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-
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 confirmed 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 Mg2+, 2 μl dNTP, 0.3 μl Taq DNA polymerase, 2.5 μl 10x buffer and 14.7 μl ddH2O. 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 3730xl 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-
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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

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

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

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