymorphisms in the VDR gene and susceptibility to BC in a sample of Southeastern Iranian population.
Methods: This case-control study was conducted on 180 women with BC and 178 age-matched healthy women. RFLP-PCR method was used for analysis of *Bsm1* (*rs 1544410*), *Apa1* (*rs 7975232*), *Fok1* (*rs 2228570*) and *Taq1* (*rs 731236*) and also TETRA-ARMS method for *Cdx2* (*rs 11568820*).
Results: No significant correlation was found between polymorphisms of *Taq1*, *Fok1* and Apa1 with BC, but was for *Bsm1* (odds ratio (OR) = 3.452, 95% CI 1.769 - 6.738; P < 0.001). Also, there was a significant correlation between the case and control groups for *Cdx2* (OR = 3.720, 95% CI 2.224 - 6.225; P < 0.001) and allele A in *Cdx2* had just significant correlation with BC.
Conclusions: The present study findings showed that there were significant correlations between *Bsm1* and *Cdx2* polymorphisms with BC in women of Sistan and Baluchestan Province (southeastern Iran). Also, signals of *Rs1544410-Bsm1* and *Rs11568820-Cdx2* positions were difference with routes of estrogen and progesterone per person and they probably act independently.

Keywords: Breast Cancer, Polymorphism, VDR, Southeastern Iran

1. Background

Breast cancer (BC) is the most frequent malignancy among women (1) that is the second leading cause in low and middle income countries (2). Inherited genetic risk factors contribute toward BC onset and the discovery of new BC susceptibility genes is critical for improved risk assessment and to provide insight toward disease mechanisms for the development of more effective therapies (3). As in Iran, since the onset of the disease is at low age, in spite of the relatively high survival rate as compared to other cancers, prevention and screening programs at early age for early stage diagnosis seem necessary (4). A combination of family- and population-based approaches indicated that genes involved in DNA repair are associated with moderate BC risk (5). The genetic factors known to be involved in BC risk comprise about 30 genes (6), the risk of some of them has been reported in Iranian people with BC (7-9). Vitamin D (1, 25-dihydroxyVitamin D3) has been shown experimentally to have anti-carcinogenic effects and is thought to inhibit BC (10). Vitamin D is hypothesized to lower the risk of BC by inhibiting cell proliferation via the nuclear vitamin D receptor (VDR) (11). Therefore, the actions of Vitamin D are mediated via the VDR, and the polymorphisms at 3'UTR region (four important single nucleotide polymorphisms (SNPs) in exon 2 including *VDR-Fok1* (*rs 2228570*), *VDR-Bsm1* (*rs 1544410*), *VDR-Taq1* (*rs 731236*) and *VDR-Apa1* (*rs 7975232*) (12) of this gene are associated with the risk and progression of breast carcinoma (10). Also, the VDR is a key nuclear receptor that binds nutritionally derived ligands and exerts bio-effects that contribute to bone mineral homeostasis, detoxification of exogenous and endogenous compounds, cancer prevention, and mammalian hair cycling (13). *VDR-Cdx2* is another polymorphism of the VDR. There are limited studies on the re-

lationship between it and BC's unfavorable biopathological characteristics (14). Therefore, these polymorphisms change the codons that alter the function of VDR protein.

2. Objectives

In the present study, we investigated the *Fok1*, *Bsm1*, *Taq1* and *Cdx2* polymorphisms in the VDR gene and susceptibility to BC in a sample of Southeastern Iranian population.

3. Methods

3.1. Patients

This study was approved by the ethical committee of Zahedan University of Medical Sciences (Grant number: 6796 and Ethical Code: IR.ZAUMS.REC1393.6796). In a cross-control study, 180 BC and 178 control women (age-matched) who referred to Ali-ibn Abi Talib hospital and private centers, Zahedan, Iran were chosen. The controls did not have any relationship with patients and had no history of cancer.

3.2. Immunohistochemical (IHC) Analysis

Estrogen receptor (*ER*) and progesterone receptor (*PR*) positivity, defined as $\geq$ 10% positive tumor cells with nuclear staining (15). Also, for *HER2* 2+ based on IHC, chromogenic in situ hybridization (*CISH*) identified *HER2* gene amplification for determination of *HER2* status.

3.3. VDR Genotype Analysis

Blood samples of the controls and patients were gathered in tubes with EDTA, and DNA was extracted with salting out method (16). RFLP-PCR method was used for analysis of *rs 1544410*, *rs 7975232*, *rs 2228570*, and *rs 731236* while TETRA-ARMS method was used for rs11568820. Primer sequence and reaction conditions have been shown in Table 1. The amplified PCR products were digested with *Taq1*, *Apa1*, *Bsm1* and *Fok1* restriction endonuclease enzymes (Thermo Scientific Company, USA) overnight (16 hours) at temperatures 65°C, 37°C, 37°C and 55°C respectively. The PCR conditions for VDR polymorphisms (*Taq1*, *Fok1*, *Apa1* and *Bsm1*) were: The initial denaturation in 95°C for 5 minutes and after that, thirty cycles in 95°C for 30 seconds, 68°C for 30 seconds, 72°C for 30 seconds and at last, 72°C for 5 minutes. Then, products of PCR with 2% agarose gel and 0.5 μg/mL Ethidium bromide were loaded and observed under UV light. At last, each site was digested with specific enzyme. The PCR conditions for *Cdx2* was: The initial denaturation in 95°C for 5 minutes and after that, thirty cycles in 95°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds and at last, 72°C for 5 minutes.

3.4. Statistical Analysis

The analysis was done using SPSS 22 software (*IBM*, SPSS Inc., Chicago, IL, USA). The logistic regression analyses were assessed by computing the odds ratio (OR) and 95% confidence intervals (CI) for association between genotypes and BC. Also, a p-value < 0.05 was considered to be statistically significant.

4. Results

The mean age of the case and control groups were 47.93 years and 48.28 years, respectively. Table 2 shows a number of variables in the patients. The prevalence of genotypes in two groups has been shown in Table 3. There was no significant correlation between polymorphisms of *Taq1*, *Fok1* and *Apa1* with BC, but there was for Bsm1 (OR = 3.452, 95% CI 1.769 - 6.738; $P < 0.001$). Also, there was a significant correlation between the case and control groups for *Cdx2* (OR = 3.720, 95% CI 2.224 - 6.225; $P < 0.001$) and allele A in *Cdx2* had just significant correlation with BC.

The correlation between five genotypes and three receptors in BC patients have been shown in Table 4. There was just a significant correlation between *Fok1* and *HER2* status (P = 0.025).

5. Discussion

This study showed that there were significant correlations between polymorphisms of VDR, such as *Bsm1* and *Cdx2*, and risk of BC in women of Sistan and Baluchestan province (southeastern Itan). These polymorphisms, based on their position at the beginning of VDR gene, impacted translation and ultimately levels of expression of these protein. The OR for BC in association with *Bsm1* and *Cdx2* was (OR = 0.4, 95% CI 0.222 - 0.721; $P < 0.05$) and (OR = 0.29, 95% CI 0.148 - 0.565; $P < 0.05$), respectively. Guy et al. (17) reported that VDR polymorphisms are associated with BC risk and may be associated with disease progression in United Kingdom Caucasian population and Chandler et al. (3) showed that they are associated with BC in African-Americans, but not in Hispanic/Latinas and that the *Fok1FF* genotype is linked with poor prognosis in African-American women with BC. The results of one study (18) suggested that *Cdx2* polymorphism was a potential biomarker for vitamin D treatment in BC, independent of the VDR receptor expression, and another study reported the *Bsm1* associated with BC risk, with a trend for increasing risk with increasing number of *Bsm1* B alleles in Latina women (19) and the b allele in Pakistani women (20). In addition, *Bsm1* genotype significantly modified the association between dietary vitamin D and BC overall (21). The

Table 1. Primer Sequence and Reaction Conditions

SNP	Primer sequence	Restriction enzyme	Product size (bp)	Annealing
rs 1544410	Forward: 5-AACCAAGACTACAAGTACCGCGTCAGTGA-3 (30bp)	*Bsm1*	GG 650 + 175	68°C
			AG 825 + 650 + 175	
	Reverse: 5-AACCAGCGGAAGAGGTCAAGGG-3 (22bp)		AA 825	
rs 7975232	Forward: 5-GCAACTCCTCATGGCTGAGGTCTCA-3 (25bp)	*Apa1*	TT 745	68°C
	Reverse: 5-AGAGCATGGACAGGGAGCAAG-3 (21bp)		GT 745 + 528 + 217	
			GG 528 + 217	
rs 2228570	Forward:5-ATGGAAACACCTTGCTTCTTCTCCCTC-3 (27bp)	*Fok1*	FF 272	68°C
			Ff 272 + 198 + 74	
	Reverse: 5-ATGCCAGCTGGCCCTGGCACTG-3 (22bp)		Ff 198 + 74	
rs 731236	Forward: 5-GCAACTCCTCATGGCTGAGGTCTCA-3 (25bp)	*Taq1*	CC 294 + 251 + 201	68°C
			TC 493 + 294 + 251 + 201	
	Reverse: 5-AGAGCATGGACAGGGAGCAAG-3 (21bp)		TT 493 + 251	
rs 11568820	F1: 5'-AGGATAGAGAAAATAATAGAAAACATT-'3 (27bp)	*Cdx2*	GG 297 + 110	58°C
	R1: 5'-AACCCATAATAAGAAATAAGTTTTTAC-'3 (27bp)		AG 297 + 235 + 110	
	F2: 5'-TCCTGAGTAAACTAGGTCACAA-'3 (22bp)		AA 297 + 235	
	R2: 5'-ACGTTAAGTTCAGAAAGATTAATTC-'3 (25bp)			

Table 2. Demographic Variables in Breast Cancer Patients (n = 180)[a]

Variable	Patients group
Age	
≥ 50	67 (39.4)
> 50	103 (60.6)
TNM Stage	
I	25 (14)
II	75 (41.9)
III	50 (27.9)
IV	29 (16.2)
Grade	
I	28 (19)
II	92 (62.6)
III	27 (18.4)
ER status	
Positive	105 (61)
Negative	67 (39)
HER2 status	
Positive	88 (49.4)
Negative	90 (50.6)
PR status	
Positive	97 (56.7)
Negative	74 (43.3)

[a]Values are expressed as N. (%).

Pakistani authors (22) offered that the GG genotype of *Cdx2*-VDR gene polymorphism may increase the risk of developing BC in young female patients in South Pakistan. The authors of one research concluded that the common genetic variants in vitamin D genes (*Bsm1*, *Apo1*, *Fok1* and *Taq1*) were not risk factors for BC in Chinese women (23). Also, the current analysis suggested that they may not be associated with BC risk in Caucasian women (24) and a meta-analysis study confirmed this result in Caucasian population (25). The results of Tang et al. (26) showed that there were not significant associations between the *Bsm1*, *Apa1* and *Taq1* variants and risk of BC. *Apa1* and *Taq1* and *Fok1* were tested for association with BC risk in 135 females with sporadic BC and 110 cancer-free female controls (27) where allele frequencies of Apa1 polymorphism showed a significant association, while the *Taq1* showed a similar trend, but the *Fok1* polymorphism were not significantly different in the study population. Chen et al. (28) observed a significantly increased risk of BC among carriers of the ff genotype of *Fok1* compared with those with FF, but did not observe an association between polymorphisms in BsmI and BC risk for BB versus bb. Therefore, the results suggested that the VDR may be a mediator of BC risk and could represent a target for cancer prevention efforts. Shahbazi et al. (29) concluded that statistically significant association between *Fok1* genotypes and BC risk was not observed, but there was an increased risk of BC associated with the BsmI polymorphism (*Bsm1* bb or even Bb genotype) in Tehran (Central Iran).

In conclusion, the present study findings showed that there were significant correlations between Bsm1 and Cdx2 polymorphisms, and BC in women of Sistan and Baluches-

Table 3. The Exact Prevalence of Genotypes in Two Groups

Variabes	Case Group[a]	Control Group[a]	OR	P Value
Rs1544410-Bsm1				
GG	14 (7.8)	35 (19.7)	1	< 0.001
AG	145 (80.6)	105 (59)	3.452 (1.769 - 6.738)	< 0.001
AA	21 (11.6)	38 (21.3)	1.382 (0.610 - 3.129)	0.438
Allele				
G	157 (45.63)	175 (49.15)	1	-
A	187 (54.36)	181 (50.85)	1.15 (0.86 - 1.55)	0.364
Rs7975232-Apa1				
TT	45 (25)	52 (29.2)	1	0.263
GT	124 (68.9)	121 (68)	0.393 (0.127 - 1.218)	0.106
GG	11 (6.1)	5 (2.8)	0,466 (0.157 - 1.380)	0.168
Allele				
T	214 (59.45)	225 (63.21)	1	-
G	146 (40.55)	131 (36.79)	1.17 (0.87 - 1.58)	0.319
Rs2228570-Fok1				
FF	98 (54.4)	88 (49.4)	1	0.297
Ff	72 (40)	84 (47.2)	0.668 (0.233 - 1.914)	0.453
Ff	10 (5.6)	6 (3.4)	0.514 (0.178 - 1.484)	0.219
Allele				
F	268 (74.45)	260 (73.04)	1	-
F	92 (25.55)	96 (26.96)	0.93 (0.67 - 1.29)	0.672
Rs731236-Taq1				
TT	79 (43.9)	83 (46.6)	1	0.253
TC	90 (50)	77 (43.3)	1.558 (0.692 - 3.504)	0.284
CC	11 (6.1)	18 (10.1)	1.913 (0.851 - 4.297)	0.116
Allele				
T	248 (68.88)	243 (68.25)	1	-
C	112 (31.12)	113 (31.75)	0.97 (0.71 - 1.33)	0.872
Rs11568820-Cdx2				
GG	26 (14.4)	69 (38.8)	1	< 0.001
AG	150 (83.4)	107 (60.1)	3.720 (2.224 - 6.225)	< 0.001
AA	4 (2.2)	2 (1.1)	5.308 (0.917 - 30.736)	0.06
Allele				
G	202 (56.12)	245 (68.82)	1	-
A	158 (43.88)	111 (31.18)	1.73 (1.27 - 2.34)	< 0.001

[a]Values are expressed as N. (%).

tan province (southeastern Itan). Also, signals of *Rs1544410-Bsm1* and *Rs11568820-Cdx2* positions were different with routes of ER and PR per person and they probably act independently. Therefore, studies with more sample sizes and in different ethnicities and long-term follow-up are required to confirm our finding.

Table 4. The Correlation Between Genotypes and Receptors in Breast Cancer Patients

Variables		Bsm1		P Value
	GG, N = 14	AG, N = 137	AA, N = 21	
ER, Positive	8 (57.1)	87 (63.5)	10 (47.6)	0.362
PR, Positive	9 (64.3)	77 (56.6)	11 (52.4)	0.783
HER2, Positive	7 (50)	68 (47.6)	13 (61.9)	0.470
		Cdx2		
	GG, N = 26	AG, N = 148	AA, N = 4	
ER, Positive	13 (50)	73 (49.3)	2 (50)	0.998
PR, Positive	13 (54.2)	83 (58)	1 (25)	0.406
HER2, Positive	16 (64)	86 (60.1)	3 (75)	0.791
		Fok1		
	FF, N = 93	Ff, N = 69	ff, N = 10	
ER, Positive	55 (59.1)	43 (62.3)	7 (70)	0.796
PR, Positive	53 (57.6)	39 (56.5)	5 (50)	0.898
HER2, Positive	53 (54.6)	34 (47.9)	1 (10)	0.025
		Taq1		
	TT, N = 78	TC, N = 89	CC, N = 11	
ER, Positive	43 (55.1)	55 (66.3)	7 (63.6)	0.345
PR, Positive	45 (58.4)	46 (55.4)	6 (54.5)	0.918
HER2, Positive	34 (43.6)	50 (56.2)	4 (36.4)	0.179
		Apa1		
	TT, N = 44	GT, N = 117	GG, N = 11	
ER, Positive	28 (63.6)	71 (60.7)	6 (54.5)	0.850
PR, Positive	21 (47.7)	70 (60.3)	6 (54.5)	0.351
HER2, Positive	23 (51.1)	53 (51.6)	2 (18.2)	0.101

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Footnotes

Authors' Contribution: Seyed Mehdi Hashemi and Mohammad Hashemi were supervisor and designed the study. Narges Arbabi was the corresponding author; wrote the article, prepared the proposal and extracted the gene polymorphisms of blood samples. Mohammad Ali Mashhadi analyzed the data, checked the gene polymorphisms and the proposal. Abolghasem Allahyari and Masoud Sadeghi revised the article.

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References

1. Madani SH, Payandeh M, Sadeghi M, Motamed H, Sadeghi E. The correlation between Ki-67 with other prognostic factors in breast cancer: A study in Iranian patients. *Indian J Med Paediatr Oncol.* 2016;**37**(2):95–9. doi: 10.4103/0971-5851.180136. [PubMed: 27168707].
2. Payandeh M, Sadeghi M, Sadeghi E. Differences in Prognostic Factors between Early and Late Recurrence Breast Cancers. *Asian Pac J Cancer Prev.* 2015;**16**(15):6575–9. doi: 10.7314/APJCP.2015.16.15.6575. [PubMed: 26434877].
3. Chandler MR, Bilgili EP, Merner ND. A Review of Whole-Exome Sequencing Efforts Toward Hereditary Breast Cancer Susceptibility Gene Discovery. *Hum Mutat.* 2016;**37**(9):835–46. doi: 10.1002/humu.23017. [PubMed: 27226120].
4. Rahimzadeh M, Pourhoseingholi MA, Kavehie B. Survival Rates for Breast Cancer in Iranian Patients: a Meta- Analysis. *Asian Pac J Cancer Prev.* 2016;**17**(4):2223–7. doi: 10.7314/APJCP.2016.17.4.2223. [PubMed: 27221922].
5. Filippini SE, Vega A. Breast cancer genes: beyond BRCA1 and BRCA2. *Front Biosci (Landmark Ed).* 2013;**18**:1358–72. doi: 10.2741/4185. [PubMed: 23747889].

6. Collins A, Politopoulos I. The genetics of breast cancer: risk factors for disease. *Appl Clin Genet.* 2011;**4**:11–9. doi: 10.2147/TACG.S13139. [PubMed: 23776363].
7. Hashemi M, Amininia S, Ebrahimi M, Hashemi SM, Taheri M, Ghavami S. Association between hTERT polymorphisms and the risk of breast cancer in a sample of Southeast Iranian population. *BMC Res Notes.* 2014;**7**:895. doi: 10.1186/1756-0500-7-895. [PubMed: 25491902].
8. Hashemi M, Amininia S, Ebrahimi M, Hashemi SM, Yousefi J, Eskandari-Nasab E, et al. Association between LAPTM4B gene polymorphism and breast cancer susceptibility in an Iranian population. *Med Oncol.* 2014;**31**(8):111. doi: 10.1007/s12032-014-0111-8. [PubMed: 25001088].
9. Hashemi M, Yousefi J, Hashemi SM, Amininia S, Ebrahimi M, Taheri M, et al. Association between Programmed Cell Death 6 Interacting Protein Insertion/Deletion Polymorphism and the Risk of Breast Cancer in a Sample of Iranian Population. *Dis Markers.* 2015;**2015**:854621. doi: 10.1155/2015/854621. [PubMed: 26063962].
10. Chakraborty A, Mishra AK, Soni A, Regina T, Mohil R, Bhatnagar D, et al. Vitamin D receptor gene polymorphism(s) and breast cancer risk in north Indians. *Cancer Detect Prev.* 2009;**32**(5-6):386–94. doi: 10.1016/j.canep.2009.04.012. [PubMed: 19588543].
11. McKay JD, McCullough ML, Ziegler RG, Kraft P, Saltzman BS, Riboli E, et al. Vitamin D receptor polymorphisms and breast cancer risk: results from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. *Cancer Epidemiol Biomarkers Prev.* 2009;**18**(1):297–305. doi: 10.1158/1055-9965.EPI-08-0539. [PubMed: 19124512].
12. Mishra DK, Wu Y, Sarkissyan M, Sarkissyan S, Chen Z, Shang X, et al. Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic women. *PLoS One.* 2013;**8**(3):e57967. doi: 10.1371/journal.pone.0057967. [PubMed: 23554871].
13. Haussler MR, Haussler CA, Bartik L, Whitfield GK, Hsieh JC, Slater S, et al. Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. *Nutr Rev.* 2008;**66**(10 Suppl 2):S98–112. doi: 10.1111/j.1753-4887.2008.00093.x. [PubMed: 18844852].
14. Pulito C, Terrenato I, Di Benedetto A, Korita E, Goeman F, Sacconi A, et al. Cdx2 polymorphism affects the activities of vitamin D receptor in human breast cancer cell lines and human breast carcinomas. *PLoS One.* 2015;**10**(4):e0124894. doi: 10.1371/journal.pone.0124894. [PubMed: 25849303].
15. Payandeh M, Sadeghi M, Sadeghi E, Madani SH. Expression of p53 Breast Cancer in Kurdish Women in the West of Iran: a Reverse Correlation with Lymph Node Metastasis. *Asian Pac J Cancer Prev.* 2016;**17**(3):1261–4. doi: 10.7314/APJCP.2016.17.3.1261. [PubMed: 27039757].
16. Eskandari-Nasab E, Hashemi M, Amininia S, Ebrahimi M, Rezaei M, Hashemi SM. Effect of TP53 16-bp and beta-TrCP 9-bp INS/DEL polymorphisms in relation to risk of breast cancer. *Gene.* 2015;**568**(2):181–5. doi: 10.1016/j.gene.2015.05.048. [PubMed: 26003292].
17. Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Peckitt C, Bliss J, et al. Vitamin D receptor gene polymorphisms and breast cancer risk. *Clin Cancer Res.* 2004;**10**(16):5472–81. doi: 10.1158/1078-0432.CCR-04-0206. [PubMed: 15328186].
18. Zhou ZC, Wang J, Cai ZH, Zhang QH, Cai ZX, Wu JH. Association between vitamin D receptor gene Cdx2 polymorphism and breast cancer susceptibility. *Tumour Biol.* 2013;**34**(6):3437–41. doi: 10.1007/s13277-013-0919-4. [PubMed: 23821301].
19. Ingles SA, Garcia DG, Wang W, Nieters A, Henderson BE, Kolonel LN, et al. Vitamin D receptor genotype and breast cancer in Latinas (United States). *Cancer Causes Control.* 2000;**11**(1):25–30. doi: 10.1023/A:1008979417618. [PubMed: 10680726].
20. Rashid MU, Muzaffar M, Khan FA, Kabisch M, Muhammad N, Faiz S, et al. Association between the BsmI Polymorphism in the Vitamin D Receptor Gene and Breast Cancer Risk: Results from a Pakistani Case-Control Study. *PLoS One.* 2015;**10**(10):e0141562. doi: 10.1371/journal.pone.0141562. [PubMed: 26517870].
21. Rollison DE, Cole AL, Tung KH, Slattery ML, Baumgartner KB, Byers T, et al. Vitamin D intake, vitamin D receptor polymorphisms, and breast cancer risk among women living in the southwestern U.S. *Breast Cancer Res Treat.* 2012;**132**(2):683–91. doi: 10.1007/s10549-011-1885-4. [PubMed: 22130867].
22. Iqbal M, Khan TA, Maqbool SA. Vitamin D receptor Cdx-2 polymorphism and premenopausal breast cancer risk in southern Pakistani patients. *PLoS One.* 2015;**10**(3):e0122657. doi: 10.1371/journal.pone.0122657. [PubMed: 25799416].
23. Dorjgochoo T, Delahanty R, Lu W, Long J, Cai Q, Zheng Y, et al. Common genetic variants in the vitamin D pathway including genome-wide associated variants are not associated with breast cancer risk among Chinese women. *Cancer Epidemiol Biomarkers Prev.* 2011;**20**(10):2313–6. doi: 10.1158/1055-9965.EPI-11-0704. [PubMed: 21828234].
24. Yang B, Liu S, Yang X, Wang Y, Zhao X, Zheng D, et al. Current evidence on the four polymorphisms of VDR and breast cancer risk in Caucasian women. *Meta Gene.* 2014;**2**:41–9. doi: 10.1016/j.mgene.2013.09.008. [PubMed: 25606388].
25. Lu D, Jing L, Zhang S. Vitamin D Receptor Polymorphism and Breast Cancer Risk: A Meta-Analysis. *Medicine (Baltimore).* 2016;**95**(18):e3535. doi: 10.1097/MD.0000000000003535. [PubMed: 27149457].
26. Tang C, Chen N, Wu M, Yuan H, Du Y. FokI polymorphism of vitamin D receptor gene contributes to breast cancer susceptibility: a meta-analysis. *Breast Cancer Res Treat.* 2009;**117**(2):391–9. doi: 10.1007/s10549-008-0262-4. [PubMed: 19145484].
27. Curran JE, Vaughan T, Lea RA, Weinstein SR, Morrison NA, Griffiths LR. Association of A vitamin D receptor polymorphism with sporadic breast cancer development. *Int J Cancer.* 1999;**83**(6):723–6. doi: 10.1002/(SICI)1097-0215(19991210)83:6<723::AID-IJC4>3.3.CO;2-V. [PubMed: 10597185].
28. Chen WY, Bertone-Johnson ER, Hunter DJ, Willett WC, Hankinson SE. Associations between polymorphisms in the vitamin D receptor and breast cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2005;**14**(10):2335–9. doi: 10.1158/1055-9965.EPI-05-0283. [PubMed: 16214913].
29. Shahbazi S, Alavi S, Majidzadeh AK, Ghaffarpour M, Soleimani A, Mahdian R. BsmI but not FokI polymorphism of VDR gene is contributed in breast cancer. *Med Oncol.* 2013;**30**(1):393. doi: 10.1007/s12032-012-0393-7. [PubMed: 23277283].