Brief Report

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No association between *Vitamin D receptor* gene polymorphisms and nasopharyngeal carcinoma in a Chinese Han population

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Summary **An abundance of candidate genes have been reported as susceptibility factors for the risk of nasopharyngeal carcinoma (NPC). Vitamin D receptor (VDR) plays an important role in cellular differentiation and the control of proliferation in a variety of cell types. To our knowledge, however, no study has reported the relationship between the VDR and NPC. The purpose of this study is to explore the potential correlation between single-nucleotide polymorphisms of the VDR gene (*VDR*) *Fok* I and *Bsm* I and NPC. A total of 171 patients with NPC and 176 age- and sex-matched controls were involved in this study. Genotypes were determined by using polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing. There were no significant differences in the genotype and allele frequencies of *VDR Fok* I and *Bsm* I polymorphisms between the group of patients with NPC and the control group in a Chinese Han population (for *VDR Fok* I: adjusted OR 1.03, 95% CI: 0.76-1.41; for *VDR Bsm* I: adjusted OR 0.80, 95% CI: 0.48-1.33). Further studies will be needed to explore the complicated gene-gene interaction and gene-environmental interactions in the susceptibility to NPC, especially in ethnically disparate populations in cohort study samples.**

Keywords: Vitamin D receptor, gene polymorphism, nasopharyngeal carcinoma

1. Introduction

Nasopharyngeal carcinoma (NPC) is a disease with a remarkable racial differentiation. It occurs relatively rare in the United States with an incidence rate generally less than 1/100,000, but it is autochthonic in Southern China and Southeast Asia with an aged-adjusted incidence rate shown to be 30-50/100,000 (*1*). NPC is a malignancy arising from the epithelial cells lining the nasopharynx and it is a leading cause of cancer deaths in the southern China and also is the 8th greatest cause of cancer mortality around China (*2*). Previous studies show that the incidence of NPC is associated with the combined effects of environmental carcinogens, such as tobacco smoking, consumption of salted fish, and biologic factors, including Epstein-Barr virus (EBV) infection and genetic susceptibility (*1,3,4*). However, the molecular mechanism of NPC pathogenesis is not yet well known. An abundance of recent research has shown single-nucleotide polymorphisms (SNP), such as cytochrome P450 2E1 (CYP2E1) (*5*), cyclin D1 (*6*) and a series of cytokines involved in the development of NPC (*7,8*), which indicated that NPC carcinogenesis may be due to genetic differences such as SNP.

For more than a century, Vitamin D has been recognized as playing a key role in the normal development and mineralization of a healthy skeleton. However, recent studies showed that more extensive roles for vitamin D were discovered in tissues through the vitamin D receptor (VDR), which is a member of the steroid hormone receptor superfamily. VDR has been found in most tissues and cells and it could arise in a wide variety of biologic responses to $1_{\alpha},25(OH)_2D_3$. The functional roles of VDR suggest that vitamin D plays additional roles in cellular differentiation and the control of proliferation in a variety of cell types (*9*).The VDR gene (*VDR*) is located on chromosome

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12q12-q14. Two common *VDR* SNPs were investigated intensively for association with various human traits, including *Fok* I (in exon 2) and *Bsm* I (in intron 8), (rs10735810, and rs1544410, respectively) (*10,11*). Recent studies suggested that polymorphisms of the VDR gene have been associated with several forms of cancer, such as breast cancer (*12*), prostate cancer (*13*), and colorectal cancer (*14*).

In our previous studies, we have found that gene polymorphisms of some cytokines may contribute to the development of NPC (*7,8*), such as *interleukin-1B*, *interleukin-8*, *interleukin-10*, and *transforming growth factor-b1*. Therefore, we hypothesized that VDR gene polymorphisms may also be involved in modulating susceptibility to NPC. Therefore, we conducted a hospital-based case-control study to explicate the potential association between *VDR Fok* I and *Bsm* I polymorphisms and the risk of NPC in a Chinese Han population.

2. Materials and Methods

2.1. *Study population*

171 NPC patients and 176 non-cancer controls were recruited in this study. All the patients and controls were unrelated Chinese people, and were selected from the same population living in China between July 2005 and March 2007 (Table 1). All the patients were recruited from West China hospital, Sichuan University. Pathologically confirmed NPC diagnosis was the only selection criterion for patients. The case group (131 males and 40 females) had a mean age (S.D.) of 46.4 (13.2) years. The control group consisted of 176 healthy volunteers who visited the general health check-up division at West China Hospital, Sichuan University. The mean age (S.D.) of the control group (126 males and 50 females) was 44.4 (13.4) years. Selection criteria for controls were no evidence of any personal or family history of cancer or other serious diseases. There was no significant difference between patients and control subjects in terms of gender and age distribution. Written informed consent was obtained from all subjects, and the study was performed with the approval of the ethics committee of Chinese Human Genome.

Table 1. Characteristics of the study population

Variables	NPC patients (n = 171)	Controls (n = 176)
Age(yrs)	46.4 ± 13.2	44.4 ± 13.4
Sex		
Male	131 (76.6)[a]	126 (71.6)
Female	40 (23.4)	50 (28.4)
Clinical stages		
stages I and II	21 (12.3)	–
stages III and IV	150 (87.7)	–
Histologic type (%)		
Poorly differentiated SCC	141 (82.5)	–
Undifferentiated cancer	22 (12.9)	–
Others	8 (4.6)	–

Abbreviation: SCC, squamous cell carcinoma. [a] Numbers in parentheses denote percentage; others include poorly differentiated adenocarcinoma (n = 2) and moderately differentiated SCC(n = 6).

2.2. *Genotyping*

Genomic DNA was extracted from circulating blood with an extraction kit (Bioteke Corporation, Beijing, China). The *VDR Fok* I (ref SNP ID: rs10735810) and *VDR Bsm* I (ref SNP ID: rs1544410) polymorphisms were detected with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) analysis, as described previously (*15,16*). Approximately 10% of the samples were sequenced to validate the results. In the absence of the restriction site, *Fok* I and *Bsm* I genotypes were defined by capital letters (F and B, respectively), and where the restriction site was present the two genotypes were defined by small letters (f and b, respectively).

2.3. *Statistical analysis*

The SPSS statistical software package (version 11.5) was used for all the statistical analyses. Genotype and allele frequencies of *VDR Fok* I and *Bsm* I were compared between NPC cases and controls using the chi square test and Fisher's exact test when appropriate, and odds ratio (OR) and 95% confidence intervals (CI) were calculated to assess the relative risk conferred by a particular allele and genotype. Demographic and clinical data between groups were compared using the chi square test and Student's t test. Hardy-Weinberg equilibrium was tested with a goodness of fit the for chi square test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. Statistical significance was assumed at the $p < 0.05$ level.

3. Results and Discussion

The genotype and allele frequencies of *VDR Fok* I and *Bsm* I polymorphisms between the controls and the cases are shown in Table 2. Genotype distributions of the two polymorphisms were found to be in accordance with the Hardy-Weinberg equilibrium expectation. The frequencies of the FF, Ff and ff genotypes of *VDR Fok* I were 31.3, 44.3, and 24.4% in controls, and 29.2, 46.8, and 24.0% in the cases. The frequencies of F and f alleles of *VDR Fok* I were 53.4 and 46.6% in controls and 52.6 and 47.4% in the cases. The frequencies of the bb and BB carrier genotypes of *VDR Bsm* I were 81.2 and 18.8 % in controls, and 84.2 and 15.8% in the cases. The frequencies of b and B alleles of *VDR Bsm* I were 89.8 and 10.2% in controls, and 91.8 and 8.2% in the cases. No significant differences were observed in the genotype distributions and allele frequencies of the

Table 2. The genotype and allele frequencies of *Fok* I and *Bsm* I in the VDR gene between patients with NPC and controls

Polymorphisms	Patients $n = 171$ (%)	Controls $n = 176$ (%)	Adjusted[b] OR (95% CI)	p
Fok I				
FF	50 (29.2)	55 (31.3)	1.00 (Ref)	0.63
Ff	80 (46.8)	78 (44.3)	1.11 (0.68-1.83)	0.87
ff	41 (24.0)	43 (24.4)	1.00 (0.56-1.79)	
alleles				
F[a]	180 (52.6)	188 (53.4)	1.00 (Ref)	0.84
f	162 (47.4)	164 (46.6)	1.03 (0.76-1.41)	
Bsm I				
genotypes				
bb	144 (84.2)	143 (81.2)	1.00 (Ref)	0.47
bB + BB	27 (15.8)	33 (18.8)	0.87 (0.49-1.53)	
alleles				
b	314 (91.8)	316 (89.8)	1.00 (Ref)	0.35
B	28 (8.2)	36 (10.2)	0.80 (0.48-1.33)	

[a] F, f, B, and b revealed C, T, A, and G, respectively. [b] Adjusted for sex and age by the logistic regression model.

VDR Fok I and *VDR Bsm* I polymorphisms between the cases and controls (for *VDR Fok* I: adjusted OR 1.03, 95% CI: 0.76-1.41; for *VDR Bsm* I: adjusted OR 0.80, 95% CI: 0.48-1.33).

As a polymorphism in the *VDR* start code, the frequencies of *VDR Fok* I genotype and allele between our control group in the present study and those of previous reports in different countries were also compared. Among the studies with Asian controls, mainly Chinese (*17*) and Japanese controls (*18*), the frequencies of F and f alleles were 47.2-60.5% and 39.5-52.8%. The allele distribution of *VDR Fok* I in this study had no significant difference from that found in Asians from other studies. Among the studies with Caucasian controls, the frequencies of F and f alleles were 65.6-68.8% and 31.2-34.4% (*19*). When compared with this study's controls, however, the Caucasian controls showed significantly higher allele F and lower allele f frequencies ($p < 0.05$). This result was in agreement with previous studies, which concluded the prevalence of the f allele of *VDR Fok* I polymorphism was 36% and 46% in controls of European and Asian descent, respectively (*20*). Furthermore, we compared the *VDR Fok* I and *Bsm* I allele frequency in this study's controls with HapMap (the International HapMap Project) genomes data in the National Institutes of Health Single Nucleotide Polymorphism database (dbSNP) (*http://www.ncbi.nlm.nih.gov/projects/SNP/*). It was shown that the distribution of the *VDR Fok* I allele frequency in this study's controls was in agreement with Chinese and European results. However, the frequency of F allele was higher in Japanese (73.3%) and in Sub-Saharan Africans (83.3%) than this study's controls (53.4%). The distribution of *VDR Bsm* I allele frequency in this study's controls was in agreement with Asians, but differed from Europeans and Sub-Saharan Africans. The frequency of B allele was significantly higher in Europeans (43.8%) and Sub-Saharan Africans (27.9%) than this study's controls (10.2%).

The present study, to our knowledge, is the first to investigate the potential correlation between the *VDR Fok* I and *VDR Bsm* I polymorphisms and NPC. In this pilot study, no significant association was found between *VDR Fok* I and *Bsm* I polymorphisms and the risk of development of NPC. The result implies that *VDR Fok* I and *VDR Bsm* I polymorphisms may not directly contribute to the susceptibility to NPC in the Chinese Han population.

Most of the biologic activities of $1_{\alpha},25(OH)_2D_3$ are mediated by a high-affinity receptor, VDR, which plays a role as a ligand-activated transcription factor. The main steps involved in the modulation of gene transcription by the *VDR* include binding of ligand, heterodimerization with retinoid X receptor (RXR), binding of the heterodimer to vitamin D receptor elements (VDREs), and recruitment of other nuclear proteins into the transcriptional pre-initiation complex. Therefore, genetic changes of the *VDR* gene could induce serious defects of gene activation, influencing calcium metabolism, cell proliferation, and immune function (*9*). Previous studies also implied that polymorphisms in the gene of *VDR* were associated with disease susceptibility, such as cancer, immune dysfunction and chronic disease. *VDR Fok* I polymorphism is a C-to-T transition within exon 2 of the *VDR* gene, defined by endonuclease Fok I. The *VDR Bsm* I genotype is at the 3' end of the VDR gene (intron 8), it has been proved that it does not lead to any change in either the transcribed mRNA or the translated protein. However, the two polymorphisms have been reported to be associated with malignancy (*12-14*), including breast cancer, prostate cancer, primary parathyroid tumors, and colorectal cancer. NPC is a malignancy arising from the epithelial cells lining the nasopharynx and it often occurs in Southern China and Southeast Asia. Our previous studies and other studies have shown that a range of cytokines (*7,8*), including *IL-1B*, *interleukin-8*, *interleukin-10*, and *transforming growth factor-b1* gene polymorphisms, may contribute to the development of NPC. Therefore, in combination with the correlation with *VDR* gene alteration and cancer risk, we postulate that *VDR Fok* I and *Bsm* I gene polymorphisms may modulate susceptibility to NPC. No significant association, however, was found between *VDR Fok* I and *Bsm* I polymorphisms and the risk of NPC in the current study.

In agreement with our findings, some studies have shown that the *VDR Fok* I polymorphism was not associated with breast cancer (*12*), colorectal adenomas (*19*), prostate cancer (*21*), and systemic lupus erythematosus (SLE) (*17*). In addition, some authors reported no associations were found between *VDR Bsm* I polymorphism in breast cancer (*22*), and prostate cancer (*23*). However, the association between

VDR polymorphisms and studied types of tumors is still controversial. Bodiwala *et al*. showed that the *VDR Fok*1 ff genotype was associated with an increased prostate cancer risk (OR = 2.91) (*24*). Hutchinson *et al*, figured that *VDR Fok*1 polymorphisms were correlated to an altered risk for malignant melanoma (MM) (p = 0.014) (*25*). Also Ingles *et al*. found that the Bb and BB genotypes had a 1.6-fold and 2.2-fold increased breast cancer risk than the bb genotype, respectively (OR = 1.6, OR = 2.2, respectively) (*26*). Furthermore, Habuchi *et al*, reported that the Bb and BB genotypes of *VDR Bsm*1 were associated with a significantly reduced risk for prostate cancer (OR = 3.31; 95% CI: 2.05-5.32) and with one-half the risk for benign prostate hyperplasia (BPH) (OR = 2.07; 95% CI: 1.33-3.22) when compared with the male controls (*27*).

These debatable results mentioned above may be due to various reasons, including a limited number of cases, or the analysis of different ethnic groups, or environment factors which the individuals were exposed to, or even gene-gene and gene-environment interactions. Most case-control studies reported no association with *VDR Fok*1 polymorphism and breast caner risk (*12*). However, when the samples were matched up to cohorts (1,234 cases, 1,676 controls), a significantly increased risk of breast cancer was observed (*28*). Chaimuangraj *et al*. focused on the Chinese Han population and suggested that variations in the distribution of *VDR Bsm* I in different ethnic groups might contribute to the racial difference in prostate cancer risk (*29*). Also there is a significant increase of prostate cancer risk found for African-Americans with the ff genotype but not for US Caucasians (*23*). Moreover, Michelle Guy found that when analyzed independently, *Fok* I was not associated with breast cancer risk ($p > 0.05$). However, when it was analyzed combined with *VDR Bsm1* and poly (A) polymorphism, *Fok* I did modulate the increased risk associated with the bb/LL genotype, and one or more F alleles together with the bb/LL genotype enhanced breast cancer risk (*12*). Furthermore, taking environmental factors into consideration, Li *et al*. concluded that the *VDR Fok*1 polymorphism is not an independent risk factor for MM, but it could modulate melanoma risk when it interacts with skin color ($p = 0.029$), moles ($p = 0.017$) and number of first-degree relatives with any cancer ($p = 0.013$) (*30*).

This study also has some limitations. One is that the detailed information on the survival from NPC is unavailable, which restricted our further analysis on the role of *VDR Fok* I and *Bsm* I in cancer prognosis. Another is that the association between *VDR Fok* I and *Bsm* I gene polymorphisms and NPC should be analyzed in different ethnic populations.

In conclusion, we did not find that *VDR Fok* I and *Bsm* I gene polymorphisms were associated with the risk of NPC in a Chinese Han population. Nevertheless, due to complex reasons, further studies will be needed to explore the complicated gene-gene interactions and gene-environmental interactions in the susceptibility to NPC, especially in ethnically disparate populations in cohort study samples.

References

1. McDermott AL, Dutt SN, Watkinson JC. The aetiology of nasopharyngeal carcinoma. Clin Otolaryngol Allied Sci. 2001; 26:82-92.
2. Li LD, Rao KQ, Zhang SW, Lu FZ, Zhou XN. Statistical analysis of data from 12 cancer registries in China, 1993-1997. Bull Chin Cancer. 2003; 11:497-507. (in Chiese)
3. Chang ET, Adami HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev. 2006; 15:1765-1777.
4. Hsu WL, Chen JY, Chien YC, Liu MY, You SL, Hsu MM, Yang CS, Chen CJ. Independent effect of EBV and cigarette smoking on nasopharyngeal carcinoma: A 20-year follow-up study on 9,622 males without family history in Taiwan. Cancer Epidemiol Biomarkers Prev. 2009; 18:1218-1226.
5. Guo X, O'Brien SJ, Zeng Y, Nelson GW, Winkler CA. GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. Cancer Epidemiol Biomarkers Prev. 2008; 17:1760-1763.
6. Jia WH, Pan QH, Qin HD, Xu YF, Shen GP, Chen L, Chen LZ, Feng QS, Hong MH, Zeng YX, Shugart YY. A Case-control and a family-based association study revealing an association between CYP2E1 polymorphisms and nasopharyngeal carcinoma risk in Cantonese. Carcinogenesis. 2009; 30:2031-2036.
7. Zhu Y, Xu Y, Wei Y, LiangW, Liao M, Zhang L. Association of IL-1B gene polymorphisms with nasopharyngeal carcinoma in a Chinese population. Clin Oncol (R Coll Radiol). 2008; 20:207-211.
8. Wei YS, Lan Y, Tang RG, Xu QQ, Huang Y, Nong HB, Huang WT. Single nucleotide polymorphism and haplotype association of the interleukin-8 gene with nasopharyngeal carcinoma. Clin Immunol. 2007; 125:309-317.
9. Mullin GE, Dobs A. Vitamin D and its role in cancer and immunity: A prescription for sunlight. Nutr Clin Pract. 2007; 22:305-322.
10. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004; 338:143-156.
11. Zmuda JM, Cauley JA, Ferrell RE. Molecular epidemiology of vitamin D receptor gene variants. Epidemiol Rev. 2000; 22:203-217.
12. Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Peckitt C, Bliss J, Wilson RG, Thomas V, Colston KW. Vitamin D receptor gene polymorphisms and breast cancer risk. Clin Cancer Res. 2004; 10:5472-5481.
13. Xu Y, Shibata A, McNeal JE, Stamey TA, Feldman D, Peehl DM. Vitamin D receptor start codon polymorphism (Fok1) and prostate cancer progression. Cancer Epidemiol Biomarkers Prev. 2003; 12:23-27.
14. Slattery ML, Herrick J, Wolff RK, Caan BJ, Potter JD, Sweeney C. CDX2 VDR Polymorphism and Colorectal Cancer. Cancer Epidemiol Biomarkers Prev. 2007;

16:2752-2755.
15. Gross C, Eccleshall TR, Malloy PJ, Villa ML, Marcus R, Feldman D. The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican-American women. J Bone Miner Res. 1996; 11:1850-1855.
16. Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PN, Eisman JA. Prediction of bone density from vitamin D receptor alleles. Nature. 1994; 367:284-287.
17. Huang CM, Wu MC, Wu JY, Tsai FJ. No association of vitamin D receptor gene start codon fok 1 polymorphisms in Chinese patients with systemic lupus erythematosus. J Rheumatol. 2002; 29:1211-1213.
18. Ban Y, Taniyama M, Ban Y. Vitamin D receptor gene polymorphism is associated with Graves' disease in the Japanese population. J Clin Endocrinol Metab. 2000; 85:4639-4643.
19. Peters U, McGlynn KA, Chatterjee N, Gunter E, Garcia-Closas M, Rothman N, Sinha R. Vitamin D, calcium, and vitamin D receptor polymorphism in colorectal adenomas. Cancer Epidemiol Biomarkers Prev. 2001; 10:1267-1274.
20. Ntais C, Polycarpou A, Ioannidis JP. Vitamin D receptor gene polymorphisms and risk of prostate cancer: A meta-analysis. Cancer Epid Biomarkers Prev. 2003; 12:1395-1402.
21. Cheteri MB, Stanford JL, Friedrichsen DM, Peters MA, Iwasaki L, Langlois MC, Feng Z, Ostrander EA. Vitamin D receptor gene polymorphism and prostate cancer risk. Prostate. 2004; 59:409-418.
22. Buyru N, Tezol A, Yosunkaya-Fenerci E, Dalay N. Vitamin D receptor gene polymorphisms in breast cancer. Exp Mol Med. 2003; 35:550-555.
23. Oakley-Girvan I, Feldman D, Eccleshall TR, Gallagher RP, Wu AH, Kolonel LN, Halpern J, Balise RR, West DW, Paffenbarger RS, Whittemore AS. Risk of early-onset prostate cancer in relation to germline polymorphisms of the vitamin D receptor. Cancer Epidemiol Biomarkers Prev. 2004; 13:1325-1330.
24. Bodiwala D, Luscombe CJ, French ME, Liu S, Saxby MF, Jones PW, Fryer AA, Strange RC. Polymorphisms in the vitamin D receptor gene, ultraviolet radiation, and susceptibility to prostate cancer. Environ Mol Mutagen. 2004; 43:121-127.
25. Hutchinson PE, Osborne JE, Lear JT, Smith AG, Bowers PW, Morris PN, Jones PW, York C, Strange RC, Fryer AA. Vitamin D receptor polymorphisms are associated with altered prognosis in patients with malignant melanoma. Clin Cancer Res. 2000; 6:498-504.
26. Ingles SA, Garcia DG, Wang W, Nieters A, Henderson BE, Kolonel LN, Haile RW, Coetzee GA. Vitamin D receptor genotype and breast cancer in Latinas (United States). Cancer Causes Control. 2000; 11:25-30.
27. Habuchi T, Suzuki T, Sasaki R, Wang L, Sato K, Satoh S, Akao T, Tsuchiya N, Shimoda N, Wada Y, Koizumi A, Chihara J, Ogawa O, Kato T. Association of vitamin D receptor gene polymorphism with prostate cancer and benign prostatic hyperplasia in a Japanese population. Cancer Res. 2000; 60:305-308.
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; 14:2335-2339.
29. Chaimuangraj S, Thammachoti R, Ongphiphadhanakul B, Thammavit W. Lack of association of VDR polymorphisms with Thai prostate cancer as compared with benign prostate hyperplasia and controls. Asian Pac J Cancer Prev. 2006; 7:136-139.
30. Li C, Liu Z, Wang LE, Gershenwald JE, Lee JE, Prieto VG, Duvic M, Grimm EA, Wei Q. Haplotype and genotypes of the VDR gene and cutaneous melanoma risk in non-Hispanic whites in Texas: A case-control study. Int J Cancer. 2008; 122:2077-2084.

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