Original Article
Association of vitamin D receptor-a gene polymorphisms with coronary heart disease in Han Chinese

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Abstract: Objective: To assess the association between coronary heart disease (CHD) and vitamin D receptor (VDR) gene polymorphisms in Han Chinese adults. Methods: A total of 215 CHD patients and 67 controls were recruited. In both groups, the VDR gene single nucleotide polymorphisms (SNP) of Tru9I (rs757343), ApaI (rs7975232), TaqI (rs731236) and FokI (rs2228570) were detected, and the frequencies of VDR genotypes were compared between patients and controls. The relationship between VDR FokI genotype and risk for CHD was assessed by logistic regression analysis after adjusting for age and sex. In addition, the clinical parameters and biochemical characteristics of CHD subgroups were compared according to the VDR FokI polymorphism. Results: The frequencies of FokI genotypes in CHD patients were 23.7% for AA, 47.9% for AG, and 28.4% for GG. The frequency of FokI-GG genotype significantly decreased in CHD patients as compared to control group (\( P = 0.039 \)). No significant differences were observed in other VDR SNPs (rs7975232, rs731236 and rs757343) (\( P > 0.05 \)) between groups. FokI-A allele carriers had a 2.61-fold increase in the odds (95% CI: 1.116-6.102, \( P = 0.027 \)) as compare to CHD subjects with FokI mutation. In CHD subgroup, patients with GG genotype had a significantly higher concentration of high-density lipoprotein cholesterol than those with AG genotype or A* genotype (\( P = 0.001 \), respectively). Conclusion: VDR FokI polymorphisms appear to be associated with CHD. GG genotype predicts a higher HDL-cholesterol in CHD adults.

Keywords: Vitamin D receptor, coronary heart disease, gene polymorphism

Introduction

Coronary heart disease (CHD) is the most common cause of death in developed countries and the second most common cause of death in developing countries [1]. The mortality of CHD patients and the risk factors of CHD have substantially increased and continue to rise rapidly in China [2]. Although environmental factors play important roles in the pathogenesis of CHD [3, 4], genetic factors (such as single nucleotide polymorphism; SNP) also affect the occurrence of CHD [5, 6]. However, the exact mechanism underlying the influence of SNP on the pathogenesis of CHD is poorly understood. Some common variants of coronary diseases show allelic heterogeneity or copy number variation.

The vitamin D endocrine system is involved in a wide variety of biological processes including bone metabolism, regulation of cell proliferation and differentiation and modulation of immune responses [7]. The role of vitamin D and vitamin D receptor (VDR) in the skeletal metabolism is well known. VDR gene plays an important role in the vitamin D pathway, and belongs to the steroid hormone family of nuclear receptors which are responsible for the transcriptional regulation of a number of hormone responsive genes. Polymorphisms within the VDR gene may potentially influence the vitamin D expression and the stability of VDR mRNA. Recent studies have well-characterized four VDR polymorphisms Fok1, Bsm1, Apa1 and Taq1 [7]. More recent attention has been focused on the possible role of VDR gene polymorphisms in the development of a range of diseases, including osteoarthritis, psoriasis, diabetes, as well as CHD [8]. However, some results are conflicting. Van Schooten et al. [9] reported an association between VDR Bsm1 polymorphism and coronary artery disease (CAD) of European white in the Netherlands,
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Endocrinology and Metabolism Research Institute approved the whole study protocol. On recruitment, all participants provided written informed consent and were voluntary to participate in this study and receive DNA genotyping.

Coronary angiography

An experienced cardiologist performed angiography on each subject if angiography was indicated. Following angiography, subjects were divided to two groups. In CHD group, more than 50% stenosis was observed. In control group, coronary artery was normal.

Genetic analyses

Fasting blood samples were collected from all the subjects into EDTA-coated tubes for genotyping and DNA was extracted using a Flexi-Gen DNA kit (QIAGEN kit) according to the manufacturer’s instructions. The Tru9I, ApaI, TaqI and FokI polymorphisms of VDR gene were detected by polymerase chain reaction. Genotyping was done by sequencing total PCR products.

Statistical analysis

Hardy-Weinberg equilibrium was tested by a \(x^2\) goodness-of-fit test. The chi-square test was employed to compare categorical variables. Descriptive data are presented as mean ± standard deviation (SD). Paired t test was per-
**Table 3.** Clinical and biochemical characteristics of CHD subgroups

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>A*</th>
<th>GG</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>62.11 ± 9.36</td>
<td>62.23 ± 9.57</td>
<td>0.933</td>
</tr>
<tr>
<td>TG</td>
<td>1.46 ± 0.98</td>
<td>1.38 ± 0.94</td>
<td>0.566</td>
</tr>
<tr>
<td>TC</td>
<td>4.23 ± 0.90</td>
<td>4.34 ± 1.18</td>
<td>0.425</td>
</tr>
<tr>
<td>HDL</td>
<td>1.16 ± 0.33</td>
<td>1.34 ± 0.41</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL</td>
<td>2.63 ± 0.79</td>
<td>2.73 ± 0.95</td>
<td>0.397</td>
</tr>
<tr>
<td>FPG</td>
<td>5.91 ± 2.62</td>
<td>5.71 ± 1.78</td>
<td>0.597</td>
</tr>
<tr>
<td>CR</td>
<td>77.53 ± 24.81</td>
<td>79.28 ± 28.00</td>
<td>0.654</td>
</tr>
</tbody>
</table>

Coronary artery integral: 51.85 ± 32.03, 53.53 ± 35.79, 0.737

Note: TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; CR: creatinine; FPG: fasting plasma glucose.

**Table 4.** Logistic regression of traits associated with VDR FokI polymorphism in the study population

<table>
<thead>
<tr>
<th>B</th>
<th>Sig.</th>
<th>95.0% CI for EXP (B)</th>
<th>Exp (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>CA</td>
<td>-5.414</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Step 2</td>
<td>FPG</td>
<td>0.405</td>
<td>0.002</td>
</tr>
<tr>
<td>CA</td>
<td>-6.102</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Step 3</td>
<td>Smoking</td>
<td>-7.26</td>
<td>0.022</td>
</tr>
<tr>
<td>FPG</td>
<td>0.388</td>
<td>0.004</td>
<td>1.136</td>
</tr>
<tr>
<td>CA</td>
<td>-5.931</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Step 4</td>
<td>Smoking</td>
<td>-8.18</td>
<td>0.012</td>
</tr>
<tr>
<td>FPG</td>
<td>0.394</td>
<td>0.004</td>
<td>1.132</td>
</tr>
<tr>
<td>CA</td>
<td>-6.081</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>FokI</td>
<td>0.959</td>
<td>0.027</td>
<td>1.010</td>
</tr>
<tr>
<td>FokI-AA</td>
<td>0.688</td>
<td>0.047</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: CA: serum calcium; FPG: Fasting plasma glucose.

No significant differences in the age and sex. The genotype and allele frequencies of Apal, TaqI, Tru9I and FokI polymorphisms of patients and controls are shown in Table 2. The genotype distributions were in Hardy-Weinberg equilibrium in both groups. The genotype frequencies of FokI in CHD patients were 23.7% for AA, 47.9% for AG, and 28.4% for GG. Statistical analysis showed the proportion of CHD patients with GG genotype reduced significantly when compared with controls (P = 0.039, OR = 0.552, 95% CI 0.312-0.974). No significant differences were observed in the genotype and allele frequencies of Apal, TaqI and Tru9I polymorphisms between patients and controls (ApaI: = 0.646, OR = 1.103, 95% CI 0.726-1.673; TaqI: P = 0.404, OR = 1.585, 95% CI 0.532-4.722, Tru9I: P = 0.364, OR = 1.23, 95% CI 0.786-1.924). The association of FokI-A allele genotype and CHD was then assessed within all individuals with a binary logistic regression model after adjusting for age and sex. In CHD subgroups, GG genotype predicted a higher HDL-cholesterol as compared to AG genotype and A* genotype (Table 3; P = 0.001, respectively). Interestingly, FokI-A allele carriers had a 2.61-fold increase in the odds (95% CI: 1.116-6.102, P = 0.027) as compared to CHD subjects with FokI mutation (Table 4).

**Discussion**

Vitamin D is initially metabolized to the intermediate compound 25-hydroxyvitamin D in the liver which subsequently binds to the intracellular receptors to regulate gene expression. Results from cross-sectional studies examining the relation between vitamin D and CAD in the general population are conflicting [12]. In type 1 diabetic patients, vitamin D deficiency has...
been shown to independently predict both prevalence and development of CAD [13]. However, a study in type 2 diabetic patients with a history of cardiovascular disease (CVD) found a strong inverse association between low vitamin D level and prevalence of coronary, cerebrovascular, and peripheral CVD [14]. Furthermore, a low vitamin D level is associated with increased cardiovascular morbidity and mortality in the general population [15].

VDR is an important regulator of vitamin D pathway, which involves the conversion of serum 25-hydroxyvitamin D into the active hormone, 1,25-dihydroxyvitamin D. VDR is required for the functions of vitamin D [16]. VDR is an intracellular hormone receptor that specifically binds the biologically active form of calcitriol or vitamin D, 1,25-dihydroxyvitamin D and interacts with specific nucleotide sequences of target genes to produce a variety of biologic effects [17].

VDR gene plays an important role in the vitamin D pathway. VDR protein is known to display polymorphic variation and belongs to the steroid hormone family of nuclear receptors which are responsible for the transcriptional regulation of a number of hormone responsive genes. As VDR is expressed in a large number of tissues, it is not surprising that ligand-activated VDR modulates the expression of multiple targeted genes [18], which is consistent with the fact that vitamin D deficiency has been associated with risk factors for cardiovascular disease, metabolic syndrome and even with overall mortality [19]. VDR harbors several known functional polymorphisms and several of these polymorphisms have been commonly investigated [7]. The human VDR gene is mapped to chromosome 12q12-q14, and five common polymorphisms have been typically associated with VDR activity [20-22], namely VDR Tru9I (rs757343), FokI (rs2228570), TaqI (rs731236), BsmI (rs1544410) and ApaI (rs7975232). CAD is the leading cause of death worldwide. Although environmental factors play important roles in the pathogenesis of CAD [4], genetic factors also affect the occurrence of CAD [5-7].

We focused on four SNPs of VDR: Tru9I (rs757343), FokI (rs2228570), ApaI (rs7975232) and TaqI (rs731236). FokI restriction enzyme gene has a polymorphic site in the exon 2 at the 5' end of VDR gene. Three other polymorphisms are identified by their restriction endonuclease cleavage sites (Tru9I, ApaI and TaqI) [23]. According to our results, FokI-GG genotype, a mutant SNP, showed significant difference between patients (36.1%) and controls (47.5%). GG genotype frequency of CHD patients was significantly lower. This was inconsistent with the findings of Pan et al. [29], which may be ascribed to the different regions and different types of CHD. As we known that the genetic characteristics of different types of CHD are likely to present obvious difference. In addition, sample size may be another contributing factor causing this discrepancy [30]. A binary logistic regression model revealed that FokI-A allele carriers had a 2.61-fold increase in the odds (95% CI: 1.116-6.102, P = 0.027) as compared to CHD patients with FokI mutation. VDR FokI polymorphisms were independent factors
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affecting CHD. In addition, the clinical parameters and biochemical characteristics of CHD subgroups were compared on the basis of VDR FokI polymorphism. Results showed that patients with FokI-GG genotype had a higher HDL (P = 0.001 < 0.05) as compared to those with FokI-As genotype, which was consistent with the findings of Natielen et al [31]. However, Tru9I, Apal and TaqI polymorphisms were not related to the increased risk for CHD. Tru9I, Apal and TaqI polymorphisms are promising SNPs causing CHD. In Chinese, the frequencies of Tru9I-AA and TaqI-CC genotypes are low. Thus, it is necessary to increase the sample size for further investigation. A few studies conducted in CAD patients have investigated the distribution of VDR polymorphisms. Arash et al [32] investigated the relationship between VDR FokI polymorphism and collateralization in CAD patients. They found there was no relationship between VDR genotype and severity of CAD. Consistent with this result, Pan et al. [29] found no association between FokI polymorphism and CAD, but they did not investigate other VDR gene polymorphisms.

In conclusion, our findings support the hypothesis that VDR FokI-GG genotype may predict a low risk for CHD. VDR FokI polymorphism appears to be associated to CHD. However, due to a small sample size, further studies with elegant study design are needed to confirm our findings and investigate the potential mechanisms underlying this association.

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Disclosure of conflict of interest

None.

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