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Original Article

Genetic association of *VDR* polymorphisms and multiple myeloma susceptibility: a case control study

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Abstract: Objective: The aim of this study was to investigate whether the vitamin D receptor (*VDR*) gene polymorphisms were associated with multiple myeloma (MM) susceptibility in the Chinese Han population. Methods: Two polymorphisms rs2228570 (FokI) and rs731236 (TaqI) in *VDR* gene were genotyped in 113 MM patients and 117 healthy controls. Chi-square test was employed to compare the genotype and allele distributions of each polymorphism between case and control groups. The strength of the association between *VDR* gene polymorphisms and MM risk was evaluated based on the odds ratio (OR) with corresponding 95% confidence interval (CI). Results: Significant association was found between *VDR* gene polymorphisms and MM risk. The TT genotype frequency of rs2228570 significantly increased in case group compared with controls, revealing that rs2228570 TT genotype positively associated with MM risk (*P*=0.031, OR=2.407, 95% CI=1.075-5.393). Individuals carrying T allele showed higher risk to be affected by MM by 1.463 fold versus the C allele carriers (*P*=0.043, OR=1.463, 95% CI=1.011-2.118). Mutant C allele of rs731236 was a risk factor for the onset of MM (*P*=0.012, OR=2.407696, 95% CI=1.213-5.990). Conclusion: All results suggested that *VDR* gene two polymorphisms rs2228570 and rs731236 might be important genetic factors in MM susceptibility in the Chinese Han population.

Keywords: *VDR*, multiple myeloma, polymorphism

Introduction

Multiple myeloma (MM), is a kind of plasma cells cancer, which is characterized by bone marrow plasmacytosis and presence of monoclonal immunoglobulin [1]. MM always occurs in the elderly, and it seems to be more common in men than in women [2]. Epidemiological studies have shown that MM accounts for approximately 10% of hematologic malignancies, being the second most common hematological cancer, behind the lymphoma but ahead the leukemia [3]. MM is regarded as a multifactorial disease, that can be influenced by the interaction between various environmental and promoter factors [4, 5]. Various risk factors have been identified to be involved in the development of MM, including increased age, positive family history, tobacco smoking, alcohol consumption, ionizing radiation, industrial occupation, and obesity [6, 7]. Recent evidences have suggested that genetic predisposition would participant in MM carcinogenesis, and several genetic polymorphisms have been reported to be associated with the susceptibility of MM [8, 9].

The calcitriol receptor, also known as the vitamin D receptor (VDR), is a member of the nuclear receptor family of transcription factors [10]. Vitamin D regulates many human biological processes such as bone metabolism, innate immune response, and cell proliferation and differentiation via binding to its receptor VDR. VDR can maintain the stability of the calcium and phosphorus in the serum, regulate the cell proliferation, differentiation and immune regulating function. Previous evidences have considered lower levels of vitamin D to be a risk factor for human cancers [11], in which the anticancer effect of vitamin D is reported to be activated mainly through the VDR [12].

The *VDR* gene is located on chromosome 12q13.11, with a full length of 70495 bp, containing 11 exons and 11 introns. Numbers of single nucleotide polymorphisms (SNPs) have been identified in *VDR* gene, which may influ-

Table 1. Primer sequences of *VDR* gene two polymorphisms rs2228570 and rs731236

SNP	Primer sequences		Tm (°C)
rs2228570	Sense	5'-CTGGCACTGACTCTGGCTCT-3'	55.3
	Reverse	5'-CGGTCAAAGTCTCCAGGGTC-3'	
rs731236	Sense	5'-AGAGCATGGACAGGGAGCAAG-3'	60.8
	Reverse	5'-GCAACTCCTCATGGCTGAGGTCTCA-3'	

ence the expression of *VDR* and further change the quantity and activity of receptor proteins. Recent studies have reported that *VDR* gene polymorphisms are correlated with several human cancers, such as breast, colorectal, prostate and skin [13-16]. Furthermore, Shafia S et al. have found a significant association between *VDR* gene polymorphism and MM susceptibility in the ethnic Kashmiri population [17]. All data suggests the potential role of *VDR* gene polymorphism in the development of MM.

Therefore, we performed a case control study to evaluate the association of *VDR* gene polymorphism rs2228570 (FokI) and rs731236 (TaqI) and MM risk in a Chinese Han population.

Materials and methods

Subjects

A cohort of 230 individuals were enrolled in this case control study, including 113 patients with MM and 117 healthy controls. The 113 patients were histologically conformed MM who were admitted to the Affiliated Hospital of Guangdong Medical University from February 2011 to November 2014. The inclusion criteria of patients group was according to the Blood Disease Diagnostics and Curative Standard edited by Zhinan Zhang. Another 117 healthy individuals were recruited as control group, who attending the clinic for annual health check-ups in the Affiliated Hospital of Guangdong Medical University during the same period. All controls involved in this study had no history of cancer. The control group was matched to the patients with MM by age, gender. And all participants were Chinese Han population and had no blood relationship with each other.

This case control study was received and consented by Ethics committee of Affiliated Hospital of Guangdong Medical University. All subjects participated in this research were required to complete a questionnaire to collect epidemiological data and provided signed informed consent. The process of sample collection was performed according to the ethnic criteria of national genome research.

Sample collection

5 ml of venous blood were collected from each participant into the anticoagulative tube with EDTA-disodium salt. The genomic DNA was extracted by TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering CO., LTD, China). Then the extracted DNA samples were solved in sterile and distilled water and stored at -20°C for standby application.

SNP genotyping

The target fragments for VDR gene rs2228570 and rs731236 were directly amplified using the polymerase chain reaction (PCR). Primers for amplification of the two SNPs were designed by Primer Premier 5.0 software, and synthesized by Sangon Biothch (Shanghai, China) (**Table 1**). The PCR reaction was performed in a total volume of 25 μl, containing 2 μl genomic DNA, 2 μl primer (1 μl each of upstream and downstream), 1.5 μl Mg^{2+}, 2 μl dNTP, 0.3 μl Taq DNA polymerase, 2.5 μl 10× buffer and 14.7 μl ddH_2O. The PCR procedures were carried out at 94°C for 5 min, followed by 35 cycles at 94°C for 45 s, annealing at different temperature for 60 s (55°C for rs2228570 and 61°C for rs731236), 60 s of extension at 72°C, and a final extension at 72°C for 10 min.

Following amplification, the PCR products of rs2228570 and rs731236 were then purified with a purification kit, and were directly sequenced by automated DNA sequencing with an Applied Biosystems 3730×l automated sequencer (Applied Biosystems, Foster City, CA, USA), and sequence analysis was performed using Vector NTI software.

Statistical analysis

All statistical analyses in this study were performed with the PASW Statistics 18.0 statistical software. The genotype and allele frequen-

Table 2. Genotype and allele distributions of *VDR* gene two polymorphisms rs2228570 and rs731236 in case and control groups

Genotype/ Allele	Case *n*=113 (%)	Control *n*=117 (%)	χ^2	*P*	OR (95% CI)
rs2228570					
CC	27 (23.89)	39 (33.33)	-	-	1
CT	61 (53.98)	63 (53.85)	1.189	0.276	1.399 (0.765-2.558)
TT	25 (22.13)	15 (12.82)	4.646	0.031	2.407 (1.075-5.393)
C	115 (50.88)	141 (60.26)	-	-	1
T	111 (49.12)	93 (39.74)	4.091	0.043	1.463 (1.011-2.118)
rs731236					
TT	93 (82.30)	108 (92.31)	-	-	1
TC	18 (15.93)	9 (7.69)	3.964	0.046	2.323 (0.996-5.417)
CC	2 (1.77)	0 (0)	2.296	0.130	0.979 (0.951-1.008)
T	204 (90.27)	225 (96.15)	-	-	1
C	22 (9.73)	9 (3.85)	6.342	0.012	2.696 (1.213-5.990)

cies of VDR gene rs2228570 and rs731236 polymorphisms were estimated by direct counting. Hardy-Weinberg equilibrium (HWE) test was tested via chi-square test to assess the representativeness of our study sample. Chi-square test was also employed to compare the genotype and allele distribution differences between groups. The strength of association between VDR gene polymorphisms and MM susceptibility was calculated by odds ratio (OR) and 95% confidence interval (CI). All statistical tests were two-sided with a significance level of P<0.05.

Results

HWE test

Table 2 presented the genotype and allele frequencies of VDR gene rs2228570 and rs731236 polymorphisms between the case and control groups. The chi-square test results showed that the genotype distribution of each SNP did not deviate from HWE in both case and control groups (P>0.05), suggesting our study sample were from the same group.

Genetic association of VDR gene with MM

The assessment of the correlation between VDR gene two polymorphisms rs2228570 and rs731236 and MM susceptibility were presented in **Table 2**. Rs2228570 showed significant differences in both genotype and allele distributions between groups. The TT genotype frequency significantly increased in case group compared with controls (22.13% vs. 12.82%), while the CC genotype frequency decreased (23.89% vs. 33.33%), and the difference reached significant level (P=0.031). This difference present a significant association between rs2228570 TT genotype and increased MM susceptibility (OR=2.407, 95% CI= 1.075-5.393). But there was no obvious difference in the CT genotype distribution between groups (P>0.05). Additionally, the T allele also showed significantly increased trend in MM patients group (49.12% vs. 39.74%), and individuals carrying T allele showed higher risk to be affected by MM (OR=1.463, 95% CI= 1.011-2.118). These results suggested that VDR gene rs2228570 polymorphism was associated with MM risk in the Chinese Han population, and the T allele acted as a risk factor for the onset of MM.

For rs731236, only two genotypes of TT and TC were detected in control group with the frequency of 92.31% and 7.69%, and no CC genotype was found in control group. But the mutant homozygous CC genotype was detected in the case group with a frequency of 1.77%. Besides, Heterozygous TC genotype showed higher frequency in MM patients group than that in controls (15.93% vs. 7.69%), the difference was statistically significant (P<0.05), but it had no significant association with AA risk. Mutant C allele also significantly increased in case groups (9.73% vs. 3.85%, P<0.05). We speculated that the VDR gene rs731236 polymorphism was associated with MM susceptibility, and individuals with C allele were easier to be affected by MM (OR=2.696, 95% CI=1.213-5.990).

Discussion

MM is a kind of plasma cells cancer, which always occurs in the elderly. The Cytogenetic analysis reveals that MM is caused by the combination of multiple genes and environment. But only a small part of individuals exposed to the same environment will suffer from MM, suggesting that the possibility of developing MM

largely depends on their genetic predisposition [18]. MM is considered as a multifactorial disease, containing different kinds of risk factors that span numerous life aspects [19]. MM is also regarded as a genetically heterogeneous disease, and recent years several candidate genes have been identified to be associated with MM susceptibility [20-23].

During the previous research, vitamin D has been reported to have anticancer effect in the development of various cancers. And the anticancer effect of vitamin D is activated by VDR, which specifically binds to 1,25-dihydroxyvitamin D3 for the regulation of skeletal development, maintenance of skeletal architecture, hormone secretion and immune function [24, 25]. Vitamin D has been found to has the inhibitory effects on the MM cells [26, 27], and the anticancer effect of vitamin D is reported to be activated mainly through the VDR. The human *VDR* gene polymorphisms, which may influence the expression of *VDR* and further change the quantity and activity of receptor proteins, have been widely reported to be involved in the development of various cancers [28]. Furthermore, a major study has reported the significant association between *VDR* gene polymorphism and MM susceptibility in Kashmiri population.

The present study presented a case control study, and explore the potential association of *VDR* gene polymorphisms with MM susceptibility in a Chinese Han population. We noted that *VDR* gene rs2228570 polymorphism showed significant association with MM risk. The TT genotype carriers showed higher risk to suffer from MM by 2.407 fold versus the CC genotype carriers. Besides, the mutant T allele frequency was significantly higher in MM patients group than that in controls, suggesting T allele to be a risk factor for the onset of MM in the Chinese Han population. In the previous study, rs2228570 polymorphism is reported to be involved in the susceptibility to development and progression in MM in the ethnic Kashmiri population, which was in accordance with our study results [17]. Rs731236 is another common SNP in *VDR* gene, which has been found to be associated with an increased risk for colorectal cancer [29]. In the present study, significant association was also identified between rs731236 polymorphism and MM risk, and the mutant C allele acted as a risk factor for the onset of MM in the Chinese Han population. All results suggested the crucial role of *VDR* gene polymorphisms in the development of cancer.

In summary, results from this case control study all suggested that *VDR* gene two polymorphisms might be important genetic factors in MM susceptibility in the Chinese Han population. Of course, several limitations still presented in this research, such as the small study sample and the single race. Thus, further studies in other larger or different populations should be replicated to confirm our results. Furthermore, to substantiate the results, further functional studies of VDR regulation are still required.

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Disclosure of conflict of interest

None.

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References

[1] Smith D and Yong K. Multiple myeloma. BMJ 2013; 346: f3863.
[2] Nagy Z. [Multiple myeloma and other plasma cell dyscrasias]. Magy Onkol 2016; 60: 154-163.
[3] Rajkumar SV. Multiple myeloma: 2016 update on diagnosis, risk-stratification, and management. Am J Hematol 2016; 91: 719-734.
[4] Becker N. Epidemiology of multiple myeloma. Recent Results Cancer Res 2011; 183: 25-35.
[5] Munshi NC and Avet-Loiseau H. Genomics in multiple myeloma. Clin Cancer Res 2011; 17: 1234-1242.
[6] Lope V, Perez-Gomez B, Aragones N, Lopez-Abente G, Gustavsson P, Plato N, Zock JP and Pollan M. Occupation, exposure to chemicals, sensitizing agents, and risk of multiple myeloma in Sweden. Cancer Epidemiol Biomarkers Prev 2008; 17: 3123-3127.
[7] Wallin A and Larsson SC. Body mass index and risk of multiple myeloma: a meta-analysis of prospective studies. Eur J Cancer 2011; 47: 1606-1615.
[8] Martino A, Campa D, Jamroziak K, Reis RM, Sainz J, Buda G, Garcia-Sanz R, Lesueur F, Marques H, Moreno V, Jurado M, Rios R, Szem-

raj-Rogucka Z, Szemraj J, Tjonneland A, Overvad K, Vangsted AJ, Vogel U, Mikala G, Kadar K, Szombath G, Varkonyi J, Orciuolo E, Dumontet C, Gemignani F, Rossi AM, Landi S, Petrini M, Houlston RS, Hemminki K and Canzian F. Impact of polymorphic variation at 7p15.3, 3p22.1 and 2p23.3 loci on risk of multiple myeloma. Br J Haematol 2012; 158: 805-809.

[9] Morgan GJ, Johnson DC, Weinhold N, Goldschmidt H, Landgren O, Lynch HT, Hemminki K and Houlston RS. Inherited genetic susceptibility to multiple myeloma. Leukemia 2014; 28: 518-524.

[10] Heyne K, Heil TC, Bette B, Reichrath J and Roemer K. MDM2 binds and inhibits vitamin D receptor. Cell Cycle 2015; 14: 2003-2010.

[11] Galunska B, Gerova D, Kosev P, Anakievski D and Hinev A. Serum 25-hydroxy vitamin D levels in Bulgarian patients with prostate cancer: a pilot study. Clin Lab 2015; 61: 329-335.

[12] Chakraborti CK. Vitamin D as a promising anticancer agent. Indian J Pharmacol 2011; 43: 113-120.

[13] Lee YH and Song GG. Vitamin D receptor FokI, BsmI, ApaI, and TaqI polymorphisms and the susceptibility to breast cancer: ameta-analysis. Neoplasma 2014; 61: 607-616.

[14] Budhathoki S, Yamaji T, Iwasaki M, Sawada N, Shimazu T, Sasazuki S, Yoshida T and Tsugane S. Vitamin D receptor gene polymorphism and the risk of colorectal cancer: a nested case-control study. PLoS One 2016; 11: e0164648.

[15] Kang S, Zhao Y, Liu J, Wang L, Zhao G, Chen X, Yao A, Zhang L, Zhang X and Li X. Association of Vitamin D receptor Fok I polymorphism with the risk of prostate cancer: a meta-analysis. Oncotarget 2016; 7: 77878-77889.

[16] Burns EM, Elmets CA and Yusuf N. Vitamin D and skin cancer. Photochem Photobiol 2015; 91: 201-209.

[17] Shafia S, Qasim I, Aziz SA, Bhat IA, Nisar S and Shah ZA. Role of vitamin D receptor (VDR) polymorphisms in susceptibility to multiple myeloma in ethnic Kashmiri population. Blood Cells Mol Dis 2013; 51: 56-60.

[18] Nikesitch N and Ling SC. Molecular mechanisms in multiple myeloma drug resistance. J Clin Pathol 2016; 69: 97-101.

[19] Sergentanis TN, Zagouri F, Tsilimidos G, Tsagianni A, Tseliou M, Dimopoulos MA and Psaltopoulou T. Risk factors for multiple myeloma: a systematic review of meta-analyses. Clin Lymphoma Myeloma Leuk 2015; 15: 563-577, e561-563.

[20] Manier S, Salem KZ, Park J, Landau DA, Getz G and Ghobrial IM. Genomic complexity of multiple myeloma and its clinical implications. Nat Rev Clin Oncol 2017; 14: 100-113.

[21] Zhang Q, Wang LQ, Wong KY, Li ZY and Chim CS. Infrequent DNA methylation of miR-9-1 and miR-9-3 in multiple myeloma. J Clin Pathol 2015; 68: 557-561.

[22] Campa D, Martino A, Varkonyi J, Lesueur F, Jamroziak K, Landi S, Jurczyszyn A, Marques H, Andersen V, Jurado M, Brenner H, Petrini M, Vogel U, Garcia-Sanz R, Buda G, Gemignani F, Rios R, Vangsted AJ, Dumontet C, Martinez-Lopez J, Moreno MJ, Stepien A, Watek M, Moreno V, Dieffenbach AK, Rossi AM, Butterbach K, Jacobsen SE, Goldschmidt H, Sainz J, Hillengass J, Orciuolo E, Dudzinski M, Weinhold N, Reis RM and Canzian F. Risk of multiple myeloma is associated with polymorphisms within telomerase genes and telomere length. Int J Cancer 2015; 136: E351-358.

[23] Martino A, Campa D, Jurczyszyn A, Martinez-Lopez J, Moreno MJ, Varkonyi J, Dumontet C, Garcia-Sanz R, Gemignani F, Jamroziak K, Stepiel A, Jacobsen SE, Andersen V, Jurado M, Landi S, Rossi AM, Lesueur F, Marques H, Dudzinski M, Watek M, Moreno V, Orciuolo E, Petrini M, Reis RM, Rios R, Sainz J, Vogel U, Buda G, Vangsted AJ and Canzian F. Genetic variants and multiple myeloma risk: IMMEnSE validation of the best reported associations--an extensive replication of the associations from the candidate gene era. Cancer Epidemiol Biomarkers Prev 2014; 23: 670-674.

[24] Wacholder S, Hartge P, Prentice R, Garcia-Closas M, Feigelson HS, Diver WR, Thun MJ, Cox DG, Hankinson SE, Kraft P, Rosner B, Berg CD, Brinton LA, Lissowska J, Sherman ME, Chlebowski R, Kooperberg C, Jackson RD, Buckman DW, Hui P, Pfeiffer R, Jacobs KB, Thomas GD, Hoover RN, Gail MH, Chanock SJ and Hunter DJ. Performance of common genetic variants in breast-cancer risk models. N Engl J Med 2010; 362: 986-993.

[25] Bouillon R, Okamura WH and Norman AW. Structure-function relationships in the vitamin D endocrine system. Endocr Rev 1995; 16: 200-257.

[26] Park WH, Seol JG, Kim ES, Binderup L, Koeffler HP, Kim BK and Lee YY. The induction of apoptosis by a combined 1,25(OH)2D3 analog, EB1089 and TGF-beta1 in NCI-H929 multiple myeloma cells. Int J Oncol 2002; 20: 533-542.

[27] Kumagai T, O'Kelly J, Said JW and Koeffler HP. Vitamin D2 analog 19-nor-1,25-dihydroxyvitamin D2: antitumor activity against leukemia, myeloma, and colon cancer cells. J Natl Cancer Inst 2003; 95: 896-905.

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

[29] Serrano D, Gnagnarella P, Raimondi S and Gandini S. Meta-analysis on vitamin D receptor and cancer risk: focus on the role of TaqI, ApaI, and Cdx2 polymorphisms. Eur J Cancer Prev 2016; 25: 85-96.