Clinical Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2018, Vol. 46(5) 1801–1814

DOI: 10.1177/0300060518761304
journals.sagepub.com/home/imr
SAGE

Association of low penetrance vitamin D receptor *Tru*9I (rs757343) gene polymorphism with risk of premenopausal breast cancer

Mehir un Nisa Iqbal[1], Syed Amir Maqbool[2] and Taseer Ahmed Khan[3]

Abstract

Objective: The aim of this study was to determine whether a novel polymorphism (*Tru*9I) in the low penetrance vitamin D receptor *(VDR)* gene is associated with risk of premenopausal breast cancer (BC).

Methods: This case-control study included 228 patients with BC and 503 healthy women living in Pakistan who were analyzed for the *VDR Tru*9I (rs757343) single nucleotide polymorphism. BC cases were histopathologically confirmed, and all healthy controls were age-matched with patients (age range, 20–45 years). DNA was extracted, and the polymerase chain reaction and restriction fragment length polymorphism assays were performed.

Results: The *VDR Tru*9I polymorphism was not significantly associated with premenopausal BC. However, the risk of BC was associated with the 'uu' genotype (odds ratio [OR], 1.141; 95% confidence interval [95% CI], 0.206–6.317). Further, mutant *Tru*9I was significantly associated with Grade IV carcinoma (OR, 5.36; 95% CI, 1.181–24.338).

Conclusion: The *VDR Tru*9I 'uu' genotype may increase the risk of premenopausal BC.

Keywords

Vitamin D receptor, Tru9I, rs757343, premenopausal, breast cancer, Pakistan

Date received: 14 November 2017; accepted: 25 January 2018

[1]Department of Physiology, University of Karachi, Karachi, Pakistan
[2]Department of Clinical Oncology, Karachi Institute of Radiotherapy and Nuclear Medicine (KIRAN) hospital, Karachi, Pakistan
[3]Department of Physiology, University of Karachi, Karachi, Pakistan

Corresponding author:
Taseer Ahmed Khan, Department of Physiology, University of Karachi, Karachi, Pakistan.
Email: takhan@uok.edu.pk

Introduction

Breast cancer (BC) is the most prevalent cancer and a major cause of death among women worldwide. In Pakistan, the incidence of BC is approximately 34.6% in women.[1] Among risk factors, lifestyle, alcoholism, high birth weight, and abdominal adiposity intensify the risk of BC.[2–4] Diet may modify the incidence of BC,[5] although many dietary components such as vitamin D are categorized as "Limited or no conclusion."[6] Vitamin D plays a major role in mineral homeostasis;[6] however, evidence indicates that its deficiency exacerbates the risk of BC.[7,8] Moreover, higher vitamin D levels are weakly associated with the low BC risk".[9,10]

Vitamin D derived from diet and sun exposure of the skin undergoes activation within the liver through 25 hydroxylation, and in the kidney through 1-α hydroxylation, to form 1,25(OH)2D3 (active vitamin D). The classical role of vitamin D is to regulate calcium and phosphate metabolism to maintain bone mineral density (BMD). However, there are wide ranges of nonclassical functions of vitamin D within the body. Vitamin D is activated by forming a complex with the vitamin D receptor (VDR) that is involved in the regulation of many target genes that contribute to cell differentiation, cell growth, programmed cell death, angiogenesis, inflammation, and immune responses.[11,12] Vitamin D, in concert with growth factors, arrests the cell cycle by preventing entry into S phase.[13] Vitamin D induces apoptosis through down-regulation of the antiapoptotic protein B-cell lymphoma 2 (BCL2),[13] inhibits the expression of P-cadherin[14] and increases the expression E-cadherin,[15] which may contribute to the anti-invasive or antimetastatic effects of vitamin D. Further, vitamin D induces the expression of the tumor suppressor BRCA1, because the binding sites for the vitamin D/VDR/retinoid X receptor heterodimer complex are present within the *BRCA1* promoter region.[16] Although VDR is present in many body tissues, including normal and cancerous breast tissues, its expression is low in mammary cancers cells,[17] which may be caused by variations in *VDR*.

There are many polymorphic regions in *VDR*, among which *Fok*1, *Bsm*1, *Apa*1, and *Taq*1 are the most extensively studied in American (Hispanic white, nonhispanic white, and Caucasian) and Asians (Iranian and Egyptian) populations.[19–24] Attention focuses on the role of single nucleotide polymorphisms (SNPs) in *VDR* that are associated with the development of a wide range of pathological conditions, including type 2 diabetes mellitus, prostate cancer, and BC[18–20]. However, limited studies are available on the Pakistani population.[25] Studies of Jordanian populations reveal a statistically significant difference between *VDR Taq*1 variants and 25(OH)D among women with BC.[21] *Bsm*1 and *Cdx*2 polymorphisms are significantly associated with women with BC living in Southeastern Iran.[20] The *Fok1* SNP in BC plays a protective role, whereas the *Bsm1* SNP is not associated with BC among women living in the Iranian city of Urmia.[22] Among Egyptian women, the *Bsm*1 "B" allele and "Bb" variants of *VDR* may represent a risk factor for susceptibility to the development of BC.[23] In contrast, a meta-analysis shows no association of *VDR* SNPs (*Fok*1, *Bsm*1, *Taq*1, and *Apa*1) with risk of BC in a general population or in a Caucasian population.[24] Among Pakistanis who are BRCA1/2 noncarriers, the Bsm1 "b" allele is associated with an increased risk of BC".[25] Although the associations of *VDR Fok*1 and *Bsm*1 SNPs with the risk of BC have been investigated, no study, to our knowledge, investigated the relationship between the *VDR Tru*9I polymorphism and the

premenopausal risk of BC. Therefore, the present study was designed detect an association between the novel low penetrance *VDR Tru*9I SNP and BC among premenopausal women of Pakistan.

Materials and methods

Ethics statement

The study was approved by the Bioethics Committee, Board of Advance Studies & Research, University of Karachi (ethics approval number [10(27) 28032012] and conducted in accordance with the Helsinki Declaration. Each subject provided written informed consent before providing a blood sample.

Study design and study population

The study included 228 patients with BC and 503 control subjects who were premenopausal ethnic Pakistanis aged 20–45 years. The sample size was calculated using OpenEpi (http://www.openepi.com/Menu/OE_Menu.htm) with 80% statistical power and 95% confidence intervals (95% CIs). Patients with BC, who were randomly selected from two tertiary hospitals in Sindh, Pakistan from 2012 to 2015, were histopathologically diagnosed. The medical information (types, grades, estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2, and TNM classification) of patients with BC was obtained from histopathological reports and hospital records. All healthy volunteers were age-matched (1:2) to premenopausal healthy subjects acquired from the general population. Whole blood (3 mL) was collected from each subject. We used a self-structured questionnaire to acquire along with information about personal demographics, detailed history, and BC-related risk factors.

DNA isolation

DNA was extracted using a DNA isolation kit (Gene JET Genomic DNA Purification Kit, Thermo Fisher Scientific Baltics UAB, and Vilnius, Lithuania) following the manufacturer's protocols. Samples were stored at –86°C.

Primer selection and genotyping

*VDR Tru*9I (rs757343) genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism analysis. The forward primer F: 5'-GCAGGGTACAAAACTTTGGAG-3' and the reverse primer R: 5'-CCTCATCACCGA CATCATGTC-3' were used[26] to amplify the isolated DNA. Amplification was performed using a thermal cycler (Veriti; Applied Biosystems, Foster City, CA, USA) in a final reaction volume (50 μL) containing 25 μL of 2X GoTaq Green Master Mix (Promega, Madison, WI, USA), 4 μL of 12 pmol of both primers, 5 μL of DNA, and 12 μL of nuclease-free water. Polymerase chain reaction analysis was performed as follows: 94°C, initial denaturation (5 minutes); 40 cycles of denaturation at 94°C (30 s); annealing at 59°C (30 s), and extension at 72°C (30 s). The final extension was performed at 72°C for 7 minutes. The 177-bp amplicons were digested using 5 units of Mse1 (37°C for 1 hour). Amplicons and restriction fragment length polymorphism fragments, which were separated using 2.5% agarose gel electrophoresis, were detected using ethidium bromide and visualized using a ChemiDoc-It2 imaging system with VisionWorks LS Analysis Software (version 7.1) (UVP, Cambridge, UK). The results were coded as U-u, where the Upper case letter is denoted as the absence and lowercase letter is designated as the presence of the restriction site, respectively. The call and concordance rates of the *VDR* genotypes were 98% and 100%, respectively. The accuracy of the genotypes

was determined by subjecting samples (15 cases and 15 controls) to nucleotide sequence analysis.

Data analysis

Statistical analysis was performed using SPSS software version 22 (IBM Corporation, Armonk, NY, USA) with significance indicated by $P < 0.05$. The Shapiro–Wilk and Kolmogorov–Smirnov tests were used to determine whether the variables were normally distributed. The goodness-of-fit (χ^2) test was used to test whether the controls exhibited Hardy–Weinberg equilibrium to determine deviation of their genotype distribution. Comparisons between groups of *VDR Tru*9I genotypic and allelic distributions were calculated using Person's χ^2 test. Odds ratios (ORs) along with 95% CIs were determined using binary and multinomial logistic regression analysis of the *Tru*9I SNP to determine the risk of BC. The significance of ORs was determined using Wald's statistics and defined as $P < 0.05$.

Results

Characteristics of patients with BC

Patients' characteristics are presented in Table 1, which shows that the parameters were not normally distributed among

Table 1 Characteristics of patients with BC (N = 228).

Characteristics	Patients n (%)
Affected area	
Right*	123 (53.9)
Left*	99 (43.2)
Both*	6 (2.6)
Types of BC	
Invasive ductal carcinoma (IDC)*	197 (93.4)
Invasive lobular carcinoma (ILC)*	13 (5.7)
Ductal carcinoma in situ (DCIS)*	6 (2.6)
Others*	12 (5.2)
Grades of BC	
I*	6 (2.6)
II*	164 (71.9)
III*	45 (19.7)
IV*	7 (3)
Both II &III*	2 (1.3)
None*	4 (1.7)
Immunohistochemical classification	
Estrogen receptor status	
Positive (0 to +9)*	126 (55.3)
Negative (−1 to −2)*	34 (14.9)
Unknown	68 (29.8)
Progesterone receptor status	
Positive (0 to +8)*	114 (5)
Negative (−1 to −2)*	38 (16.6)
Unknown	76 (33.33)
Human epidermal growth factor receptor 2 status	
Positive (0 to +3)*	98 (42.9)
Negative (−1 to −2)*	20 (8.7)
Unknown	110 (48.2)
TNM classification (For invasive cases only)	
Size of primary tumor (T stage)	
Tx*	2 (0.8)
Tis*	4 (1.7)
T1*	0 (0)
T2*	35 (15.3)
T3*	31 (13.5)
T4*	26 (11.4)
No involvement*	199 (56.5)
Metastasis to regional lymph node (N stage)	
Nx*	6 (2.6)
N0*	23 (10)
N1*	36 (15.7)
N2*	19 (8.3)
N3*	15 (6.5)
No involvement*	129 (56.5)
Distant metastasis (M stage)	
Mx*	39 (17.1)
M0*	48 (21.0)
M1*	12 (5.2)
Unknown	129 (56.5)

BC, breast cancer. *Statistically significant difference ($P < 0.05$, Shapiro–Wilk or Kolmogorov–Smirnov tests).

Table 2 *VDR Tru*9I genotype frequencies and associations with the risk of premenopausal BC.

Genotypes	Alternate designation	BC cases n (%)	Controls n (%)	X^2	*P* value	UOR (95% CI)	AOR (95% CI)
GG	UU	179 (78.5)	398 (80)	0.238	0.888	Ref	Ref
GA	Uu	47 (20.6)	95 (19.1)			1.1 (0.744–1.627)	1.085 (0.731–1.611)
AA	uu	2 (0.88)	4 (0.8)			1.112 (0.202–6.125)	1.141 (0.206–6.317)
Total		228	497				
GG	UU	179 (78.5)	398 (80)			Ref	Ref
GA+AA	Uu+uu	49 (21.4)	99 (19.6)			1.101 (0.749–1.617)	1.087 (0.737–1.604)
Total		228	497				
G allele	U allele	405 (88.8)	891 (89.6)	0.223	0.637	Ref	Ref
A allele	u allele	51 (11.1)	103 (10.3)			1.089 (0.763–1.554)	1.086 (0.76–1.55)
Total		456	994				

DNA was amplified from 228/228 patients' samples and 497/503 control subjects' samples. BC, breast cancer; UOR, unadjusted odds ratio; AOR, odds ratio adjusted for age and time of blood collection, CI, confidence interval.

patients according to the Shapiro–Wilk and Kolmogorov–Smirnov tests ($P < 0.01$).

Association of the VDR Tru9I SNP with BC

The genotype distributions of *Tru*9I among patients (228/228) and healthy controls (497/503) are presented in Table 2. The distribution of *Tru*9I in the control population did not deviate ($\chi^2 = 0.416$, $P > 0.05$) from the Hardy–Weinberg equilibrium. There was no significant difference ($\chi^2 = 0.238$) between the *VDR Tru*9I genotype among patients and controls. However, an 11.2% increase in OR (1.112; 95% CI, 0.202–6.125) was observed among 'uu' carriers compared with 'UU and Uu' carriers. After adjusting for age, the *VDR Tru*9I 'uu' genotype exhibited a 14.1% increase in the OR (1.141; 95% CI, 0.206–6.317) (Table 2).

Association of the VDR Tru9I SNP with specific pathological characteristics of patients with BC

We next investigated the association of the *VDR Tru*9I SNP with BC risk through immunohistochemical subtyping of BC tissues and TNM classification (Table 3). A direct association with risk of BC was found for at least one 'u' allele, which was more evident and significant for G-IV stage carcinoma (OR, 5.36; 95% CI, 1.181–24.338).

Relationship of the VDR Tru*9I SNP and risk factors associated with BC*

The possible relationship of the *Tru*9I SNP and other contributing factors to the risk of BC are presented in Table 4. Those with the mutant (Uu+uu) genotype along with Sindhi and Baluchi ethnicity and marital age greater than 20 years were at significantly increased risk of developing BC; however, there were no significant associations with other contributing factors and BC.

Nine models of contributing factors were prepared in which the *Tru*9I genotype was adjusted by sequentially adding individual contributing factors (Appendix 1). This analysis shows that the *Tru*9I 'uu' genotype in model 8 significantly increased the risk of BC (OR, 1.288; 95% CI, 0.16–10.398).

Discussion

Among Asian countries, Pakistan has the highest incidence of BC,[27] which is a

Table 3 Associations of the *VDR Tru*9I single nucleotide polymorphism and risk of premenopausal BC according to the immunohistochemical and pathological characteristics of patients with BC.

	*Tru*9I genotype			
	UU (GG)		Uu+uu (GA+AA)	
BC characteristics	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
Types of BC				
IDC	152/398	Ref	45/99	1.19 (0.799–1.774)
ILC	13/398	Ref	0/99	1.67×10^{-9} (1.67×10^{-9}–1.67×10^{-9})
DCIS	5/398	Ref	1/99	0.804 (0.93–6.96)
Other	9/398	Ref	3/99	1.34 (0.356–5.042)
Grades of BC				
I	5/398	Ref	1/99	0.804 (0.093–6.96)
II	131/398	Ref	33/99	1.02 (0.66–1.578)
III	35/398	Ref	10/99	1.149 (0.55–2.399)
IV	3/398	Ref	4/99	5.36 (1.181–24.338)*
II & III	2/398	Ref	0/99	12×10^{-9} (12×10^{-9}–12×10^{-9})
Unknown	3/398	Ref	1/99	1.34 (0.138–13.02)
Immunohistochemical classification				
Estrogen receptor status				
Positive (0 to +9)	103/398	Ref	23/99	0.898 (0.543–1.484)
Negative (−1 to −2)	25/398	Ref	9/99	1.447 (0.655–3.199)
None	51/398	Ref	17/99	1.34 (0.742–2.421)
Progesterone receptor status				
Positive (0 to +8)	92/398	Ref	22/99	0.961 (0.575–1.608)
Positive (−1 to −2)	30/398	Ref	8/99	1.072 (0.477–2.411)
None	57/398	Ref	19/99	1.34 (0.762–2.355)
Human epidermal growth factor receptor 2 status				
Positive (0 to +3)	78/398	Ref	20/99	1.031 (0.602–1.766)
Negative (−1 to −2)	18/398	Ref	2/99	0.447 (0.102–1.957)
None	83/398	Ref	27/99	1.308 (0.804–2.128)
TNM classification (For invasive cases)				
Size of primary tumor (T stage)				
Tx	1/398	Ref	1/99	4.681 (0.277–79.215)
Tis	3/398	Ref	1/99	1.281 (0.13–12.598)
T1	0/398	Ref	0/99	NC
T2	27/398	Ref	9/99	1.412 (0.639–3.121)
T3	25/398	Ref	6/99	0.896 (0.354–2.267)
T4	23/398	Ref	3/99	0.521 (0.152–1.783)
No involvement	100/398	Ref	29/99	1.135 (0.706–1.823)
Metastasize to regional lymph node (N stage)				
Nx	4/398	Ref	2/99	1.821 (0.325–10.218)
N0	20/398	Ref	3/99	0.593 (0.172–2.048)

(continued)

Table 3 Continued.

BC characteristics	*Tru*9I genotype			
	UU (GG)		Uu+uu (GA+AA)	
	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
N1	25/398	Ref	11/99	1.796 (0.847–3.806)
N2	16/398	Ref	3/99	0.737 (0.209–2.598)
N3	14/398	Ref	1/99	0.297 (0.038–2.304)
No involvement	100/398	Ref	29/99	1.134 (0.706–1.822)
Distant metastasis (M stage)				
Mx	29/398		10/99	1.386 (0.654–2.94)
M0	39/398	Ref	9/99	0.928 (0.435–1.979)
M1	11/398	Ref	1/99	0.365 (0.047–2.864)
No involvement	100/398	Ref	29/99	1.166 (0.73–1.862)

BC, breast cancer; CI, confidence interval; OR, odds ratio. *VDR Tru*9I "Uu" and "uu" genotypes were combined, because there were very few subjects with "uu." ORs were calculated using multinomial logistic regression analysis adjusted for age and time of blood collection. $^{*}P < 0.05$.

heterogeneous group of diseases associated with various risk factors. The most common risk factor is exposure to endogenous hormones such as estradiol.[28] Approximately 15% of BC is caused by genetic changes,[29] including those associated with high penetrance genes such as *BRCA* 1/2,[30] and low-to-moderate penetrance genes such as checkpoint kinase-2 (*CHEK*2) and *VDR*.[20,31] VDR contributes to the prevention of BC, and the vitamin D/VDR complex induces apoptosis, reduces angiogenesis, proliferation, and invasion and increases cell differentiation.[32]

Evidence indicates that the VDR mediates most of the activities of vitamin D. Analyses of common *VDR* SNPs (*Fok*1, *Bsm*1, *Apa*1, *Taq*1, and *Poly*-A) show associations between *VDR* SNPs and BC, although the results are inconsistent among different types of exposure.[20,22,33,34] The *Fok*1 'ff' genotype may increase the risk of BC.[35,36] However, evidence suggests that *Fok*1 significantly correlates with human epidermal growth factor receptor 2 status[20] and is significantly associated with estrogen receptor status of patients with BC.[37] One study shows that the *Bsm*1 'b' allele is associated with risk of BC;[20] although another study reports the opposite results.[22] To our knowledge, the present study is the first to determine the association of the low penetrance *VDR Tru*9I polymorphism with premenopausal BC. The *VDR Tru*9I is caused by an A to G substitution that was first identified in 2000.[38] *VDR Tru*9I resides in intron 8, +443-bp from the end of exon 8, and may be involved in the regulation of gene expression through its affect on enhancer affinity and target area.[39]

The present study shows that the *Tru*9I mutant 'uu' genotype was associated with an increased risk of developing premenopausal BC. There are few association studies of *Tru*9I in other diseases such as coronary heart disease (CHD), colorectal adenoma, and prostate cancer.[26,39,40] However, *Tru*9I is not associated with CHD[39] and prostate cancer[40] among the Chinese Han population, whereas *Tru*9I lowers the risk of colorectal carcinoma

Table 4 Combined associations of the *VDR Tru*9I single nucleotide polymorphism and factors contributing to the risk of premenopausal BC.

	Combined		UU		Uu+uu	
BC risk factors	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
Age (years)#						
20–26 (Younger)	20/58	Ref	16/51	Ref	4/5	Ref
27–33	34/78	1.264 (0.661–2.418)	23/53	1.383 (0.667–2.914)	11/23	0.598 (0.134–2.675)
34–40	110/225	1.418 (0.812–2.475)	83/179	1.478 (0.796–2.745)	27/44	0.767 (0.189–3.109)
41–45 (Older)	64/142	1.307 (0.726–2.352)	57/115	1.58 (0.829–3.012)	7/27	0.324 (0.068–1.535)
Total	228/503		179/398		49/99	
Ethnicity/Race						
Urdu-Speaking	94/252	Ref	78/199	Ref	16/51	Ref
Sindhi	41/12	9.41 (4.719–18.767)*	29/9	8.456 (3.809–18.772)*	12/3	14.934 (3.573–62.418)*
Panjabi	20/104	0.522 (0.305–0.891)*	16/86	0.493 (0.272–0.897)*	4/17	0.675 (0.193–2.335)
Pukhtoon	18/47	0.996 (0.549–1.806)	15/39	0.946 (0.492–1.821)	3/8	0.97 (0.22–4.283)
Baluchi	19/4	12.926 (4.265–39.171)*	12/3.	10.875 (2.963–39.908)*	7/1	19.323 (2.15–173.641)*
Others	36/84	1.17 (0.738–1.854)	29/62	1.188 (0.709–1.992)	7/19	1.179 (0.408–3.41)
Total	228/503		179/398		49/99	
Marital status						
Yes	208/363	Ref	162/286	Ref	45/75	Ref
No	20/140	0.139 (0.071–0.273)*	16/112	0.15 (0.071–0.318)*	4/24.	0.111 (0.024–0.524)*
Total	228/503		179/398		49/99	
Marital age (years)						
<20	105/244	Ref	84/186	Ref	21/56	Ref
20 to 30	95/117	3.343 (2.157–5.18)*	71/98	2.48 (1.525–4.033)*	24/19	10.645 (3.473–32.623)*
>30	8/2.	20.996 (4.15–106.236)*	8/2.	15.914 (3.138–80.698)*	0/0	NC
Total	208/363		163/286		45/75	
Parity						
Parous	191/342	Ref	149/267	Ref	40/71	Ref
Nulliparous	37/161	0.347 (0.216–0.557)*	30/131	0.366 (0.216–0.619)*	9/28	0.395 (0.145–1.071)
Total	228/503		179/398		45/99	

(continued)

Table 4 Continued.

BC risk factors	Combined		UU		Uu+uu	
	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
Age at first child birth (years)						
Below 20	49/63	Ref	41/55	Ref	8/8	Ref
20 to 24	81/118	0.528 (0.33–0.845)*	64/87	0.515 (0.302–0.879)*	17/30	0.582 (0.208–1.627)
25 to 29	39/115	0.621 (0.328–1.179)	30/88	0.458 (0.215–0.977)*	9/26	1.533 (0.41–5.731)
>29	17/43	1.218 (0.753–1.969)	11/36	1.086 (0.639–1.846)	6/7	2.006 (0.604–6.661)
Total	186/339		146/266		40/71	
History of breast feeding						
Yes	186/339	Ref	146/266	Ref	40/71	Ref
No	22/24	1.674 (0.901–3.107)	17/20	1.54 (0.773–3.068)	5/4	2.269 (0.528–9.748)
Total	208/363		163/286		45/75	
Age at menarche (years)						
12 to 14	186/423	Ref	148/334	Ref	38/83	Ref
>14	28/45	0.913 (0.479–1.74)	13/29	0.995 (0.5–1.978)	1/6	0.306 (0.035–2.662)
<12	14/35	1.389 (0.838–2.303)	18/35	1.111 (0.606–2.035)	10/10	2.567 (0.946–6.966)
Total	228/503		179/398		49/99	
Use of hormone replacement therapy						
No	209/481	Ref	168/382	Ref	41/93	Ref
Yes	19/22	1.954 (1.034–3.691)*	11/16.	1.547 (0.702–3.412)	8/6	3.114 (0.99–9.798)
Total	228/503		179/398		49/99	
Use of oral contraceptive						
No	197/454	Ref	155/361	Ref	42/87	Ref
Yes	31/49	1.473 (0.91–2.383)	24/37	1.521 (0.878–2.635)	7/12	1.17 (0.423–3.234)
Total	228/503		179/398		49/99	
Waist to hip ratio						
Good (<0.8)	27/92	Ref	20/71	Ref	7/19	Ref
Average (0.8–0.85)	15/173	0.296 (0.15–0.585)*	12/134	0.317 (0.146–0.685)*	3/37	0.252 (0.057–1.12)
High (>0.85)	186/238	2.687 (1.672–4.317)*	147/193	2.727 (1.58–4.707)*	39/43	2.568 (0.949–6.944)
Total	228/503		179/398		49/99	

(continued)

Table 4 Continued.

BC risk factors	Combined		UU		Uu+uu	
	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)	Cases/ Controls	OR (95% CI)
BMI (kg/m^2)						
Normal/healthy weight (18.6–23)	56/157	Ref	43/127	Ref	13/29	Ref
Underweight (>18.5)	22/64	0.966 (0.544–1.717)	19/48	1.196 (0.633–2.261)	3/14	0.383 (0.09–1.637)
Overweight (23.1–27)	79/158	1.381 (0.909–2.097)	63/132	1.374 (0.858–2.2)	16/23	1.689 (0.654–4.364)
Obese (>27)	71/124	1.589 (1.028–2.456)	54/91	1.708 (1.036–2.815)*	17/33	1.183 (0.474–2.953)
Total	228/503		179/398		49/99	
Family history of BC						
No	191/455		149/361	Ref	42/88	Ref
Yes	37/48	1.83 (1.153–2.906)*	30/37	1.947 (1.156–3.28)*	7//11	1.348 (0.473–3.841)
Total	228/503		179/398		49/99	

VDR Tru9I "Uu" and "uu" genotypes were combined because there were very few subjects in the "uu" category. DNA was amplified from 228/228 patients' samples and 497/503 control subjects' samples. ORs were adjusted for age and time of blood collection. *P <0.05.
#Crude/unadjusted ORs for age were calculated. BC, breast cancer; BMI, body mass index; NC, not calculated, OR, odds ratio.

among women living in North Carolina.[26] We show further that mutant *Tru*9I may be associated with the development of Grade IV carcinoma, whereas another study shows the opposite relationship with colorectal adenoma, which is more pronounced for multiple, larger, and sessile adenomas, and perhaps with dysplasia.[26]

This study has certain limitations. First, the sample size was relatively small. Large-scale studies are therefore required to support our conclusions. Second, we did not study environmental factors including diet and lifestyle, which may modify the association between the *VDR Tru*9I polymorphism and BC. Finally, other *VDR* SNPs and serum vitamin D levels were not studied. However, further research is required to confirm our findings and explore the possible mechanisms of BC underlying the association of *VDR Tru*9I with oncogenesis to facilitate the development of more effective treatment.

We conclude that the *VDR Tru*9I 'uu' genotype may predict a high risk for premenopausal BC.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

ORCID iD

Taseer Ahmed Khan http://orcid.org/0000-0003-3844-4750

References

1. Shaukat U, Ismail M and Mehmood N. Epidemiology, major risk factors and genetic predisposition for breast cancer in the Pakistani population. *Asian Pac J Cancer Prev* 2013; 14: 5625–5629.
2. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136: E359–E386. DOI: 10.1002/ijc.29210.
3. Hirko KA, Chen WY, Willett WC, et al. Alcohol consumption and risk of breast cancer by molecular subtype: Prospective analysis of the nurses' health study after 26 years of follow-up. *Int J Cancer* 2016; 138: 1094–1101. DOI: 10.1002/ijc.29861.
4. Nagrani R, Mhatre S, Rajaraman P, et al. Central obesity increases risk of breast cancer irrespective of menopausal and hormonal receptor status in women of South Asian Ethnicity. *Eur J Cancer* 2016; 66: 153–161. DOI: http://dx.doi.org/10.1016/j.ejca.2016.07.022.
5. Bassett JK, Hodge AM, English DR, et al. Plasma phospholipids fatty acids, dietary fatty acids, and breast cancer risk. *Cancer Causes Control* 2016; 27: 759–773. DOI: 10.1007/s10552–016-0753–2.
6. Eisman JA and Bouillon R. Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. *BoneKEy Rep* 2014; 3: 499. DOI: 10.1038/bonekey.2013.233.
7. Bauer SR, Hankinson SE, Bertone-Johnson ER, et al. Plasma vitamin D levels, menopause, and risk of breast cancer: dose-response meta-analysis of prospective studies. *Medicine (Baltimore)* 2013; 92: 123–131. DOI: 10.1097/MD.0b013e3182943bc2.
8. Williams JD, Aggarwal A, Swami S, et al. Tumor autonomous effects of Vitamin D deficiency promote breast cancer metastasis. *Endocrinology* 2016; 157: 1341–1347. DOI: 10.1210/en.2015–2036.
9. Kim Y and Je Y. Vitamin D intake, blood 25 (OH)D levels, and breast cancer risk or mortality: a meta-analysis. *Br J Cancer* 2014; 110: 2772–2784. DOI: 10.1038/bjc.2014.175.
10. Kuhn T, Kaaks R, Becker S, et al. Plasma 25-hydroxyvitamin D and the risk of breast cancer in the European prospective investigation into cancer and nutrition: a nested case-control study. *Int J Cancer* 2013; 133: 1689–1700. DOI: 10.1002/ijc.28172.

11. Lin R and White JH. The pleiotropic actions of vitamin D. *BioEssays* 2004; 26: 21–28. DOI: 10.1002/bies.10368.
12. Rai V, Abdo J, Agrawal S, et al. Vitamin D Receptor Polymorphism and Cancer: An Update. *Anticancer Research* 2017; 37:3991–4003. DOI: 10.21873/anticanres.11784. Available at: https://www.ncbi.nlm.nih.gov/pubmed/28739681
13. Deeb KK, Trump DL and Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. *Nat Rev Cancer* 2007; 7: 684–700. DOI: 10.1038/nrc2196.
14. Pendas-Franco N, Gonzalez-Sancho JM, Suarez Y, et al. Vitamin D regulates the phenotype of human breast cancer cells. *Differentiation* 2007; 75: 193–207. DOI: 10.1111/j.1432-0436.2006.00131.x.
15. Lopes N, Carvalho J, Duraes C, et al. 1Alpha,25-dihydroxyvitamin D3 induces de novo E-cadherin expression in triple-negative breast cancer cells by CDH1-promoter demethylation. *Anticancer Res* 2012; 32: 249–257.
16. Campbell MJ, Gombart AF, Kwok SH, et al. The anti-proliferative effects of 1alpha,25 (OH)2D3 on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. *Oncogene* 2000; 19: 5091–5097. DOI: 10.1038/sj.onc.1203888.
17. Lopes N, Sousa B, Martins D, et al. Alterations in Vitamin D signalling and metabolic pathways in breast cancer progression: a study of VDR, CYP27B1 and CYP24A1 expression in benign and malignant breast lesions. *BMC Cancer* 2010; 10: 483. DOI: 10.1186/1471–2407-10–483.
18. Bertoccini L, Sentinelli F, Leonetti F, et al. The vitamin D receptor functional variant rs2228570 (C>T) does not associate with type 2 diabetes mellitus. *Endocr Res* 2017; 42: 1–5. DOI: 10.1080/07435800.2017.1305965.
19. Torkko KC, van Bokhoven A, Mai P, et al. VDR and SRD5A2 polymorphisms combine to increase risk for prostate cancer in both non-Hispanic White and Hispanic White men. *Clin Cancer Res* 2008; 14: 3223–3229. DOI: 10.1158/1078–0432.ccr-07–4894.
20. Hashemi SM, Arbabi N, Hashemi M, et al. 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. *Int J Cancer Manag* 2017; 10: e8807.
21. Atoum MF and Al-Khatib YM. Association between Serum 25-hydroxy Vitamin D Concentration and TaqI Vitamin D Receptor Gene Polymorphism among Jordanian females with breast cancer. *Chin Med J (Engl)* 2017; 130: 1074–1078. DOI: 10.4103/0366–6999.204933.
22. Talaneh S, Ghorbani A, Oghabi Bakhshaiesh T, et al. FokI and BsmI Polymorphisms of the VDR Gene and Breast Cancer Risk. *MCI* 2017; 1: 21–25.
23. Elzehery RR, Baiomy AA, Hegazy MAF, et al. Vitamin D status, receptor gene BsmI (A/G) polymorphism and breast cancer in a group of Egyptian females. *Egyptian Journal of Medical Human Genetics* 2017; 18: 269–273. DOI: https://doi.org/10.1016/j.ejmhg.2016.11.003.
24. Lu D, Jing L and Zhang S. Vitamin D Receptor Polymorphism and Breast Cancer Risk: A Meta-Analysis. *Medicine* 2016; 95: e3535. DOI: 10.1097/md.0000000000003535.
25. Rashid MU, Muzaffar M, Khan FA, 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: e0141562. DOI: 10.1371/journal.pone.0141562.
26. Gong YL, Xie DW, Deng ZL, et al. Vitamin D receptor gene Tru9I polymorphism and risk for incidental sporadic colorectal adenomas. *World J Gastroenterol* 2005; 11: 4794–4799.
27. Sarwar MR and Saqib A. Cancer prevalence, incidence and mortality rates in Pakistan in 2012. *Cogent Medicine* 2017; 4: 1288773. DOI: 10.1080/2331205X.2017.1288773.
28. Wu H, Chen Y, Liang J, et al. Hypomethylation-linked activation of PAX2 mediates tamoxifen-stimulated endometrial carcinogenesis. *Nature* 2005; 438: 981–987. DOI: doi:10.1038/nature04225
29. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for

breast screening with MRI as an adjunct to mammography. *CA Cancer J Clin* 2007; 57: 75–89.
30. Peterlongo P, Chang-Claude J, Moysich KB, et al. Candidate genetic modifiers for breast and ovarian cancer risk in BRCA1 and BRCA2 mutation carriers. *Cancer Epidemiol Biomarkers Prev* 2015; 24: 308–316. DOI: 10.1158/1055–9965.epi-14–0532.
31. Desrichard A, Bidet Y, Uhrhammer N, et al. CHEK2 contribution to hereditary breast cancer in non-BRCA families. *Breast Cancer Res* 2011; 13: R119. DOI: 10.1186/bcr3062.
32. Krishnan AV and Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu Rev Pharmacol Toxicol* 2011; 51: 311–336. DOI: 10.1146/annurev-pharmtox-010510–100611.
33. Guo B, Jiang X, Hu X, et al. Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population. *Int J Clin Exp Med* 2015; 8: 8020–8024.
34. Reimers LL, Crew KD, Bradshaw PT, et al. Vitamin D-related gene polymorphisms, plasma 25-hydroxyvitamin D, and breast cancer risk. *Cancer Causes Control* 2015; 26: 187–203. DOI: 10.1007/s10552–014-0497–9.
35. Engel LS, Orlow I, Sima CS, et al. Vitamin D receptor gene haplotypes and polymorphisms and risk of breast cancer: a nested case-control study. *Cancer Epidemiol Biomarkers Prev* 2012; 21: 1856–1867. DOI: 10.1158/1055–9965.epi-12–0551.
36. Mishra DK, Wu Y, Sarkissyan M, et al. Vitamin D receptor gene polymorphisms and prognosis of breast cancer among African-American and Hispanic women. *PloS One* 2013; 8: e57967. DOI: 10.1371/journal.pone.0057967.
37. Nemenqani DM, Karam RA, Amer MG, et al. Vitamin D receptor gene polymorphisms and steroid receptor status among Saudi women with breast cancer. *Gene* 2015; 558: 215–219. DOI: 10.1016/j.gene.2014.12.065.
38. Ye WZ, Reis AF and Velho G. Identification of a novel Tru9 I polymorphism in the human vitamin D receptor gene. *J Hum Genet* 2000; 45: 56–57. DOI: 10.1007/s100380050011.
39. He L and Wang M. Association of vitamin d receptor-a gene polymorphisms with coronary heart disease in Han Chinese. *Int J Clin Exp Med* 2015; 8: 6224–6229.
40. Bai Y, Yu Y, Yu B, et al. Association of vitamin D receptor polymorphisms with the risk of prostate cancer in the Han population of Southern China. *BMC Med Gen* 2009; 10: 125–125. DOI: 10.1186/1471–2350-10–125.

Appendix 1 Association of the Tru9I single nucleotide polymorphism with early onset BC after adjustment for multiple contributing factors.

Models	Contributing factors	Beta coefficient (β)	Wald	*P* value	OR	95% CI	
						Lower	Upper
Model 8	*Tru*9I uu	0.253	0.057	0.812	1.288	0.16	10.398
	*Tru*9I Uu	0.074	0.078	0.78	1.076	0.642	1.805
	Age 27–33 years	−21.417	0.000	0.999	0.000	0.000	NC
	Age 34–40 years	−21.577	0.000	0.999	0.000	0.000	NC
	Age 41–45 years	−22.593	0.000	0.998	0.000	0.000	NC
	Marital age 20–30 years	0.854	10.332	0.001*	2.349	1.396	3.955
	Marital age >30 years	2.917	10.134	0.001*	18.492	3.068	111.444

(continued)

Continued.

Models	Contributing factors	Beta coefficient (β)	Wald	*P* value	OR	95% CI	
						Lower	Upper
	Age at first live birth 20–24 years	−0.435	2.616	0.106	0.647	0.382	1.097
	Age at first live birth 25–29 years	−0.457	1.546	0.214	0.633	0.308	1.301
	Age at first live birth >29 years	−0.044	0.025	0.873	0.957	0.554	1.653
	No lactation	NC	NC	NC	NC	NC	NC
	Age at menarche >14 years	0.025	0.004	0.951	1.025	0.46	2.287
	Age at menarche <12 years	0.236	0.541	0.462	1.266	0.676	2.372
	BMI >18.5 kg/m^2	0.527	1.493	0.222	1.694	0.727	3.944
	BMI 23.1–27 kg/m^2	0.149	0.282	0.595	1.16	0.67	2.009
	BMI >27 kg/m^2	0.329	1.217	0.27	1.39	0.774	2.496
	WHR 0.8–0.85	−1.315	9.386	0.002*	0.269	0.116	0.623
	WHR >0.85	0.813	6.885	0.009*	2.254	1.228	4.137
	OC use	0.437	1.761	0.185	1.548	0.812	2.953

BC, breast cancer; BMI, body mass index; WHR, waist to hip ratio; OC, oral contraceptive use; HRT, hormone replacement therapy. *Significant association (Wald's statistics); $P < 0.05$. Data are shown only for model 8.