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Association of vitamin D receptor gene polymorphisms in Chinese patients with generalized aggressive periodontitis

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Background and Objective: The clinical features suggest that genetic factors may have a strong influence on susceptibility to aggressive periodontitis. The aim of this study was to investigate the association of vitamin D receptor gene polymorphisms with generalized aggressive periodontitis in Chinese patients.

Material and Methods: A restriction fragment length polymorphism (RFLP) for 10,438,141 C to T (rs1544410, *Bsm*I), 10,382,063 A to G (rs731236, *Taq*I), 10,382,143 C to A (rs7975232, *Apa*I) and 10,416,201 A to G (rs2228570, *Fok*I) of vitamin D receptor gene was analysed by polymerase chain reaction, followed by digestion with restriction enzymes and gel electrophoresis. The genotypes of 51 generalized aggressive periodontitis patients and 53 periodontally healthy control subjects were analysed. The genotypic and allelic frequencies of each polymorphism site for the patients and control subjects were compared.

Results: The distribution of vitamin D receptor *Fok*I genotypes and alleles between the two groups was significantly different ($p = 0.043$ and $p = 0.012$, respectively). The F allele seemed to increase the susceptibility of aggressive periodontitis (odds ratio = 2.02, 95% confidence interval = 1.16–3.50) in Chinese patients. There was no significant difference in the genotype distribution or the allele frequencies of vitamin D receptor *Bsm*I, *Apa*I and *Taq*I between two groups.

Conclusion: The study indicates that *Fok*I polymorphism of vitamin D receptor gene might be associated with generalized aggressive periodontitis in Chinese patients. In addition, the carriage of F allele increases the risk of developing generalized aggressive periodontitis.

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Generalized aggressive periodontitis (GAgP) represents a group of inflammatory diseases with common pathogenic features, such as onset primarily during early adult years and generalized rapid destruction of periodontal tissue, leading to loss of teeth. The aetiology of GAgP involves complex interactions between exogenous factors, in particular periodontal bacteria, and genetic factors (1). Although periodontopathogenic bacteria may indeed constitute an initiator of the condition, its clinical features have indicated that genetic factors may have a strong influence on susceptibility to GAgP.

Vitamin D has special roles in regulating the metabolism of calcium and phosphorus, and in the immune system. The vitamin D receptor (VDR) is involved in a variety of biological

processes, including bone metabolism and the modulation of the immune response. It is clear that mutations in functionally critical areas of the VDR gene can have profound effects on mineral metabolism and bone mineral density (2,3). Vitamin D receptor is also an important nuclear receptor of vitamin D_3, which is known to be essential for the maintenance of mineral homeostasis and for bone structural integrity. Vitamin D_3 and its receptor have also been demonstrated to act as upregulating agents during innate immunity (4). Vitamin D receptor mediates these effects via a variety of mechanisms, including transcription regulation, mRNA stability and post-translational modifications.

The aim of this study was to determine the prevalence of *Bsm*I, *Apa*I, *Taq*I and *Fok*I VDR genotypes in Chinese GAgP patients and to evaluate the association of VDR gene polymorphisms with GAgP.

Materials and methods

Subjects

A total of 51 patients with GAgP were recruited from the periodontic clinics at the Affiliated Stomatology Hospital, Nanjing University. The GAgP patient group contained 28 males and 23 females, with an age range from 16 to 49 years (mean 35.7 years). Generalized aggressive periodontitis was diagnosed on the basis of clinical attachment loss and radiographic patterns of alveolar bone loss, using the criteria defined by the American Academy of Periodontology (5). The main clinical criteria of GAgP patients recruited in this study were a clinical attachment loss of at least 6 mm or alveolar bone resorption of one-third of the length of the root, affecting at least 10 teeth, four or more of which were not incisors or first molars. In addition, 53 healthy control subjects with no previous or existing periodontal disease were selected from the volunteers. The healthy control subjects ranged in age from 24 to 45 years (mean 39.1 years). All subjects were Chinese Han nationality, and subjects with a history of systemic disease or smoking were excluded from the study. The nature of the study was explained to all subjects verbally and in writing and all signed a content form.

Sample collection and DNA extraction

Buccal swab samples were taken from each subject. The DNA was extracted from the buccal swab samples with a buccal swab DNA extraction reagent (Tiangen, Beijing, China), following the manufacturer's instructions. The concentration of each DNA sample was determined by ultraviolet spectrophotometry.

Genotype analysis

Four single-nucleotide polymorphisms (SNPs) of the VDR gene were analysed in this study, at the following positions (according to GenBank accession no. NT_029419): 10,438,141 C to T (rs1544410, *Bsm*I), 10,382,063 A to G (rs731236, *Taq*I), 10,382,143 C to A (rs7975232, *Apa*I) and 10,416,201 A to G (rs2228570, *Fok*I). The four sites were analysed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR reaction primers were designed by Primer Premier 5 (Premier Biosoft International, Palo Alto, CA, USA). All primers and their amplifying fragment length are shown in Table 1.

Reaction conditions and cycling parameters were as follows: 200 ng of genomic DNA was used for PCR amplification in a reaction mixture containing 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 1.5 μM of each primer, reaction buffer and 2.5 U Taq polymerase. The amplification consisted of 94°C for 4 min, 35 cycles of 94°C for 45 s, 55°C for 45 s (*Bsm*I, 58°C for *Taq*I and *Apa*I; 60°C for *Fok*I), 72°C for 45 s, and a final extension of 72°C for 5 min,

Ten microlitres of the four PCR fragments generated were subjected to restriction enzyme digestion using *Bsm*I, *Taq*I, *Apa*I and *Fok*I restriction endonuclease, respectively. The digested PCR products were electrophoresed on a 2% gelose gel and were stained with ethidium bromide to visualize DNA fragments.

Statistical methods

The genotypic distributions of the four SNP sites were all tested for Hardy–Weinberg equilibrium by using Stata 9.0. The distributions of genotypes and allele frequencies in the disease and control groups were compared using the chi-squared test. The risk associated with individual alleles or genotypes was calculated as the odds ratio (OR) with 95% confidence intervals (95% CI). Statistical significance in all tests was determined at $p < 0.05$.

Results

The genotype distributions of all four SNP sites were in accordance with Hardy–Weinberg equilibrium for the control subjects and patients. The PCR-RFLP results showed that no BB or tt genotype was detected in any subject. Genotypic and allelic frequencies involving VDR *Bsm*I, *Taq*I and *Apa*I evidenced no differences between the GAgP patients and the control subjects (Table 2).

There was, however, significant difference in the distribution of VDR *Fok*I genotypes and the allelic frequencies between the GAgP group and healthy control group. The detected frequency of FF genotype was signifi-

Table 1. The primers for analysing SNPs of the VDR gene (according to GenBank accession no. NT_029419)

SNP	Primers	Fragment
*Bsm*I	5′-ATACCTACTTTGCTGGTTTGC-3′ 5′-AGCCCATCTCCATTCCTTG-3′	500 bp
*Taq*I, *Apa*I	5′-GGAGAAGTCACTGGAGGGC-3′ 5′-GGATCATCTTGGCATAGAGC-3′	307 bp
*Fok*I	5′-AGCTGGCCCTGGCACTGACTCTGGCTC-3′ 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′	267 bp

Table 2. Distribution of VDR genotypes and alleles in GAgP patients and control subjects

SNP	Control subjects (%)	GAgP patients (%)	χ^2	OR
*Bsm*I				
Genotype N	$n = 53$	$n = 51$		
BB	0 (0)	0 (0)	—	—
Bb	4 (7, 55)	3 (5, 88)	$\chi^2 = 0.11$	—
bb	49 (92,45)	48 (94 12),	$p = 0.73$	—
Allele N	$n = 106$	$n = 102$		
B	4 (3, 77)	3 (2, 94)	$\chi^2 = 0.11$	—
b	102 (96,23)	99 (97 06),	$p = 0.74$	—
*Taq*I				
Genotype N	$n = 53$	$n = 51$		
TT	46 (86, 79)	45 (88,24)	—	—
Tt	7 (13,21)	6 (11, 76)	$\chi^2 = 0.05$	—
tt	0 (0)	0 (0)	$p = 0.82$	—
Allele N	$n = 106$	$n = 102$		
T	99 (93,40)	96 (94 12),	$\chi^2 = 0.05$	—
t	7 (6, 60)	6 (5, 88)	$p = 0.83$	—
*Apa*I				
Genotype N	$n = 53$	$n = 51$		
AA	16 (30 19),	15 (29,41)	—	—
Aa	29 (54,72)	25 (49 02),	$\chi^2 = 0.76$	—
aa	8 (09,15)	11 (21, 57)	$p = 0.68$	—
Allele N	$n = 106$	$n = 102$		
A	61 (57,55)	55 (53,92)	$\chi^2 = 0.28$	—
a	45 (42,45)	47 (46 08),	$p = 0.60$	—
*Fok*I				
Genotype N	$n = 53$	$n = 51$		
FF	9 (16,98)	19 (37,25)	—	FF vs. (Ff + ff)
Ff	27 (50,94)	23 (45 10),	$\chi^2 = 6.32$	OR = 2.90
ff	17 (32 08),	9 (17,65)	$p = 0.04$	95% CI = 1.16–7.24
Allele N	$n = 106$	$n = 102$		F vs. f
F	45 (42,45)	61 (59,80)	$\chi^2 = 6.26$	OR = 2.02
f	61 (57,55)	41 (40 20),	$p = 0.01$	95% CI = 1.16–3.50

cantly higher in the GAgP group than in the healthy control subjects (37.25 vs. 16.98%, $p < 0.05$). The frequency of F allele was also significantly higher in the GAgP group (59.80 vs. 42.45%, $p < 0.05$). Individuals in the Chinese population with the F allele seemed to be more likely to develop GAgP (FF vs. Ff + ff, OR = 2.90, 95% CI = 1.16–7.24; F vs. f, OR = 2.02, 95% CI = 1.16–3.50; Table 2).

Discussion

Genetic polymorphisms which influence key biological processes may contribute to susceptibility to a variety of diseases, including periodontitis. In particular, the primary clinical feature of GAgP is rapid and severe irreversible alveolar bone loss. There is an increasingly prevalent point of view that periodontitis patients, especially GAgP patients, evidence more clearly defined genetic factors (6). Candidate genes which have been studied include genes for interleukin (IL)-1, IL-6 receptor, TNF-α, FcγR and MMP (7–11).

Genetic polymorphisms, such as SNPs, may influence disease in a complex way, acting with other genetic variants and environmental factors to influence disease susceptibility and progression. Many studies have revealed that certain SNPs may be associated with the susceptibility to aggressive periodontitis in Chinese population, including the IL-1A + 4845 and IL-1B −511 polymorphisms (12), TIMP2 −418 G to C gene polymorphism (11) and MMP-1 promoter region of −1607 bp (13).

In this study, the detected frequency of FF genotype was significantly higher in GAgP patients than in healthy control subjects. The frequency of allele F was also significantly higher in the GAgP group. No evidence indicated that VDR *Bsm*I, *Taq*I or *Apa*I polymorphisms were associated with GAgP in th Chinese population. These results suggest that the FF genotype might be a risk indicator for the susceptibility to GAgP of the Chinese population (OR = 2.90). Carriage of the more frequent allele F of VDR *Fok*I gene significantly increased the risk of developing GAgP (OR = 2.02). A study of the Korean population by Park came to a similar conclusion (14). In their study, they found that the VDR *Fok*I FF genotype was associated with an increased risk for GAgP (OR = 1.83), but the VDR *Bsm*I and *Taq*I polymorphisms were not associated with GAgP. In another study of a Japanese population, they found that VDR *Bsm*I polymorphism was not associated with generalized early onset periodontitis (G-EOP) either (15). There were, however, different conclusions in some other studies of Asian aggressive periodontitis (or early onset periodontitis, EOP) patients. In studies by Sun and Tachi, they found that VDR *Taq*I Tt genotype might be a risk indicator for the susceptibility to aggressive periodontitis of Chinese patients (16,17). A possible explanation for these different findings was the observed differences in the disease phenotype. There might also be variations in the frequency of the VDR minor allele in different Asian populations.

The human VDR is a ligand-regulated transcription factor that mediates the actions of the 1,25-dihydroxyvitamin D_3 hormone to effect bone mineral homeostasis. The VDR mediates the hormonal function of vitamin D and regulates a variety of downstream functions. Vitamin D receptor *Bsm*I and *Apa*I polymorphisms in this study are located at intron 8 and VDR *Taq*I polymorphism is located at extron 9, but they are synonymous mutations. None of the three SNPs can lead to a change of the protein structure. This might be the key reason why there was no association between the VDR *Bsm*I, *Apa*I and *Taq*I SNPs and GAgP. In addition, the VDR gene contains two potential translation initiation (ATG) sites. The *Fok*I polymorphism, which

occurs at the first start codon in exon 2, changes the nucleotide sequence to ACG (18). Alleles with this polymorphism initiate translation three codons downstream, resulting in a protein (424 amino acids, the F allele) that is three amino acids shorter than wild type. The short 424-amino-acid VDR interacted more efficiently transactivates with the transcription factor TFII B than the 427-amino-acid VDR *in vitro*, and the shorter VDR also transactivates vitamin D target genes more efficiently. The shorter VDR transmits stronger bone resorption and inflammation signals (19,20). We consider that is the main reason why VDR FF genotype is associated with an increased risk of GAgP.

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