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Association Study between Vitamin D Receptor Gene Polymorphism and Adult Periodontitis in Korean

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Adult periodontitis is a chronic inflammatory disease whose etiology is not well defined. Recent studies have shown that vitamin D receptor gene has been a candidate for the susceptibility of adult periodontitis. The purpose of this study is to investigate the frequency of *Taq* I restriction fragment length polymorphism (RFLP) in the vitamin D receptor gene in 141 periodontically healthy controls and 28 adult periodontitis patients. *Taq* I RFLP in the vitamin D receptor gene was detected by PCR amplification, followed by restriction enzyme digestion and 2% agarose gel electrophoresis. There was no significant difference in the distribution of *Taq* I RFLP between healthy controls and adult periodontitis group ($P > 0.05$). Thus, *Taq* I RFLP in the vitamin D receptor gene may not confer the susceptibility to adult periodontitis in Korean population. However, *t* allele distributions of this RFLP showed various frequencies among ethnic groups studied. Further studies in other ethnic groups will be required.

Adul: periodontitis is a chronic inflammatory disease affecting all populations. The main aetiology remains a bacterial infection that leads to gingival inflammation, loss of alveolar bone and tooth loss.

Bacteria are essential though probably insufficient to cause adult periodontitis. The Gram-negative dark-pigmented coccobacillus *Porphyromonas gingivalis* and the capnophylic Gram-negative rod *Actinobacillus actinomycetemcomitans* have been implicated as specific pathogens in periodontitis (Haffajee and Socransky, 1994). Cigarette smoking (Bergstrom, 1989) and diabetes mellitus (Thorstensson and Hugoson, 1993; Collin et al., 1998) are two well-documented risk factors for periodontitis. Furthermore, an individuals capacity to cope with stress has been associated with an increased severity of periodontal disease and has been suggested as a risk factor (Genco et al., 1999).

Although periodontal bacteria are the major etiological factors of this disease, there are many variations in the severity of disease among the patients who have similar stimulating factors. Many researchers have agreed that the susceptibility to periodontal disease was at least partially genetically determined (Seysour, 1991).

Periodontal disease and osteoporosis have some common pathological mechanisms involving disruption of bone homeostasis, and they may share the common genetic background. Genetic polymorphisms in genes which encode mediators of bone homeostasis have been shown to be associated with parameters of bone mineral density (BMD) and incidence of common disorders of bone metabolism in particular osteoporosis (Gross et al., 1996; Grant and Ralston, 1997; Morrison, 1997).

There are extensive studies on the associations between polymorphisms of vitamin D receptor gene and BMD. Especially, Morrison et al. (1994) have reported that polymorphisms of the vitamin D receptor gene account for 75% of the primary genetic factor for BMD. Genetic associations with early-onset periodontitis have been noted for a *Taq* I RFLP in exon 9 of the vitamin D receptor gene in Caucasian (Hennig et al., 1999) and Chinese (Sun et al., 2002) populations. However, few data have been available regarding the role of vitamin D receptor gene in adult periodontitis (Sun et al., 2002).

Case-control type association study requires pure ethnic group with homogeneous genetic component and similar environmental background. Korean population is unique in that they have a single language and a homogeneous population for a long time. To our knowledge, there is no report on the relationship between a genetic variation of vitamin D receptor gene and adult periodontitis in Korean population.

Thus, we investigated an association between *Taq*I RFLP of vitamin D receptor gene and adult periodontitis in ethnically homogeneous Koreans.

Materials and Methods

Study subjects

A total of 28 cases of adult periodontitis (21 male and 7

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female; age range 31~61 yr; mean age 48.0 yr) were recruited from Dr. Chois Dental Office, Seoul, Korea, and 141 healthy control subjects (72 male, 68 female and 1 unknown; age range 29~86 yr; mean age 56.3 yr) collected from Clinical Pathology, Seoul Hygiene Hospital, Seoul, Korea.

Biochemical assay

Blood samples were obtained in EDTA tubes from the subjects who had been fasting for 12~16 h. Serum concentrations of total cholesterol (TC), triglyceride (TG) and glucose were measured by enzymatic colorimetric methods with a commercial kit (Boehringer Mannheim) and chemistry analyzer. Serum high-density lipoprotein (HDL) cholesterol concentration was determined by measuring cholesterol in the supernatant after precipitation of the serum with $MgCl_2$ and dextran sulfate, with a Gilford Impact 400E automatic analyzer with reagents and calibrators from Boehringer Mannheim. Serum lipoprotein (a) concentration was measured by the immunoprecipitation method (SPQ test System, Incstar Corporation), and serum apolipoprotein AI concentration was determined by the immuno-turbidimetric method (Cobas Integra, Roche Diagnostics). Serum low density lipoprotein cholesterol concentration was calculated using the formula (Friedewald et al., 1972). White blood cell count, red blood cell count, hemoglobin level and hematocrit were measured by cell counter, and mean pocket depth was evaluated by several centists.

DNA analysis

Genomic DNA was extracted using Wizard® Genomic DNA purification kit (Promega) from whole blood (Bae et al., 2001; Kang et al., 2003). Polymerase Chain Reaction (PCR) techniques were used for *Taq*I RFLP of vitamin D receptor gene (Hennig et al., 1999). Briefly, total 50 μl of the reaction mixture contained 200-400 ng of genomic DNA, 100 ng of each primer, 200 mM of each dNTP, and buffers recommended by the manufacturer. The sequences of the primer for *Taq*I RFLP studied were:
sense 5'-CAGAGCATGGACAGGGAGCAAG-3', anti-sense 5'-GGATGTACGTCTGCAGTGTG-3'.

Amplification was carried out with DNA thermocycler: one cycle at 95°C for 10 min, 35 cycles at 94°C for 1 min, at 65°C for 30 sec and at 72°C for 1 min with a final polymerization at 72°C for 5 min. Following amplification, 10 μl of the PCR product were incubated with 10 units of restriction enzyme *Taq* I (Boeringer Mannheim) at 65°C for 5 h. Digested PCR products were genotyped by the electrophoresis using 2% agarose gel with 0.5 X TBE buffer.

Statistical Analysis

Differences in genotype and allele frequencies were assessed by χ^2-test. The heterozygosity and polymorphism information content (PIC) values were calculated as previously described (Bostein et al., 1980). The relative ratio of adult periodontitis associated with allelic variation was expressed in terms of an odds ratio (OR) with 95% confidence interval (CI). Differences between means were tested by students *t*-test.

A *P*-value of less than 0.05 indicated statistical significance. All statistical analyses were performed using the computer program of SPSSWIN (version 9.0).

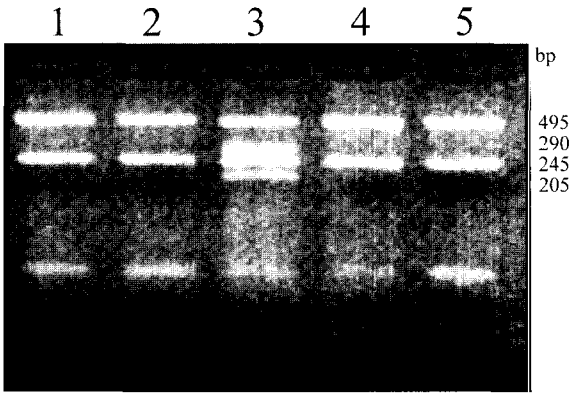

Fig. 1. Polymorphic patterns of vitamin D receptor genotype as indicated by *Taq*I restriction fragment length polymorphism. Lane 1, 2, 4 and 5, TT homozygotes; lane 3, Tt heterozygote.

Results

Polymorphic pattern

A polymorphism of vitamin D receptor gene was detected by digestion with restriction enzyme *Taq*I after PCR amplification (Figure 1). Homozygous absence of the *Taq* I restiction site (TT) yielded two bands of 495 bp and 245 bp, while heterozygote (Tt) gave four bands of 495 bp, 290 bp, 245 bp and 205 bp. Homozygous presence of restiction site (tt) with three bands (290, 245 and 205 bp) was not detected in our subjects.

Genotype distribution

Tables 1 display the gene frequencies and the values of

Table 1. Genotype and allele frequencies of the *Taq* I RFLP of the vitamin D receptor gene in controls and adult periodontitis group

	Genotype (%)			Allele (%)		H[1]	PIC[2]
	TT	Tt	tt	T	t		
Control	126(89)	15(11)	0(0)	267(95)	15(5)	0.1007	0.0957
Periodontitis	24(86)	4(14)	0(0)	52(93)	4(7)	0.1327	0.1239
χ^2	0.0532			0.0500			
P	0.8176			0.8231			
Odds ratio (CI)[3]	1.37(0.444.29)						

[1]Heterozygosity, [2]Polymorphism information content, [3]95% Confidence Interval. Frequency is given as a percentage in parenthesis.

Table 2. The comparison of the anthropometric data and intermediate phenotypes according to *Taq* I RFLP of the vitamin D receptor gene in Korean adult periodontitis group

Variables	Genotypes	
	TT (N)	Tt (N)
Age (year)	47.6 ± 8.6 (24)	50.3 ± 6.8 (4)
Total cholesterol (mg/dl)	188.2 ± 20.1 (24)	174.0 ± 26.9 (4)
Triglyceride (mg/dl)	150.3 ± 106.2 (24)	142.3 ± 51.1 (4)
HDL-cholesterol (mg/dl)	46.5 ± 9.6 (24)	40.3 ± 10.1 (4)
LDL-cholesterol (mg/dl)	114.3 ± 29.1 (24)	105.3 ± 29.5 (4)
Lipoprotein(a) (mg/dl)	19.6 ± 14.5 (24)	10.3 ± 4.8 (4)
Apolipoprotein AI (mg/dl)	140.8 ± 18.0 (24)	128.8 ± 15.8 (4)
Glucose (mg/dl)	99.6 ± 36.7 (24)	79.5 ± 14.4 (4)
WBC (count/mm^2)[1]	7,617.5 ± 2,155.4 (16)	5,340.0 ± 1,172.8 (4)
RBC (count/mm^2)[2]	4,604,375.0 ± 409,324.1 (16)	4,372,500.6 ± 493,381.2 (4)
Hematocrit (%)	44.4 ± 3.6 (16)	41.0 ± 5.6 (4)
Hemoglobin (g/dl)	14.5 ± 1.1 (16)	13.8 ± 1.3 (4)
Mean pocket depth (mm)	4.9 ± 0.8 (24)	4.7 ± 0.5 (4)

[1]white blood cell, [2]red blood cell.

heterozygosity and PIC for *Taq* I RFLP of the vitamin D receptor gene in Korean healthy controls and adult periodontitis group, respectively. The genotype and allele frequencies were not significantly different between healthy controls and adult periodontitis group, respectively ($P > 0.05$). The observed genotype distributions of this polymorphism were not significantly different from those expected for Hardy-Weinberg equilibrium ($P > 0.05$). The frequencies of TT and Tt genotypes were 89% and 11% in healthy controls, and 86% and 14% in adult periodontitis group, respectively. The heterozygosity and PIC values of *Taq* I RFLP in the vitamin D receptor gene represented the values of 0.1007 and 0.0957 for healthy controls, and 0.1327 and 0.1239 for adult periodontitis group, respectively. According to the heterozygosity and PIC values, *Taq* I RFLP in the vitamin D receptor gene indicated a relatively low degree of polymorphism in both groups.

Association with biochemical parameters

Table 2 presented the comparison of anthropometric data and intermediate phenotypes across *Taq* I RFLP in the vitamin D receptor gene. This polymorphism in the vitamin D receptor gene was not significantly associated with any anthropometrical data and biochemical parameters.

Discussion

Both genetic and environmental factors play an important roles in the pathogenesis of adult periodontitis, and considerable effects are currently directed towards identifying genetic risk factors contributing to this disease

Table 3. The distribution of *Taq* I RFLP of the vitamin D receptor gene between controls and adult periodontitis group in Chinese and Korean ethnic groups

Population		Genotype (%)			Allele (%)	
		TT	Tt	tt	T	t
Chinese[1]	Control	37(95)	2(5)	0(0)	76(97)	2(3)
	Case[2]	23(96)	1(4)	0(0)	47(98)	1(2)
Korean[3]	Control	126(89)	15(11)	0(0)	267(95)	15(5)
	Case	24(86)	4(14)	0(0)	52(93)	4(7)

[1]Sun et al., (2002), [2]Adult periodontitis group, [3]Our study. There were no significant differences in genotype and allele frequencies between normal controls and adult periodontitis patients in the two Asian ethnic groups ($P > 0.05$).

(Galbraith et al., 1999). However, the genetic variations responsible for adult periodontitis remain largely unknown, and the success to date in identifying causative genes has been very limited (Kornman et al., 1997; McDevitt et al., 2000; Holla et al., 2001a, b; Thomson et al., 2001; Holla et al., 2002; Scarel-Caminaga et al., 2002).

Vitamin D plays an important role in calcium and bone homeostasis, and has an immunoregulatory role mediated through binding to the vitamin D receptor in monocyte, macrophages and activated lymphocytes (Rigby, 1988). Vitamin D receptor is a member of the steroid/thyroid hormone nuclear receptor superfamily, and the gene encoding the vitamin D receptor is located on chromosome 12q12-q14 (Szpirer et al., 1991). *Taq* I RFLP in the vitamin D receptor gene is a silent mutation within codon 352 of the ninth exon (Hustmyer et al., 1993), and used as a genetic marker for clinical association study. Until now, *Taq* I RFLP was significantly associated with bone mineral density (Morrison et al., 1994; White et al., 1994; Eisman, 1996), prostate cancer (Taylor et al., 1996), leprosy (Roy et al., 1999), tuberculosis (Bellamy et al., 1999), and early-onset periodontitis (Hennig et al., 1999; Sun et al., 2002), although the results have not always been concordant in all populations studied.

In the present study, there were no significant differences in genotype and allele frequencies of *Taq* I RFLP in the vitamin D receptor gene between Korean healthy controls and adult periodontitis group, respectively. Also, this genetic variation was not significantly associated with any biochemical parameters. Thus, our finding of a negative association between the *Taq* I RFLP of vitamin D receptor gene and adult periodontitis was in agreement with a report by Sun et al., (2002) with Chinese population (controls 39, adult periodontitis group 24), and this genetic

Table 4. The comparison of the allele frequencies of *Taq* I RFLP in the vitamin D receptor gene among healthy ethnic groups

Allele	Negroes[1]	Negroes[2]	Gambian[3]	Caucasian[1]	Caucasian[2]	English[4]	Indian[5]	Asian[1]	Chinese[6]	Korean[7]	Korean[8]	Our study
T	0.79	0.50	0.67	0.61	0.55	0.68	0.66	0.97	0.97	0.92	0.95	0.95
t	0.21	0.50	0.33	0.39	0.45	0.32	0.34	0.03	0.03	0.08	0.05	0.05

[1]Hustmyer et al., 1993; [2]Taylor et al., 1996; [3]Bellamy et al., 1999; [4]Hennig et al., 1999; [5]Roy et al., 1999; [6]Sun et al., 2002; [7]Chung et al., 1998; [8]Lee et al., 2001.

variation are not significantly linked with adult periodontitis among Koreans as well as Chineses (Table 3).

The *t* allele frequency of Koreans (0.05~0.08) was very lower than those of Caucasians (0.32~0.45) and Negroes (0.21~0.50), but similar to that of Chinese (0.03) (Table 4). Genetic background may be an important factor for the difference in allele frequency. That is, this difference in allele frequency might be due to genetic drift by a founder effect or a complex adaptation to various environmental changes. Korean and Chinese populations indicated the similar aspects in *t* allele frequency and an association with adult periodontitis. To our knowledge, there was no report on the association between genetic variation of vitamin D receptor gene and adult periodontitis in other ethnic groups such as Caucasians and Negroes. Because the difference in genetic backgroud among ethnic groups studied may bring about the discrepancy in association studies between genetic marker and multifactorial diseases such as adult periodontitis, further research in other ethnic groups is needed to reach a precise conclusion.

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