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# Association of VDR polymorphisms with type 2 diabetes mellitus in Chinese Han and Hui populations

**J.R. Xu, Y. Yang, X.M. Liu and Y.J. Wang**

Key Laboratory of the Ministry of Education for Conservation and Utilization of Special Biological Resources, Ningxia, China

Corresponding author: Y. Wang
E-mail: wyj@nxu.edu.cn / yujiongw@gmail.com

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**ABSTRACT.** We investigated the association between vitamin D receptor (VDR) and susceptibility to type 2 diabetes mellitus (T2DM). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was conducted to examine single nucleotide polymorphisms (SNPs) of the rs1544410 (*Bsm*I, G>A), rs757343 (*Tru*9I, G>A), rs731236 (*Taq*I, T>C), and rs739837 (*Bgl*I, G>T) loci of the VDR gene in 334 healthy individuals (Hui 115, Han 219) and 355 T2DM patients (Hui 154, Han 201) living in the Ningxia Hui Autonomous Region of China. The genotypic frequency and allelic frequency distributions in the VDR gene showed no significant difference between T2DM patients and controls in the Chinese Hui population. However, statistical differences in the genotypic frequency at rs739837 and in the genotypic and allelic frequencies

 

at rs1544410 were observed between T2DM patients and controls in the Chinese Han population ($P < 0.05$). Patient-control haplotype analyses using the SHEsis online haplotype analysis software showed that the G allele frequency of rs1544410 in the T2DM group was higher than that in the control group [odds ratio (OR) = 1.738, 95% confidence interval (CI) = 1.055-2.865], suggesting that the G allele is a risk factor of T2DM in the Chinese Han population. The frequency of haplotype GGCT between cases and controls was significantly different in both Chinese Hui [OR (95%CI) = 4.714 (1.04-21.36)] and Han populations [OR (95%CI) = 1.723 (1.03-2.883)] ($P < 0.05$), implying that the haplotype GGCT of the VDR gene is associated with susceptibility to T2DM in these ethnicities.

**Key words:** Vitamin D receptor; Polymorphism; Type 2 diabetes mellitus; Chinese Hui population; Chinese Han population

## INTRODUCTION

Diabetes mellitus (DM) is a major health problem worldwide. It is estimated that the DM population will increase to 300 million by the year 2025, with type 2 diabetes mellitus (T2DM) accounting for most of these cases (King et al., 1998). The global burden of T2DM in 2010 was 285 million people, which is projected to increase by 65% to 438 million by 2030 (Raza et al., 2013), particularly in China. The epidemic of T2DM is considered to be a major public health problem, with increased risks of cardiovascular disease, kidney failure, blindness, neuropathy, and peripheral circulatory disease. Importantly, its prevalence is predicted to rapidly increase over the next decade owing to human longevity and the surge in obesity in many countries, including China (Dou et al., 2013).

T2DM is a chronic, complex, and life-long disease with a strong genetic component, which has a significant impact on quality of life, and increases the morbidity and mortality of other diseases (Liu et al., 2013). Although it is well-known that insulin resistance plays an important role in the development of T2DM, the pathogenesis of T2DM is generally considered to result from interactions between multiple susceptibility genes, as well as from interactions between genes and the environment (Mayer et al., 1996; Edwards et al., 1997; Fu et al., 2013; Li et al., 2013). Many genes, such as the vitamin D receptor gene (*VDR*; NM_001017535; GI: 7421), were shown to be involved in susceptibility to T2DM in various populations since the VDR is functionally involved in the metabolic pathway of T2DM (Ebers, 2008; Pugliatti et al., 2008; Tardieu and Mikaeloff, 2008).

Vitamin D deficiency has been associated with a number of diseases, including multiple sclerosis (Ebers, 2008; Pugliatti et al., 2008; Tardieu and Mikaeloff, 2008), rheumatoid arthritis (RA) (Maalej et al., 2005; Ghelani et al., 2011), type 1 diabetes (Fassbender et al., 2002; Audi et al., 2004; De Azevêdo Silva et al., 2013), T2DM (Boucher, 2002; Cyganek et al., 2006; Dilmec et al., 2010; Frederiksen, et al., 2013), and cancers (Garcia-Quiroz et al., 2012; Singh et al., 2013; Grant et al., 2013; Shahbazi et al., 2013), although inconsistent results have been reported among different groups. The VDR gene, which is located

on chromosome 12 (12q13.11), consists of 11 exons and spans 63,495 base pairs (bp), has long been a candidate gene involved in the pathogenesis of T2DM. This gene functionally mediates most of the effects of vitamin D on gene expression by forming a heterodimer with the retinoid X receptor (RXR) and binding to the promoter regions of many target genes (Uitterlinden et al., 2004). Most VDR gene polymorphisms have been found to be in the 3' untranslated region (3'UTR) of the VDR gene, such as the *Bsm*I, *Apa*I, and *Taq*I restriction fragment length polymorphisms (RFLPs) (Uitterlinden et al., 2004). Several observational studies have reported the association between VDR polymorphisms and T2DM, fasting glucose, glucose intolerance, insulin sensitivity, insulin secretion, and calcitriol levels (Uitterlinden et al., 2004). These findings clearly suggest that *VDR* is a novel candidate gene for both type I and type II diabetes, in which *VDR* polymorphisms may play a role in the pathogenesis of type 2 diabetes by influencing the secretory capacity of β cells. Because of the genetic variation among different populations, such as the association between polymorphisms of triosephosphate isomerase (TIM) genes with the susceptibility to RA in the Chinese Hui ethnicity (Xu et al., 2011; 2012a,b), the aim of the study was therefore to investigate whether single nucleotide polymorphisms (SNPs) at the rs1544410 (*Bsm*I, G>A), rs757343 (*Tru*9I, G>A), rs731236 (*Taq*I, T>C), and rs739837 (*Bgl*I, G>T) sites of the VDR gene are associated with the susceptibility to T2DM in Chinese Hui and Han populations living in the Ningxia Hui Autonomous Region of China.

## MATERIAL AND METHODS

### Subjects

Blood samples were taken from 201 Chinese Han patients with T2DM (124 male, 77 female) and 219 ethnically matched non-T2DM controls (131 male, 88 female) of the Chinese Han population, and from 154 Chinese Hui patients with T2DM (86 male, 68 female) and 115 ethnically matched non-T2DM controls (76 male, 39 female); patients were consecutively enrolled from the outpatient clinic of the Affiliated Hospital of Ningxia Medical University. Clinical variables were obtained for each patient, including age, alcohol consumption, body mass index (BMI), height, weight, cigarette smoking, family history, etc. Patients with overnight fasting plasma glucose (FPG) levels higher than 126 mg/dL on two consecutive events were included in the T2DM category, while patients with fasting blood glucose (FBG) levels below 110 mg/dL without a family history of diabetes were included in the study as controls. Non-T2DM controls were recruited from the general Han or Hui population and had undergone comprehensive medical screening at the hospital. All subjects were included in this study based on two criteria: pure Han or Hui descent for at least three generations and had individual ancestors living in the Ningxia Hui Autonomous Region of China for at least three generations. There was no genetic relationship among any individuals involved in the study. All samples were collected under informed consent.

### SNP selection and genotyping

Genomic DNA of leukocytes from peripheral blood was extracted using a Wizard

Genomic DNA purification kit following manufacturer instructions (Promega; Madison, WI, USA). SNPs at the rs1544410 (*Bsm*I, G>A), rs757343 (*Tru*9I, G>A), rs731236 (*Taq*I, T>C), and rs739837 (*Bgl*I, G>T) loci of the VDR gene were examined in this study. Genotyping was carried out by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) analysis. Primer sets used for PCR and the restriction endonucleases used for digestion are listed in Tables 1 and 2. PCR products were purified by using a PCR purification kit, followed by digestion with the restriction endonucleases *Bsm*I, *Tru*9I, *Taq*I, and *Bgl*I, before they were resolved on 2% agarose gel containing ethidium bromide (Table 2). The PCR reaction kit, PCR purification kit, and restriction endonucleases were purchased from Takara (Osaka, Japan).

Genotypes were scored blindly, and analysis of all ambiguous samples was repeated. All samples were examined twice to ensure the accuracy of the results.

**Table 1.** Primer sets used for amplifying the four polymorphisms in the VDR gene.

| Polymorphisms position | Primer sequence | Annealing (°C) |
|---|---|---|
| rs1544410 (G>A) | F: 5'-ATAAGGAAATACCTACTTTGCTGGTTT-3' | 58 |
| rs757343 (G>A) | R: 5'-TAGGTGCTCAATAAATTGTTGCTAAG-3' | |
| rs731236 (T>C) | F: 5'-CAGAGCATGGACAGGGAGCAA-3' | 65 |
| | R: 5'-GCAACTCCTCATGGCTGAGGTCTC-3' | |
| rs739837 (G>T) | F: 5'-GCAGGGCCTTGCCCA-3' | 64.5 |
| | R: 5'-CACTAGGCGCTGGACAAGC-3' | |

**Table 2.** Determination of the VDR genotypes by a PCR-RFLP assay.

| SNP | Size of PCR product (bp) | Restricted enzyme | Fragments of RFLP (bp) | Genotype |
|---|---|---|---|---|
| rs1544410 (G>A) | 580 | *Bsm*I | 580, 414,166 | GA |
| | | | 414,166 | GG |
| | | | 580 | AA |
| rs757343 (G>A) | 580 | *Tru*9I | 580, 322, 258 | GA |
| | | | 322, 258 | AA |
| | | | 580 | GG |
| rs731236 (T>C) | 745 | *Taq*I | 494, 293, 251, 201 | TC |
| | | | 293, 251, 201 | TT |
| | | | 494, 251 | CC |
| rs739837 (G>T) | 149 | *Bgl*I | 149, 110, 39 | GT |
| | | | 110, 39 | GG |
| | | | 149 | TT |

## Statistical analysis

Genotype and allele carrier frequencies were defined as the percentage of individuals carrying the genotype and allele of the total number of individuals, respectively. The $c^2$ and Fisher exact tests of SPSS 17.0 for Windows (SPSS Inc.; Chicago, IL, USA) were used to test for deviations from Hardy-Weinberg equilibrium (HWE). Comparisons of the frequency of discrete variables between T2DM patients and control individuals were evaluated by a 2-tailed $c^2$ test, and a Bonferoni corrected P value threshold was employed for correction of type I error. For T2DM patient-control haplotype analyses, the SHEsis online haplotype analysis

software (http://analysis.bio-x.cn/myAnalysis.php) was employed. A P value <0.05 was considered to be statistically significant.

## RESULTS

The four SNPs of the VDR gene have been investigated for their association with susceptibility to T2DM in several populations (Boucher, 2002; Ferrarezi et al., 2013). To determine whether these SNPs are also associated with susceptibility to T2DM in the Chinese Hui and Han ethnicities living in the Ningxia region, we analyzed polymorphisms in the VDR gene at the rs1544410 (*Bsm*I, G>A) (Figure 1A), rs757343 (*Tru*9I, G>A) (Figure 1B), rs731236 (*Taq*I, T>C) (Figure 1C), and rs739837 (*Bgl*I, G>T) (Figure 1D) sites in 334 Ningxia healthy individuals (Hui 115, Han 219) and 355 patients with T2DM (Hui 154, Han 201) (Figure 1 A-D). None of the genotype distributions for all SNPs showed deviations from HWE proportions. Polymorphic analysis revealed that the genotype AA at rs1544410 (G>A) and rs757343 (G>A), and genotype TT at rs731236 (T>C) and rs739837 (G>T) were either undetectable or very infrequent in both T2DM patients and controls. No statistical difference in the distribution of either VDR genotypes or alleles was detected between T2DM patients and controls in the Chinese Hui population (Table 3). Interestingly, the genotypic frequency distribution at the rs739837 site and the genotypic and allelic frequencies at the rs1544410 site showed significant differences between T2DM patients and controls in the Chinese Han population living in the Ningxia region ($P < 0.05$, Table 4); however, no difference was found for the other SNP sites evaluated in this study. The frequency of allele G in rs1544410 (G>A) in the T2DM group (93.5%) was higher than that in the control group (89.3%), with an odds ratio (OR) [95% confidence interval (CI)] of 1.738 (1.055-2.865). In contrast, allele A in the rs1544410 site was found to be a protective factor for T2DM with a higher frequency in the control group (10.7%) compared to the patient group (6.5%), with an OR (95%CI) of 0.575 (0.349-0.948) (Tables 3 and 4). This result suggests that allele G might be a risk factor for T2DM, and allele A of this SNP may be a protective factor against T2DM in the Chinese Han population (Table 4).

The haplotypes of the VDR gene were further analyzed using the SHEsis Online haplotype analysis software. Twelve and 16 haplotypes were detected in Hui and Han samples, respectively. In all samples, the haplotype GGCG was observed most frequently in both populations, with 65.7 and 65.2% in the patient group and control group of the Hui population, respectively (underlined in Table 5), and 60.9 and 63.0 % in the patient group and control group in the Han population, respectively (underlined in Table 6). GACT was the second most frequently observed haplotype, with 17.2% and 19.4% observed in the patient group and control group in the Hui population, respectively, and 18.8% and 17.6% in the patient group and control group in the Han population, respectively (Tables 5 and 6). In addition, the frequency of haplotype GGCT was significantly different between cases (Hui, 4.0% and Han, 10.0%) and control groups (Hui, 0.9% and Han, 6.1%), with an OR (95%CI) of 4.714 (1.04-21.36) in the Hui population and 1.723 (1.03-2.883) in the Han population ($P < 0.05$). These findings suggest that the haplotype GGCT of the VDR gene is associated with susceptibility to T2DM in the Chinese Hui and Han ethnicities living in the Ningxia region (Tables 5 and 6).

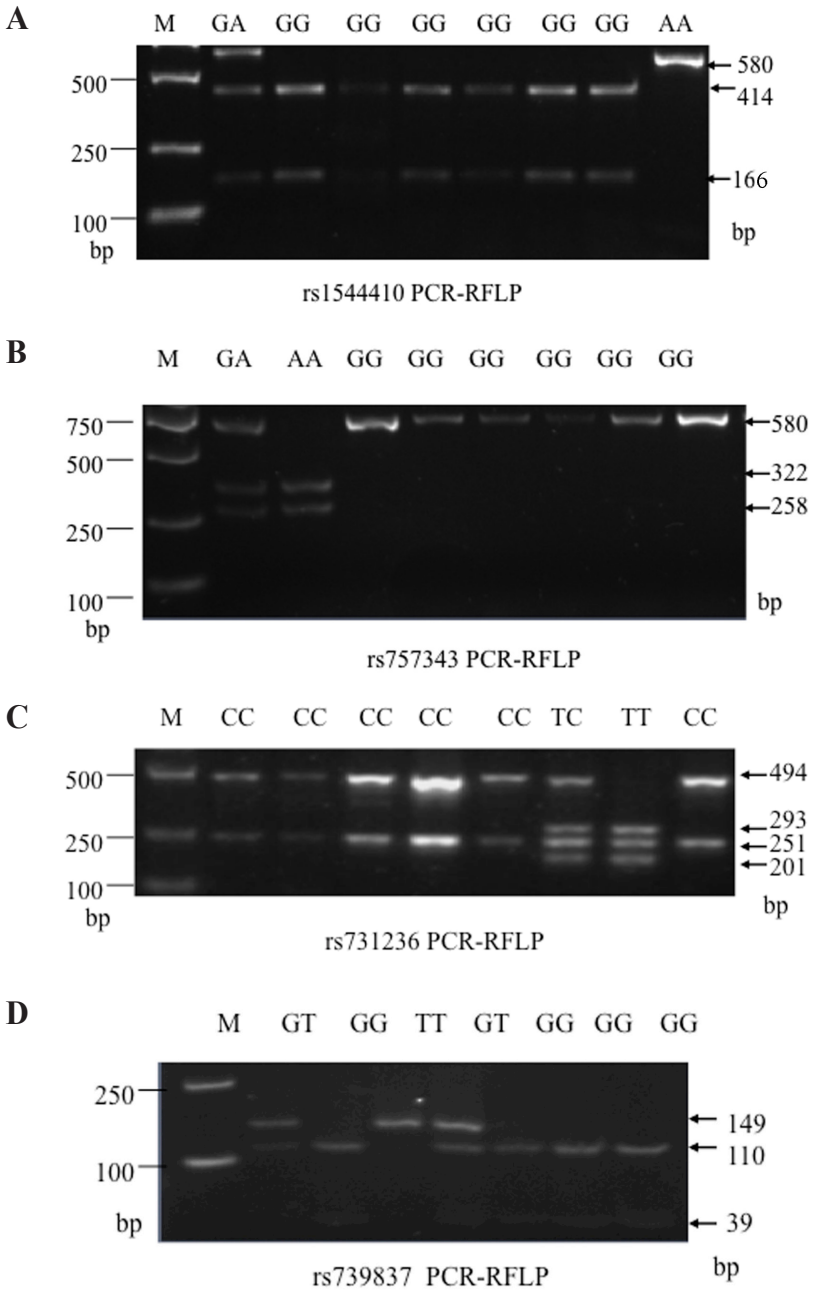


**Figure 1.** Genotype analyzed by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The PCR amplified products were digested with **A** = rs1544410 (G>A), **B** = rs757343 (G>A), **C** = rs731236 (T>C) and **D** = rs739837 (G>T) before they were resolved in an agarose gel and visualized by UV light. *Lane M* = DNA molecular ladders. The remaining lanes indicate the corresponding genotypes labeled at the top of each picture.

**Table 3.** Genotype and allele analysis of the polymorphisms of the VDR gene in T2DM patients and control of Chinese Hui population.

| Position | Genotype/allele | Control (N, %) | T2DM (N, %) | $\chi^2$ | P | OR (95%CI) |
|---|---|---|---|---|---|---|
| rs1544410 | AA | 0 (0) | 2 (1.3) | 2.325 | 0.313 | - |
| | GA | 28 (24.3) | 30 (19.5) | - | - | - |
| | GG | 87 (75.5) | 122 (79.2) | - | - | - |
| | A | 28 (12.2) | 34 (11.0) | 0.166 | 0.683 | 1.117 (0.656-1.902) |
| | G | 202 (87.8) | 274 (89.0) | - | - | 0.895 (0.526-1.524) |
| rs757343 | GG | 67 (58.3) | 99 (64.3) | 2.634 | 0.268 | - |
| | GA | 42 (36.5) | 52 (33.8) | - | - | - |
| | AA | 6 (5.2) | 3 (1.9) | - | - | - |
| | G | 176 (76.5) | 250 (81.2) | 1.725 | 0.189 | 0.756 (0.498-1.148) |
| | A | 54 (23.5) | 58 (18.8) | - | - | 1.322 (0.871-2.008) |
| rs731236 | CC | 99 (86.1) | 134 (87.0) | 2.690 | 0.261 | - |
| | TC | 16 (13.9) | 17 (11.0) | - | - | - |
| | TT | 0 (0) | 3 (2.0) | - | - | - |
| | C | 214 (93.0) | 285 (92.5) | 0.051 | 0.821 | 1.079 (0.557-2.093) |
| | T | 16 (7.0) | 23 (7.5) | - | - | 0.926 (0.478-1.796) |
| rs739837 | TT | 8 (7.0) | 13 (8.4) | 0.259 | 0.879 | - |
| | GT | 53 (46.1) | 72 (46.8) | - | - | - |
| | GG | 54 (47.0) | 69 (44.8) | - | - | - |
| | T | 69 (30.0) | 98 (31.8) | 0.203 | 0.652 | 0.918 (0.634-1.330) |
| | G | 161 (70.0) | 210 (68.2) | - | - | 1.089 (0.752-1.577) |

**Table 4.** Genotype and allele analysis of the polymorphisms of the VDR gene in T2DM patients and control in Chinese Han population.

| Position | Genotype/allele | Control (N, %) | T2DM (N, %) | $\chi^2$ | P | OR (95%CI) |
|---|---|---|---|---|---|---|
| rs1544410 | AA | 0 (0) | 1 (0.5) | 7.739 | 0.021 | |
| | GA | 47 (21.5) | 24 (11.9) | | | |
| | GG | 172 (78.5) | 176 (87.6) | | | |
| | A | 47 (10.7) | 26 (6.5) | 4.8 | 0.028 | 0.575 (0.349-0.948) |
| | G | 391 (89.3) | 376 (93.5) | | | 1.738 (1.055-2.865) |
| rs757343 | GG | 145 (66.2) | 124 (61.7) | 6.233 | 0.044 | |
| | GA | 60 (27.4) | 72 (35.8) | | | |
| | AA | 14 (6.4) | 5 (2.5) | | | |
| | G | 350 (79.9) | 320 (79.6) | 0.012 | 0.912 | 0.981 (0.701-1.374) |
| | A | 88 (20.1) | 82 (20.4) | | | 1.019 (0.728-1.427) |
| rs731236 | CC | 188 (87.9) | 182 (90.5) | 1.510 | 0.47 | |
| | TC | 25 (11.7) | 19 (9.5) | | | |
| | TT | 1 (0.5) | 0 (0) | | | |
| | C | 401 (93.7) | 383 (95.3) | 0.991 | 0.319 | 1.357 (0.742-2.481) |
| | T | 27 (6.3) | 19 (4.7) | | | 0.737 (0.403-1.347) |
| rs739837 | TT | 23 (15.5) | 35 (17.4) | 0.216 | 0.898 | |
| | GT | 55 (37.2) | 73 (36.3) | | | |
| | GG | 70 (47.3) | 93 (46.3) | | | |
| | T | 101 (34.1) | 143 (35.6) | 0.158 | 0.691 | 1.066 (0.778-1.461) |
| | G | 195 (65.9) | 259 (64.4) | | | 0.938 (0.684-1.286) |

## DISCUSSION

The Chinese Hui minor ethnicity is one of 56 nationalities and includes over 12 million people in China. The Hui are descended from Arabic and Persian merchants who came to China during the 7th century, with most of the group living in the Ningxia Hui Autonomous Region. To retain religious purity and group identity, the Hui have always segregated themselves socially from other groups, living in enclaves. Hui marriage practices tend toward endogamy in all respects, particularly in the rural part of the Ningxia Hui Autonomous Region,

**Table 5.** Haplotype analysis of the four VDR SNPs in T2DM patients and controls in Chinese Hui population.

| Haplotypes* | | | | Frequency[a] (%) | | $\chi^2$ | P[b] | OR[c] (95%CI) |
|---|---|---|---|---|---|---|---|---|
| | | | | T2DM | Control | | | |
| A | A | T | T | 0 | 0.8 | - | - | - |
| A | A | C | T | 1.6 | 2.1 | - | - | - |
| A | G | T | T | 5.4 | 4.7 | 0.112 | 0.737 | 1.143 (0.522-2.503) |
| A | G | C | T | 2.2 | 1.4 | - | - | - |
| A | G | C | G | 1.8 | 3.2 | 1.14 | 0.284 | 0.547 (0.178-1.677) |
| G | A | T | T | 0 | 0.3 | - | - | - |
| G | A | C | T | 17.2 | 19.4 | 0.466 | 0.494 | 0.857 (0.549-1.336) |
| G | A | C | G | 0 | 1.0 | - | - | - |
| G | G | T | T | 1.4 | 0.5 | - | - | - |
| G | G | T | G | 0.7 | 0.7 | - | - | - |
| G | G | C | T* | 4.0 | 0.9 | 4.876 | 0.027 | 4.714 (1.040-21.360) |
| G | G | C | G | 65.7 | 65.2 | 0.000 | 0.990 | 0.999 (0.680-1.467) |

*The order of SNPs in haplotypes: rs1544410, rs757343, rs731236, and rs739837. [a]Values were constructed by EM algorithm with genotype SNPs, [b]Values were analyzed by permutation test. [c]Values were analyzed by c2 test from 2 x 2 contingency table.

**Table 6.** Haplotype analysis of the four VDR SNPs in T2DM patients and controls in Chinese Han population.

| Haplotypes* | | | | Frequency[a] (%) | | $\chi^2$ | P[b] | OR[c] (95%CI) |
|---|---|---|---|---|---|---|---|---|
| | | | | T2DM | Control | | | |
| A | A | T | T | 0 | 0.2 | - | - | - |
| A | A | T | G | 0 | 0.2 | | | |
| A | A | C | T | 0 | 0.1 | - | - | - |
| A | A | C | G | 0 | 0.8 | | | |
| A | G | T | T | 2.1 | 4.2 | 2.756 | 0.09 | 0.503 (0.220-1.149) |
| A | G | T | G | 0 | 0.7 | | | |
| A | G | C | T | 2.7 | 1.6 | - | - | - |
| A | G | C | G | 1.6 | 3.2 | 2.237 | 0.134 | 0.492 (0.19-1.27) |
| G | A | C | T | 18.8 | 17.6 | 0.247 | 0.618 | 1.095 (0.767-1.563) |
| G | A | C | G | 1.2 | 1.1 | - | - | - |
| G | G | T | T | 1.8 | 1.0 | - | - | - |
| G | G | C | T* | 10.0 | 6.1 | 4.378 | 0.036 | 1.723 (1.03-2.883) |
| G | G | C | G | 60.9 | 63.0 | 0.263 | 0.608 | 0.925 (0.687-1.246) |
| G | A | T | T | 0.1 | 0 | | | |
| G | A | T | G | 0.3 | 0 | | | |
| G | G | T | G | 0.4 | 0 | | | |

*The order of SNPs in haplotypes: rs1544410, rs757343, rs731236, and rs739837. [a]Values were constructed by EM algorithm with genotype SNPs. [b]Values were analyzed by permutation test. [c]Values were analyzed by c2 test from 2 x 2 contingency table.

and the Hui population is culturally and religiously conservative. Our previous studies showed that the Ningxia Hui ethnicity has a distinct genetic background that shows differences in susceptibility to RA (Xu et al., 2011; 2012a,b).

There are more than 2.2 million Chinese Hui currently living in the Ningxia Hui Autonomous Region of China (Ningxia Hui population). The incidence of DM in the Ningxia Hui population is as high as 20%, which is higher than that in the Chinese Han population in this region. In this study, we first reported an association between VDR polymorphisms and susceptibility to T2DM in the Hui ethnicity, and compared allele frequencies of these SNPs with those in the Chinese Han population living in the same environment. Our results revealed genetic variation in the VDR gene that contributes to the susceptibility to T2DM between these populations.

VDR is encoded by a large gene (>100 kb), which contains 14 exons and is located on chromosome 12cen-q12 (Taymans et al., 1999). Several polymorphisms have been identified in various introns and exons of the VDR gene; *Apa*I, *Bsm*I, and *Fok*I SNPs are the most common (Baan et al., 2004). These polymorphisms are reportedly associated with both altering circulating levels of active vitamin D and *in vitro* measures of gene expression (Morrison et al., 1992). The *Bsm*I (rs1544410) and *Apa*I (rs7975232) polymorphisms are located in intron 8 at the 3'-end of VDR gene and are considered to be silent polymorphisms that do not change the amino acid sequence of the encoded protein. However, these two polymorphisms may affect gene expression by regulating mRNA stability (Jurutka et al., 2001). The *Fok*I (rs2228570) polymorphism is located at the 5'-end of the VDR gene. This polymorphism generates an alternative transcription initiation site, leading to a protein variant with three additional amino acids at the amino terminus (Arai et al., 1997). *Bsm*I (rs1544410), *Apa*I (rs7975232), and *Taq*I (rs731236) are located at the 3'-UTR of the VDR gene, which are SNPs linked to each other with high linkage disequilibrium. These SNPs do not affect the VDR protein structure, but may affect the stability of VDR mRNA or may be in high linkage disequilibrium with other functional SNPs (Jurutka et al., 2001).

Increasing evidence suggests that genetic factors are key risk factors of susceptibility to T2DM in various ethnicities, with the list of risk loci for T2DM continually expanding. In the present study, we analyzed rs1544410 (*Bsm*I, G>A), rs757343 (*Tru*9I, G>A), rs731236 (*Taq*I, T>C), and rs739837 (*Bgl*I, G>T) in the human VDR gene in the Chinese Hui and Han populations in the Ningxia region. In agreement with results in the Turkish population (Dilmec et al., 2010) and in the Polish population (Cyganek et al., 2006), *Taq*I and *Bsm*I polymorphisms were not associated with T2DM susceptibility in the Ningxia Hui population. However, individuals carrying the G allele of rs1544410 were at risk for T2DM in the Chinese Han population, while those carrying the A allele of rs1544410 of the VDR gene were less susceptible to T2DM in this population. These results suggest that genetic variations of the VDR gene contribute to T2DM susceptibility among different populations. This finding is consistent with those of previous studies (Barletta et al., 2002; Cyganek et al., 2006; Frederiksen et al., 2013). The AA/AG genotype of the VDR rs1544410 locus is reportedly associated with a lower risk of type 1 diabetes in Diabetes Autoimmunity Study in the Young (DAISY) children (Frederiksen et al., 2013). However, no correlation between *Fok*I, *Apa*I, *Bsm*I, and *Taq*I SNPs in the VDR gene and susceptibility to T2DM was observed in a Polish population (Cyganek et al., 2006). Similar to our finding in the Chinese population evaluated in this study, Barletta et al. (2002) demonstrated that the *Apa*I and *Bsm*I polymorphisms in the VDR gene may associated with glucose intolerance independently of defective insulin secretion and insulin resistance in a non-diabetic Caucasian population. Although differences were observed for the association between a single SNP in the VDR gene and T2DM between the Ningxia Hui and Han populations, haplotype analysis showed a comparable result that the haplotype GGCT of the VDR gene may be associated with susceptibility to T2DM in populations living in the Ningxia region, suggesting that haplotype analysis is more powerful than analysis of one polymorphism.

In summary, our results revealed genetic variation in the VDR gene, demonstrating an association between polymorphisms and susceptibility to T2DM in the Chinese Hui and Han populations in the Ningxia region of China. SNPs at the rs739837 and rs1544410 loci of the VDR gene were associated with susceptibility to T2DM in the Chinese Han population but

not in the Chinese Hui ethnicity in this region. In addition, the G allele of the rs1544410 locus may be a risk factor of T2DM in the Chinese Han population. Haplotype analysis further demonstrated that the frequency of haplotype GGCT was significantly different between T2DM patients and controls in both ethnicities.

## Conflicts of interest

The authors declare no conflict of interest.

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