Int J Clin Exp Med 2015;8(5):8020-8024
www.ijcem.com /ISSN:1940-5901/IJCEM0006235

Original Article

Association between vitamin D receptor gene polymorphisms and breast cancer in a Chinese population

Bingjun Guo[1], Xin Jiang[2], Xiaoqiao Hu[2], Fan Li[3], Xiaopin Chen[2]

Departments of [1]Ultrasound, [2]Oncology, [3]Endocrine and Breast Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, P. R. China

Received January 22, 2015; Accepted March 20, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Objective: The present study aimed to explore the association between vitamin D receptor (VDR) genetic polymorphisms and breast cancer risk. Methods: A total of 219 patients with breast cancer and 321 cases of females without breast cancer were enrolled for the present study. PCR-RFLP method was used to genotype 3 SNPs of the VDR gene (rs1544410, rs7975232 and rs731236). ELISA method was used to detect the amount of $1,25(OH)_2D_3$ in the plasma. The results were analyzed using SPSS 17.0 software. Results: We found rs7975232 was associated with breast cancer risk. Compared with GG genotype carriers, TT subjects had a lower risk of breast cancer (P = 0. 004, OR = 0.774, 95% CI: 0.212~0.955). However, there was no difference in $1,25(OH)_2D_3$ levels among the different genotypes. In addition, the distribution frequency of the haplotype A-G-T in the patients group was 4.4%, significantly higher than the control group (0.7%, P = 0.003; OR=2.643, 95% CI: 1.631~7.012), while the distribution of haplotype G-T-T in the patient group was significantly lower than in the control group (17.3% vs. 28.4%, P = 0.011, OR = 0.543, 95% CI: 0.325~0.854). Conclusions: The VDR gene was associated with breast cancer pathogenesis; females carrying the haplotype G-T-T had a lower breast cancer risk, while the haplotype A-G-T conferred a higher risk.

Keywords: Vitamin D receptor, haplotype, genotype, breast cancer

Introduction

Breast cancer is one of the most common malignant tumors found in females. However, its molecular pathogenesis is still not clear. It has been reported recently that vitamin D receptor (VDR) could play important roles in breast cancer pathogenesis [1, 2]. The physical and biochemical effects of active vitamin D and its derivatives on target cells include the regulation of calcium and phosphorus metabolism in bones and intestines, as well as the regulation of bodily immunity [2]; they also involve the regulation of tumor cell proliferation and apoptosis [3, 4]. Studies have shown that a low level of $1,25(OH)_2D_3$ in the serum is associated with an increased risk of breast cancer, which could be mediated through a key molecule, VDR, which induces cell apoptosis or inhibition of breast cancer cell proliferation or metastasis through the RB-E2F pathway [5]. In addition, immunohistochemistry results have shown that the VDR protein is not only expressed in 70%-90% of breast cancer tissues but is also differentially expressed in different cancer cell lines [6]. Because VDR structure and function, as well as circulating levels of vitamin D could be potentially affected by VDR gene polymorphisms, the association between VDR gene polymorphisms and breast cancer susceptibility has become a focus of attention.

In the present study, we analyzed 3 SNPs (rs1544410, rs7975232 and rs731236) of the *VDR* gene in this case-control study, and detected the levels of $1,25(OH)_2D_3$ in plasma, aiming to study their associations with breast cancer genetic susceptibility from a genetic function perspective to understand breast cancer pathogenesis.

Materials and methods

Study subjects

A total of 219 cases of breast cancer patients admitted to the First Affiliated Hospital of

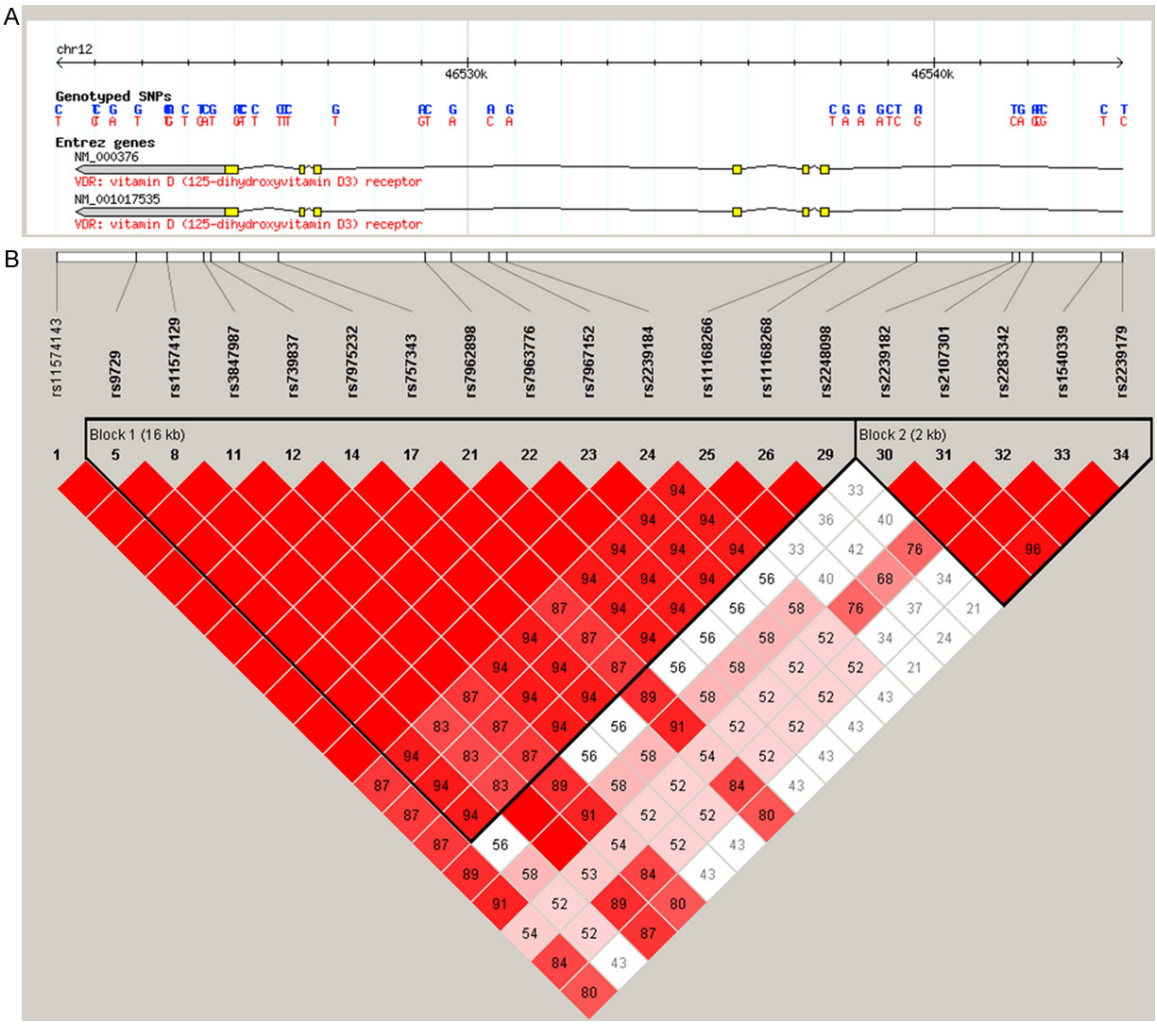

Figure 1. Genetic variations at human VDR gene. Using the Haploview 4.2 software and the HapMap phrase II database, we scanned 19 genotyped single-nucleotide polymorphisms (SNPs) in Chinese Han. Linkage disequilibrium (LD) blocks across the locus in Chinese Han. LD blocks derived by solid spline method in Haploview 4.2. LD value shown: A. |D'| × 100; |D'| colour scheme: |D'| = 0: white; 0< |D'| < 1: pink; r^2 = 1: red; B. r^2 × 100; r^2 colour scheme: r^2 = 0: white; 0 < r^2 <1: shades of grey; r^2 = 1: black.

Chongqing Medical University from Mar, 2011 to May 2014 and confirmed by pathology. Their ages ranged from 24 to 73 y, with an average age of 53.2 y. A total of 321 cases of females who received physical examinations in the same hospital were selected as the control group. The control group had no clinical symptoms, no tumors and no history of genetic diseases. The ages of the control group ranged from 22 to 86 y, with an average age of 53.5 y. All participants were Han ethnicity and were not relatives. Our study was approved by Medical Ethics Committee in the First Affiliated Hospital of Chongqing Medical University, and all study subjects or their families were informed and agreed to participate.

Methods

Sample collection, plasma isolation and total DNA extraction

EDTA·K_2 coagulant peripheral blood (5 ml) was collected from breast cancer patients and the control group, and a whole blood DNA extraction kit (Fijie Biotech, IL, USA) was used to extract the whole genomic DNA. The DNA samples were stored at -20°C for future use.

Primer design and genotyping

As shown in **Figure 1**, using of haploview 4.2 software with the minor allele frequency (MAF)

Table 1. Primers sequence of three SNPs

SNP	Primers (5'-3')	Bases variation	Annealing temperature (°C)
rs1544410 (*Bsm*I)	Sense: CAACCAAGACCTACAAGTACCGGGTCAGTGA	G > A	56
	Antisense: AACCAGCGGGAAGAGGTCAAGGG		
rs7975232 (*Apa*I)	Sense: CAGAGCATGGACAGGGAGGAA	G > T	58
	Antisense: GCAACTCCTCATGGCTGAGGTCTC		
rs731236 (*Taq*I)	Sense: CAGAGCATFFACAGGGAGCAA	T > C	58
	Antisense: GCAACTCCTCATGGCTGAGGTCTC		

Table 2. Distributions of 3 SNPs of VDR gene between breast cancer patients and control subjects

SNPs	Genotypes and allele	Breast cancer [*n* (%)]	Control subjects [*n* (%)]	OR (95% CI)*	*P value*
Rs1544410	GG	183 (83.6)	282 (88.0)	1.00 (ref)	
	GA	35 (16.0)	38 (11.8)	1. 665 (0.765~2.885)	0.143
	AA	1 (0.4)	1 (0.2)	1.121 (0.430~1.784)	0.432
	G	401 (91.6)	602 (93.9)	1.00 (ref)	
	A	37 (8.4)	40 (6.1)	1.231 (0.944~2.121)	0.086
Rs7975232	GG	138 (63.0)	148 (46.5)	1.00 (ref)	
	GT	52 (23.5)	134 (41.5)	1.132 (0.654~1.775)	0.654
	TT	29 (13.5)	39 (12.0)	0.774 (0.212~0.955)	0.004
	G	328 (74.8)	430 (52.5)	1.00 (ref)	
	T	110 (25.2)	212 (47.5)	0.843 (0.632~1.219)	0.210
Rs731236	TT	197 (89.7)	286 (89.6)	1.00 (ref)	
	CT	22 (10.1)	32 (9.9)	1.106 (0.599~2.008)	0.543
	CC	1(0.2)	3 (0.5)	0.761 (0.439~1.132)	0.443
	T	415 (94.7)	604 (94.5)	1.00 (ref)	
	C	23(5.3)	38 (5.5)	0.997 (0.557~1.432)	0.775

**Logistic* regression analyses.

> 0.05 and $r^2 \geq 0.08$ as a cut-off, we selected 3 tag SNPs for VDR gene in the present study. Primers were designed according to the literature [7] and synthesized by Shanghai Shenggong Biotech Company (Shanghai, China). The primers sequences were shown in **Table 1**.

Detection of 1,25(OH)$_2$D$_3$

The levels of 1,25(OH)$_2$D$_3$ in the plasma was detected using sandwich ELISA methods as follows. An anti-human 1,25(OH)$_2$D$_3$ monoclonal antibody (mAb) was coated onto the plate; 1,25(OH)$_2$D$_3$ in the standard and the samples binds to the mAb. An enzyme-linked antibody was added, and the immune complex formed on the plate. After adding enzyme substrate TMB, a blue color appears. After adding sulfuric acid as the stop solution, the color changes to yellow. The OD value was recorded at 450 nm. The 1,25(OH)$_2$D$_3$ concentration is proportional to the OD value. A standard curve was established to calculate 1,25(OH)$_2$D$_3$ concentrations in the samples.

Statistical methods

SPSS17.0 software was used to perform the data analyses. *Hardy-Weinberg* equilibrium was tested using χ^2. Non-conditional logistic regression analysis was used to analyze distribution difference of genotype, allele in the patients group; two-sample *t* test was used to analyze the differences in 1,25(OH)$_2$D$_3$ levels among the different genotypes. The SHEsis platform was used to evaluate difference in VDR haplotype distribution. $P < 0.05$ was considered significant.

Results

The distributions of the VDR genotype and allele in the breast cancer and control groups

The genotypes of these 3 SNPs of VDR gene in the control group were tested using the Hardy-

Table 3. Haplotype distribution between patients and control subjects [*n* (%)]

Haplotypes	Patients group	Control group	OR (95% CI)	*P*
A-G-T	14.07 (4.4)	5.11 (0.7)	2.643 (1.631~7.012)	0.003
A-T-C	12.65 (3.8)	21.09 (3.0)	1.311 (0.622~2.765)	0.421
G-G-T	211.44 (70.5)	423.12 (68.6)	1.221 (0.965~1.577)	0.328
G-T-T	50.321(17.3)	186.03 (28.4)	0.543 (0.325~0.854)	0.011

Weinberg equilibrium, and the difference between observed value and expected value was not significant, suggesting that collected samples represent the group. In addition, we found that rs731236 SNP was associated with breast cancer (as shown in **Table 2**). Compared to homozygous wild-type GG, the distribution difference of TT in the patients group and the control group was significant (P = 0. 004) and after adjustment of other confounders, the difference remains significant (OR = 0.774, 95% CI: 0.212~0.955).

The relationship between VDR genotype and plasma levels of 1,25(OH)$_2$D$_3$

1,25(OH)$_2$D$_3$ levels in the plasma were detected between patients and control subjects. The results showed that the 1,25(OH)$_2$D$_3$ levels in the patient group was 544.12 ± 312.22 pmo1/ml, which was significantly lower than the levels in the control group, which was 799.4 ± 313.11 pmol/ml; P = 0.003. However, the difference of 1,25(OH)$_2$D$_3$ amount among the different genotypes was not significant (data not shown).

VDR gene haplotype analysis

SHEsis software showed that VDR gene rs1544410, rs7975232 and rs731236 had strong linkage disequilibrium. After population analysis, it was found that VDR gene had the G-G-T haplotype the most frequently, G-T-T the next, and the haplotypes A-G-C and G-G-C the least frequently (**Table 3**). Our study showed that the distribution frequency of haplotype A-G-T in the patient group was 4.4 %, significantly higher than the distribution frequency in the control group (0.7%, P = 0.003), while the distribution frequency of haplotype G-T-T in the patient group (17.3%) was significantly lower than the control group (28.4%, P = 0.011). Haplotype results suggested that females carrying the G-T-T haplotype had a lower breast cancer risk (OR = 0.543), while the AGT haplotype resulted in a higher risk (OR = 2.643).

Discussion

In the present study, we find rs7975232 was associated with breast cancer pathogenesis in a Chinese population. And we also found haplotype of VDR gene to be associated with the breast risk. To the best of our knowledge, this is the first study to investigate the relation between haplotypes of VDR gene and breast cancer in Chinese population.

rs1544410 (*Bsm*I), rs7975232 (*Apa*I) and rs731236 (*Taq*I) loci are the most commonly observed SNPs in the VDR gene [8-11]. These loci are located at the 3' UTR of the VDR gene, and gene mutations in this area show linkage disequilibrium, potentially associated with VDR mRNA expression levels [12]. Most investigators believe that the A allele of rs1544410 locus can reduce breast cancer occurrence or metastatic risk [13, 14]. Among British and American females, individuals who carry the rs1544410 GG genotype will have 1.92 and 1.53 times increase in breast cancer risk, respectively [13, 14]. Females with serum 1,25(OH)$_2$D$_3$ levels lower than 50 nmol/L and carry the rs1544410 GG genotype have a breast cancer risk increased to 6.82 times [15]. With regard to the rs731236 locus, Ludin *et al*. [14] reported that mortality of the CC genotype patients (22%) is substantially lower than the TT genotype (41%) or the CT genotype (44%) patients; and the TT genotype patients have a significantly higher cancer metastasis to lymph nodes than patients carrying the C allele.

Our study mainly selected Han females in China and did not find that rs1544410 and rs731236 were associated with breast cancer. However, we found that rs7975232 locus was associated with breast cancer; individuals carrying TT genotype had lower risk of breast cancer than those carrying the GG genotype. Our study showed results similar to an Australian study, which showed that the G allele or GG genotype can increase breast cancer prevalence [16]. In addition, haplotype study also showed that GTT conferred a lower breast cancer risk, while the haplotype A-G-T was associated with a higher breast cancer risk.

In addition, studies have shown that 1,25 (OH)$_2$D$_3$ levels in plasma are not directly associ-

ated with genotype distribution frequency difference, suggesting that genotype differences could affect breast cancer cells through other pathways. However, our study has its limitations, such as a lack of evaluation of menopausal status, ER and PR expression levels. In addition, the sample size was small, the analyzed loci were insufficient, and no transverse comparative studies of multiple populations were performed. More accurate conclusions require further studies.

Acknowledgements

This study was supported by the Chongqing Health Bureau grant (No. 30872520) and National Natural Science Foundation of China (No. 81202089).

Disclosure of conflict of interest

None.

Address correspondence to: Fan Li and Xiaopin Chen, The First Affiliated Hospital of Chongqing Medical University, Chongqing, 400016, P. R. China. Tel: +86-23-89011463; +86-23-89011516; E-mail: lf62812@163.com (FL); cxp640910@yeah.net (XPC)

References

[1] Shen Q, Brown PH. Novel agents for the prevention of breast cancer: targeting transcription factors and signal transduction pathways. J Mammary Gland Biol Neoplasia 2003; 8: 45-73.

[2] Swami S, Raghavachari N, Muller UR, Bao YP, Feldman D. Vitamin D growth inhibition of breast cancer cells: gene expression patterns assessed by cDNA microarray. Breast Cancer Res Treat 2003; 80: 49-62.

[3] Towsend K, Trevino V, Falciani F, Stewart PM, Hewison M, Campbell MJ. Identification of VDR-responsive gene signatures in breast cancer cells. Oncology 2006; 71: 111-123.

[4] Tse AK, Zhu GY, Wan CK, Shen XL, Yu ZL, Fong WF. 1alpha,25-Dihydroxyvitamin D3 inhibits transcriptional potential of nuclear factor kappa B in breast cancer cells. Mol Immunol 2010; 47: 1728-1738.

[5] Hussain-Hakimjee EA, Mehta RG. Regulation of steroid receptor expression by 1alpha-hydroxyvitamin D5 in hormone-responsive breast cancer cells. Anticancer Res 2009; 29: 3555-3561.

[6] LaPorta E, Welsh J. Modeling vitamin D actions in triple negative/basal-like breast cancer. J Steroid Biochem Mol Biol 2014; 144: 65-73.

[7] Anderson LN, Cotterchio M, Cole DE, Knight JA. Vitamin D-related genetic variants, interactions with vitamin D exposure, and breast cancer risk among Caucasian women in Ontario. Cancer Epidemiol Biomarkers Prev 2011; 20: 1708-1717.

[8] Abbas S, Nieters A, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, Chang-Claude J. Vitamin D receptor gene polymorphisms and haplotypes and postmenopausal breast cancer risk. Breast Cancer Res 2008; 10: R31.

[9] Barroso E, Fernandez LP, Milne RL, Pita G, Sendagorta E, Floristan U, Feito M, Aviles JA, Martin-Gonzalez M, Arias JI Zamora P, Blanco M, Lazaro P, Benitez J, Ribas G. Genetic analysis of the vitamin D receptor gene in two epithelial cancers: melanoma and breast cancer case-control studies. BMC Cancer 2008; 8: 385.

[10] Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer 2001; 85: 171-175.

[11] Chakraborty A, Mishra AK, Soni A, Regina T, Mohil R, Bhatnagar D, Bhatnagar A, Chintamani C, Sharma PC, Saxena S. Vitamin D receptor gene polymorphism(s) and breast cancer risk in north Indians. Cancer Detect Prev 2009; 32: 386-394.

[12] John EM, Schwartz GG, Koo J, Wang W, Ingles SA. Sun exposure, vitamin D receptor gene polymorphisms, and breast cancer risk in a multiethnic population. Am J Epidemiol 2007; 166: 1409-1419.

[13] Curran JE, Vaughan T, Lea RA, Weinstein SR, Morrison NA, Griffiths LR. Association of A vitamin D receptor polymorphism with sporadic breast cancer development. IntJ Cancer 1999; 83: 723-726.

[14] Lundin AC, Soderkvist P, Eriksson B, Bergman-Jungestrom M, Wingren S. Association of breast cancer progression with a vitamin D receptor gene polymorphism. South-East Sweden Breast Cancer Group. Cancer Res 1999; 59: 2332-2334.

[15] Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Colston KW. Approaches to evaluating the association of vitamin D receptor gene polymorphisms with breast cancer risk. Recent Results Cancer Res 2003; 164: 43-54.

[16] Lowe LC, Guy M, Mansi JL, Peckitt C, Bliss J, Wilson RG, Colston KW. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer 2005; 41: 1164-1169.