J Clin Periodontol 2004; 31: 959–964 doi: 10.1111/j.1600-051X.2004.00587.x
Printed in Denmark. All rights reserved

Journal of Clinical Periodontology

Polymorphisms in genes coding for enzymes metabolizing smoking-derived substances and the risk of periodontitis

Jeong-Sook Kim[1], Jong Y. Park[2], Won-Yoon Chung[3], Min-Ah Choi[3,4], Kyoo-Sung Cho[1] and Kwang-Kyun Park[3,4]

[1]Department of Periodontology, Yonsei University College of Dentistry, 134 Shinchon-Dong, Seodaemoon-Ku, Seoul 120-752, South Korea; [2]Department of Interdisciplinary Oncology, H. Lee Moffitt Cancer Center & Research Institute, University of South Florida, Tampa, FL 33647, USA; [3]Department of Oral Biology, Yonsei University College of Dentistry, 134 Shinchon-Dong, Seodaemoon-Ku, Seoul 120-752, South Korea; [4]Brain Korea 21 Project for Medical Science, Yonsei University, 134 Shinchon-Dong, Seodaemoon-Ku, Seoul 120-752, South Korea

Kim J-S, Park JY, Chung W-Y, Choi M-A, Cho K-S, Park K-K: Polymorphisms in genes coding for enzymes metabolizing smoking-derived substances and the risk of periodontitis. J Clin Peridontol 2004; 31: 959–964. doi: 10.1111/j.1600–051X.2004.00587.x. © Blackwell Munksgaard, 2004.

Abstract

Objectives: Although the direct cause for periodontitis is oral bacterial infection, its progression depends upon genetic and environmental factors. Smoking, one of the environmental factors, is a risk factor for the development and severity of periodontitis. Therefore, individual susceptibility to periodontitis may be influenced by the polymorphisms of genes coding for enzymes metabolizing tobacco-derived substances. The object of this study is to investigate roles of genetic polymorphisms of these metabolizing enzymes in the risk for periodontitis.

Material and Methods: We investigated three important enzymes: cytochrome P450 (CYP) 1A1, CYP2E1 and glutathione *S*-transferase (GST) M1, involved in the metabolic activation and detoxification of tobacco-derived substances. The prevalence of the polymorphisms of these genes was examined in 115 patients with periodontitis as well as in 126 control subjects.

Results: Significantly increased risk for periodontitis was observed for subjects with the polymorphic CYP1A1 m2 allele (odds ratio (OR) = 2.3, 95% confidence interval (CI) = 1.2–4.4). A significant risk increase for periodontitis associated with the GSTM1 allele was observed (OR = 2.1, 95% CI = 1.3–3.6). However, no association was observed between the CYP2E1 *Pst1* polymorphism and risk for periodontitis (OR = 1.3, 95% CI = 0.6–2.5).

Conclusion: These results suggest that the GSTM1 and CYP1A1 polymorphisms may play an important role in risk for periodontitis.

Key words: cytochrome P450 2E1; cytochrome P450 1A1; polymorphism, periodontitis; glutathione *S*-transferase M1

Accepted for publication 2 February 2004

Periodontitis, one of the most common diseases among adults, is caused by oral bacteria and leads to irreversible bone loss and eventual tooth loss (Offenbacher 1996). Although the direct cause for periodontitis is oral bacterial infection, its progression and severity depends upon a number of genetic and environmental factors. Both environmental and genetic factors contribute to individual variations in the risk for periodontal disease (Beck & Slade 1996). Several studies were performed to elucidate the inter-individual variations of severity and progression of periodontitis (Laine et al. 2001, Kocher et al. 2002, Berglundh et al. 2003, Tachi et al. 2003). Despite epidemiological studies in different populations demonstrating a relationship between smoking and periodontal disease (Haber et al. 1993, Schenkein et al. 1995), the exact role of smoking in the pathogenesis of periodontal disease is still unknown. Because one of the risk factors for periodontitis is tobacco use (Burgan 1997), susceptibility to this disease may be linked to polymorphisms in genes coding for enzymes metabolizing tobacco-derived substances.

Cytochrome P450 (CYP) enzymes, CYP1A1 and CYP2E1, are considered to play important roles in the activation of xenobiotics, especially tobacco-derived substances such as polycyclic aromatic hydrocarbons (Kadlubar & Hammons 1987) and nitrosamines (Guengerich et al. 1991). On the other hand, glutathione *S*-transferase (GST) M1 and *N*-acetyltransferase (NAT1 and NAT2) are involved in the detoxification of these activated

metabolites. Polymorphism of CYP1A1 and CYP2E1 are associated with enhanced catalytic activities of these enzymes. In addition, the null GSTM1 genotype and the mutation in the NAT gene result in the inability to efficiently detoxify xenobiotics. Recently, it has been reported that the slow acetylator genotype of NAT2 is associated with a higher risk of periodontitis, particularly in smokers (Meisel et al. 2000, Kocher et al. 2002). Therefore, polymorphism of other xenobiotics-metabolizing enzymes, CYPs and GSTs, may also contribute to individual susceptibility to develop periodontitis.

We performed a case–control study to investigate whether the genetic polymorphisms of CYP1A1, CYP2E1 and GSTM1 are associated with the risk of periodontitis.

Material and methods

Study populations and sample processing

All cases were patients diagnosed with periodontal disease and were recruited between 1996 and 1999 from the Department of Periodontology, Yonsei University College of Dentistry (Seoul, Korea). Diagnosis of periodontal disease was made on the basis of clinical parameter and radiographic examination: probing depth and assessment of clinical attachment loss (CAL). Measurements of probing depth and attachment level were recorded at six points around each tooth. Cases for this study included only patients with $\geqslant 5$ mm CAL at at least one site on >8 teeth and $\geqslant 4$ mm probing depth. Controls without any prior diagnosis of periodontitis were comprised of adults recruited at the College of Dentistry or patients of the clinic. All subjects had more than 23 teeth in the mouth. The buccal cells ($n = 126$) or periodontal or oral tissue samples ($n = 115$) collected from all subjects during routine preventive dental screening or post-treatment at the dental clinics were used for the analysis of polymorphic genotypes. Protocols involving the analysis of buccal cell samples, periodontal specimens and oral tissues were approved by the institutional review board at the institute and informed consent was obtained from all subjects. A short questionnaire was administered to all subjects that contained questions on demographics information and lifelong smoking habits. Study subjects who smoked 100 or more cigarettes in their lifetime were categorized as smokers. Current or former smokers were included. Non-smokers are subjects who never smoked.

DNA isolation

Periodontal tissue samples were collected by biopsy from patients with periodontitis, and buccal cells were collected by mouthwash method as described previously (Laine et al. 2000). Briefly, about 1 h after the subjects brushed their teeth, the control subjects rinsed their mouth vigorously for 1 min with 10 ml of undiluted mouthwash (Listerine, Warner–Lambert Consumer Healthcare, Morris Plains, NJ, USA) and expelled it back into the 50 ml tube. The collected mouthwash was centrifuged at 2700 rpm for 15 min. The supernatant was decanted, and the pellet was washed in 25 μl of TE buffer (10 mM Tris, pH 8.0, 10 mM EDTA, pH 8.0). The suspension was centrifuged and the pellet was used for DNA extraction. Genomic DNA was isolated by incubation 2 h with proteinase K (0.1 mg/ml) in 1% sodium dodecyl sulfate at 58°C, extracted with phenol: chloroform and ethanol precipitation as previously described (Ausubel et al. 1988). To prevent contamination and cross-contamination between samples during polymerase chain reaction (PCR), careful attention was given throughout the DNA purification and isolation process. The purification of DNA samples was performed in a location distant from the workstation where PCR amplifications were performed.

Table 1. Sequences of the primers and annealing temperatures (temp) used for genotyping analysis

Genes	Sequence of primers	Annealing temp (°C)
GSTM1F	GAACTCCCTGAAAAGCTAAAGC	59
GSTM1R	GTTGGGGTCAAATATACGGTGG	
β-globinF	CAACTTCATCCACGTTCACC	59
β-globinR	GAAGAGCCAAGGACAGGTAC	
CYP1A1 *Msp1*F	TAGGAGTCTTGTCTCATGCCT	65
CYP1A1 *Msp1*R	CAGTGAAGAGGTGTAGCCGCT	
CYP2E1 *Pst1*F	CCAGTCGAGTCTACATTGTCA	55
CYP2E1 *Pst1*R	TTCATTCTGTCTTCTAACTGG	

GSTM1F, glutathione *S*-transferase M1; CYP1A1, cytochrome P450 P1A1; CYP2E1, cytochrome P450 P2E1.

Genotyping analysis

Genotyping of GSTM1 polymorphism

One hundred and fifteen patients and 126 controls were screened for the presence of the GSTM1 gene by multiplex PCR method (Deakin et al. 1996) using 20 pmol of sense and antisense primers (Table 1), homologous to the GSTM1 gene to generate a 215 bp fragment. A 268 bp fragment of the β-globin gene was co-amplified as an internal standard (Table 1). The standard PCR was performed in a 50 μl reaction volume containing 1 μg of genomic DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM of each of the dNTPs and 5.0 U of *Taq* polymerase. The reaction mixtures underwent the following incubations: 1 cycle of 94°C for 4 min, 30 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min, followed by a final cycle of 10 min at 70°C. Reactions without DNA were included as negative controls in all genotyping analysis. Ten microliter aliquots were removed from each PCR and resolved by electrophoresis in 1.5% agarose gels. PCR-amplified bands were detected after the staining of gels with 1 μg/ml ethidium bromide and photography over UV light. The GSTM1 genotypes were determined by the presence [GSTM1 (+); wild type(+/+) or heterozygote(0/+)] or absence [GSTM1(0/0), null type] of PCR product (Fig. 1).

Genotyping of CYP1A1 Msp1 polymorphism

The genotyping assays for the CYP1A1 *Msp1* polymorphism were performed by PCR-restriction fragment length polymorphism (RFLP) analysis, described previously (Hirvonen et al. 1992), with 20 pmol of primers (Table 1) homologous to sequences in the CYP1A1 gene

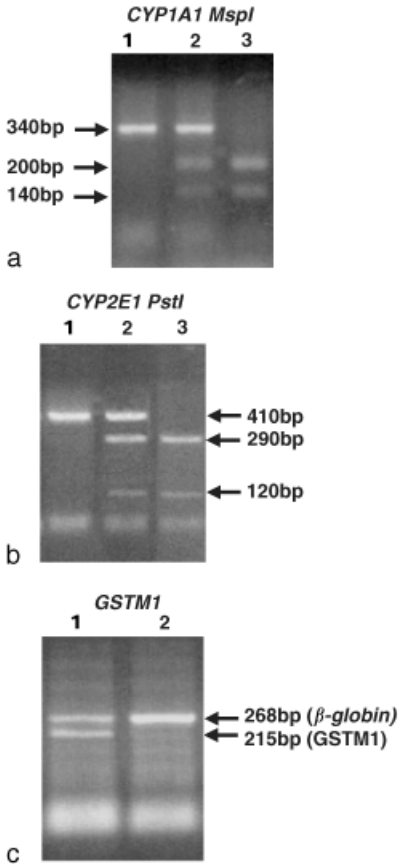

Fig. 1. Representative genotyping analysis of the cytochrome P450(CYP)1A1, CYP2E1 and glutathione *S*-transferase (GST) M1 polymorphism. (a) *MspI* polymorphism of CYP1A1. Lanes 1, 2 and 3 show genotypes m1/m1, m1/m2 and m2/m2, respectively. (b) *PstI* polymorphism of CYP2E1. Lanes 1, 2 and 3 show genotypes c1/c1, c1/c2 and c2/c2, respectively. (c) GSTM1 polymorphism. Lanes 1 and 2 indicate wild type, GSTM1(+) and null type, GSTM1(−/−), respectively. β-globin was co-amplified as an internal control.

utilized to generate a 340 bp fragment. After initial denaturation at 95°C for 4 min, amplification was carried out for 30 cycles at 95°C for 1 min, 65°C for 1 min, 72°C for 1 min, followed by final elongation at 72°C for 8 min. Differences in RFLP patterns were detected after *Msp1* restriction enzyme digestion (15 U, Promega, Madison, WI, USA) at 37°C for 2 h using 10 μl of PCR amplification. Three banding patterns were observed by RFLP analysis: 340 bp band that corresponded to the m1/m1 homozygous wild-type genotype, 340, 200 and 140 bp bands that corresponded to the m1/m2 heterozygous genotype and 200 and 140 bp bands that corresponded to the m2/m2 homozygous polymorphic genotype (Fig. 1).

Genotyping of CYP2E1 Pst1 polymorphism

The genotyping assay for the CYP2E1 *Pst1* polymorphism was performed by PCR-RFLP analysis, as described previously (Hayashi et al. 1991), with primers (Table 1) homologous to sequences in the CYP2E1 gene utilized to generate a 410 bp fragment. After initial denaturation at 95°C for 5 min, amplification was carried out for 25 cycles at 95°C for 1 min, 55°C for 1 min, 72°C for 1 min, followed by final elongation at 72°C for 7 min. Differences in RFLP patterns were detected after *Pst1* restriction enzyme digestion (15 U, Takara Shuzo Co., Shiga, Japan) at 37°C for 2 h using 10μl of PCR amplification. Three banding patterns were observed by RFLP analysis: 410 bp band that corresponded to the c1/c1 homozygous wild-type genotype, 410, 290 and 120 bp bands that corresponded to the c1/c2 heterozygous genotype and 290 and 120 bp bands that corresponded to the c2/c2 homozygous polymorphic genotype (Fig. 1).

These analyses were repeated for 10% of the specimens and selected PCR-amplified DNA samples were examined by dideoxy DNA sequencing (Sanger et al. 1977) to confirm genotyping results.

Statistical analysis

The risk of periodontal disease in relation to genotypes was estimated using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). The χ^2 test for trend was used to examine potential associations between predicted high-risk genotypes and periodontitis risk. The χ^2 or Fisher's exact tests were used to test for analysis of categorical variables, allelic prevalence, genotypes and case status, and for a deviation of genotype distribution from the Hardy–Weinberg equilibrium. The allelic frequencies were calculated from the observed genotype frequencies, assuming that alleles combine at random according to their frequencies in the set (the assumption being analogous to the Hardy–Weinberg equilibrium) during zygote formation. This assumption was verified by a reversed calculation of the expected genotype frequencies from the calculated allelic frequencies. The statistical computer software SAS (version 8e for Window, SAS Institute, Cary, NC, USA) was used to perform all statistical analyses. All statistical tests were two-sided. p-values < 0.05 were accepted as statistically significant.

Results

The genotype distribution for all polymorphisms did not differ from the Hardy–Weinberg equilibrium among controls ($p = 0.33$ for CYP2E1 and $p = 0.51$ CYP1A1). Because of design of genotyping analysis, we could not assess the allelic frequency of the GSTM1 null allele. A total of 115 cases and 126 controls were entered into this study. Informative genotyping results were available in 115 cases and 120 controls (Table 3). Among the control subjects, the allelic frequencies of the CYP1A1 m2 and CYP2E1 c2 alleles were 0.38and 0.19, respectively, and were similar to those reported in previous studies (Nakachi et al. 1993, Persson et al. 1993, Katoh et al. 1999, Bolt et al. 2003). The distribution of GSTM1 genotypes found in control subjects was similar to the one observed in earlier studies of Asian populations (Hung et al. 1997, Sato et al. 2000).

To determine whether CYP1A1 m2 and CYP2E1 c2 polymorphic alleles and the GSTM1 polymorphism contribute to change risk for periodontitis, we examined the prevalence of these polymorphisms in periodontitis patients versus control subjects. Significantly increased risk for periodontitis was observed for subjects with both the heterozygous CYP1A1 m1/m2 genotype (OR = 2.2, 95% CI = 1.1–4.2) and the homozygous CYP1A1 m2/m2 genotype (OR = 2.9, 95% CI = 1.2–7.4). These data were supported with the significant trend towards increased risk for periodontitis observed with potentially higher-risk CYP1A1 genotypes ($p = 0.009$, χ^2 trend test). Also, these data corresponded with the fact that a significantly higher prevalence of the CYP1A1 m2 allele was observed in cases (0.50) as compared with controls (0.38, $p = 0.02$). A significant increase in risk for periodontal disease was observed among subjects with the GSTM1(+) genotype (OR = 2.1, 95% CI = 1.3–3.6; Table 2). No association was observed between the CYP2E1 c2 allele and periodontal disease risk

Table 2. Relative risk associated with smoking among periodontitis patients and healthy controls

Cases	No.	Controls	No.	OR (95% CI)
smokers	57 (50)*	smokers	42 (33)	2.0 (1.2–3.4)
non-smoker	58 (50)	non-smoker	84 (67)	1.0 (referent)

OR, odds ratio; CI, confidence interval.
*Numbers in the brackets denote percentage.

Table 3. Prevalence of genotypes, smoking and risk for periodontitis

	GSTM1	No.	CYP1A1	No.	CYP2E1	No.
cases	(0/0)	43 (37)*	m1/m1	20 (18)	c1/c1	56 (57)
	[+]†	72 (63)	m1/m2	70 (64)	c1/c2	38 (39)
			m2/m2	19 (17)	c2/c2	4 (4)
controls	(0/0)	67 (56)	m1/m1	34 (34)	c1/c1	34 (63)
	[+]	53 (44)	m1/m2	54 (55)	c1/c2	20 (37)
			m2/m2	11 (11)	c2/c2	0 (0)
OR (95% CI)		2.1 (1.3 – 3.6)‡		2.3 (1.2–4.4)§		1.3 (0.6–2.5)§
non-smoker controls	(0/0)	41 (51)	m1/m1	25 (38)	c1/c1	25 (58)
	[+]	39 (49)	m1/m2	33 (51)	c1/c2	18 (42)
			m2/m2	7 (11)	c2/c2	0 (0)
OR (95% CI)		1.8 (1.0–3.1)‡		2.8 (1.4–5.6)§		1.0 (0.5–2.2)§
smoker controls	(0/0)	26 (65)	m1/m1	9 (26)	c1/c1	9 (82)
	[+]	14 (35)	m1/m2	21 (62)	c1/c2	2 (18)
			m2/m2	4 (12)	c2/c2	0 (0)
OR (95% CI)		3.1 (1.5–6.6)‡		1.6 (0.6–4.0)§		3.3 (0.7–16.5)§

GSTM1, glutathione S-transferase M1; CYP1A1, cytochrome 450 P1A1; CYP2E1, cytochrome 450 P2E1.
*Numbers in the brackets denote percentage.
†[+] = genotypes either (0/+) or (+/+).
‡Risk for subjects with GSTM1 [+] allele as compared with one with GSTM1 null genotype.
§Risk for subjects with heterozygous or homozygous polymorphic genotypes as compared with one with wild genotype.

(OR = 1.3, 95% CI = 0.6–2.5) and allelic frequencies for CYP2E1 polymorphism were not different between patients with periodontitis and the controls (0.19 for controls versus 0.23 for cases, $p = 0.32$).

To examine the relationship between risks for periodontitis and exposure to an environmental risk factor, i.e. smoking, we stratified study subjects by their smoking history. As expected, ever-smokers had increased risk for periodontitis (OR = 2.0, 95% CI = 1.2–3.4). Subjects were further stratified by genotypes to investigate the relationship between genotypes and risk for periodontal disease in different smoking history groups. We observed a risk change between smokers and non-smokers (Table 3). Study subjects with GSTM1(+) genotype had a significant increase in periodontitis risk among smokers (OR = 3.1 95%, CI = 1.5–6.6) and near significant moderate risk increase among non-smokers (OR = 1.8, 95% CI = 1.0–3.1). The risk was not changed among individuals with CYP2E1 c2 allele regardless of smoking history, although data suggested the potential of a risk increase among smokers (OR = 3.3, 95% CI = 0.7–16.5). In contrast, we observed a risk change for CYP1A1 polymorphisms between ever- and non-smokers. Study subjects with CYP1A1 m2 alleles had a significant increase in periodontitis risk among non-smokers (OR = 2.8, 95% CI = 1.4–5.6), but no risk increase was observed among ever-smokers (OR = 1.6, 95% CI = 0.6–4.0).

Discussion

Periodontal disease regarded as a chronic inflammatory oral infection presents with a wide range of clinical variability and severity. Both environmental and genetic factors are associated with the severity as well as the susceptibility for periodontal disease. Therefore, there were numerous attempts to find genetic factors responsible for periodontal disease and explain the correlation between smoking and periodontal disease (Meisel et al. 2000, Laine et al. 2001, Holla et al. 2002a, Meisel et al. 2003). In particular, polymorphisms in genes encoding molecules implicated in the host immune and inflammatory response to bacterial etiologic agents, such as cytokines, have been targeted as potential genetic factors (Kornman & di Giovine 1998, Galbraith et al. 1999). While the genetic polymorphism in genes coding for interleukin-1 (IL-1) (Parkhill et al. 2000, Walker et al. 2000, Laine et al. 2001, Greenstein & Hart 2002), IL-2 (Scarel-Caminaga et al. 2002) and IL-6 (Trevilatto et al. 2003) were associated with severe adult periodontitis and an increased risk of periodontal disease was found for IL-1 genotype-positive smokers (Meisel et al. 2003), significant associations were not observed in polymorphisms of IL-4 gene (Kang et al. 2003, Scarel-Caminaga et al. 2003) and transforming growth factor-β (Holla et al. 2002b). Also, the genotypes of Fc receptor (Kobayashi et al. 1997, Yoshihara et al. 2001, Loos et al. 2003) and vitamin D receptor (Sun et al. 2002, Tachi et al. 2003) have been found to be associated with risk change for periodontitis. However, IL-10 (Yamazaki et al. 2001, Gonzales et al. 2002, Berglundh et al. 2003) and tumor necrosis factor-α (Craandijk et al. 2002, Soga et al. 2003) have produced conflicting results in different populations.

Recent study has suggested an association of the polymorphism of NAT2 gene with periodontal disease among Caucasian population (Meisel et al. 2000). Because the NAT2 enzyme is involved in the metabolism of tobacco-derived xenobiotics and smoking is a major risk of periodontal disease, CYP1A1, CYP2E1 and GSTM1 enzymes may also contribute to an increased risk for periodontitis. The genetic polymorphisms of these enzymes have been linked with an increased risk for tobacco-related disease such as oral cavity cancer (Park et al. 1997, Sato et al. 1999, Tanimoto et al. 1999, Park et al. 2000, Sato et al. 2000, Sikdar et al. 2003).

In this study, we demonstrated that smoking is a risk factor for periodontitis (OR = 2.0, 95% CI = 1.2–3.4) as previously known. The risk increase for periodontitis among subjects with the GSTM1(+) (OR = 2.1, 95% = 1.3–3.6)

and among non-smokers with the CYP1A1 m2 allele (OR = 2.8, 95% = 1.4–5.6) is somewhat interesting. These data suggested that the GSTM1 and CYP1A1 enzymes may play a role in the pathway for developing periodontal disease in non-smokers. Another potential explanation is that glutathione-conjugated metabolites may be harmful for periodontal tissues. However, these assumptions need to be investigated. Although it is needed to be confirmed in larger study, our data suggested that CYP2E1 enzyme may increase risk for smoking-related periodontitis (OR = 3.3, 95% = 0.7–16.5). The presence of an interaction between smoking and genotypes implies that the two variables are independent of risk factors for periodontal disease. This suggests that individuals are affected by both risk factors – smoking and genotypes, separately. Higher expression of metabolic enzymes determined by genetic factors may lead to an increased damage by oxidative molecules in periodontal tissues and, as a result, the rate of tissue damage increases in association with a higher estimated relative risk of disease. In smokers, tobacco smoke induces the tissue damage. Consequently, the estimated relative risk of periodontal disease in smokers increases with tobacco use. In smokers who have increased metabolic enzyme expression, tissue damages may accumulate faster than in smokers with low-enzyme expression or in non-smokers with low-enzyme expression, leading to an overall higher estimated relative risk.

We are aware of a potential limitation of this study. There are evidences for heritability of certain forms of periodontitis, especially the aggressive form. We did not add this important family history variable in logistic regression analysis for estimating relative risk for periodontitis.

In summary, we demonstrated significant association between polymorphisms in CYP1A1 and GSTM1 genes and risk for periodontal disease in this study. These results suggest that polymorphisms of xenobiotic metabolism may contribute to individual susceptibility to the risk for periodontitis and genetically defected metabolizing capacity may be a risk factor for periodontitis among smokers. This is the first study in which polymorphisms in the CYP1A1 and GSTM1 were used with regard to the risk of periodontal disease. This information can be used for the identification of high-risk population for periodontitis risk and may affect strategy of periodontal disease prevention.

Acknowledgments

This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea. (00-PJ1-PG3-20800-0062)

References

Ausubel, F. M., Brent, R., Kingston, R., Moore, D. D., Seidman, J. G. & Smith, J. A. (1988) *Current Protocols in Molecular Biology*, **Vol. 1**. New York: John Wiley and Sons.

Beck, J. D. & Slade, G. D. (1996) Epidemiology of periodontal diseases. *Current Opinion in Periodontology* **3**, 3–9.

Berglundh, T., Donati, M., Hahn-Zoric, M., Hanson, L. A. & Padyukov, L. (2003) Association of the –1087 IL 10 gene polymorphism with severe chronic periodontitis in Swedish Caucasians. *Journal of Clinical Periodontology* **30**, 249–254.

Bolt, H. M., Roos, P. H. & Thier, R. (2003) The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. *International Archives of Occupational Environmental Health* **76**, 174–185.

Burgan, S. W. (1997) The role of tobacco use in periodontal diseases: a literature review. *General Dentistry* **45**, 449–460.

Craandijk, J., van Krugten, M. V., Verweij, C. L., van der Velden, U. & Loos, B. G. (2002) Tumor necrosis factor-alpha gene polymorphisms in relation to periodontitis. *Journal of Clinical Periodontology* **29**, 28–34.

Deakin, M., Elder, J., Hendrickse, C., Peckham, D., Baldwin, D., Pantin, C., Wild, N., Leopard, P., Bell, D. A., Jones, P., Duncan, H., Brannigan, K., Alldersea, J., Fryer, A. A. & Strange, R. C. (1996) Glutathione *S*-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. *Carcinogenesis* **17**, 881–884.

Galbraith, G. M., Hendley, T. M., Sanders, J. J., Palesch, Y. & Pandey, J. P. (1999) Polymorphic cytokine genotypes as markers of disease severity in adult periodontitis. *Journal of Clinical Periodontology* **26**, 705–709.

Gonzales, J. R., Michel, J., Diete, A., Herrmann, J. M., Bodeker, R. H. & Meyle, J. (2002) Analysis of genetic polymorphisms at the interleukin-10 loci in aggressive and chronic periodontitis. *Journal of Clinical Periodontology* **29**, 816–822.

Greenstein, G. & Hart, T. C. (2002) A critical assessment of interleukin-1 (IL-1) genotyping when used in a genetic susceptibility test for severe chronic periodontitis. *Journal of Periodontology* **73**, 231–247.

Guengerich, F. P., Kim, D. H. & Iwasaki, M. (1991) Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chemical Research in Toxicology* **4**, 168–179.

Haber, J., Wattles, J., Crowley, M., Mandell, R., Joshipura, K. & Kent, R. L. (1993) Evidence of cigarette smoking as a major risk factor for periodontitis. *Journal of Periodontology* **64**, 16–23.

Hayashi, S., Watanabe, J. & Kawajiri, K. (1991) Genetic polymorphisms in the 5′-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *Journal of Biochemistry (Tokyo)* **110**, 559–565.

Hirvonen, A., Husgafvel-Pursiainen, K., Karjalainen, A., Anttila, S. & Vainio, H. (1992) Point-mutational *Msp*I and Ile-Val polymorphisms closely linked in the CYP1A1 gene: lack of association with susceptibility to lung cancer in a Finnish study population. *Cancer Epidemiology, Biomarkers & Prevention* **1**, 485–489.

Holla, L. I., Buckova, D., Fassmann, A., Benes, P. & Znojil, V. (2002a) Plasminogen-activator-inhibitor-1 promoter polymorphism as a risk factor for adult periodontitis in non-smokers. *Genes and Immunity* **3**, 292–294.

Holla, L. I., Fassmann, A., Benes, P., Halabala, T. & Znojil, V. (2002b) 5 polymorphisms in the transforming growth factor-beta 1 gene (TGF-beta 1) in adult periodontitis. *Journal of Clinical Periodontology* **29**, 336–341.

Hung, H. C., Chuang, J., Chien, Y. C., Chern, H. D., Chiang, C. P., Kuo, Y. S., Hildesheim, A. & Chen, C. J. (1997) Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiology, Biomarkers & Prevention* **6**, 901–905.

Kadlubar, F. F. & Hammons, G. J. (1987) Cytochrome P450 in metabolism of chemical carcinogens. In: *Mammalian Cytochromes P-450*, eds. Guengerich, F. P., **Vol. 2**, pp. 81–130. Boca Raton, FL: CRC Press.

Kang, B. Y., Choi, Y. K., Choi, W. H., Kim, K. T., Choi, S. S., Kim, K. & Ha, N. J. (2003) Two polymorphisms of interleukin-4 gene in Korean adult periodontitis. *Archives of Pharmacal Research* **26**, 482–486.

Katoh, T., Kaneko, S., Kohshi, K., Munaka, M., Kitagawa, K., Kunugita, N., Ikemura, K. & Kawamoto, T. (1999) Genetic polymorphisms of tobacco- and alcohol-related metabolizing enzymes and oral cavity cancer. *International Journal of Cancer* **83**, 606–609.

Kobayashi, T., Westerdaal, N. A., Miyazaki, A., van der Pol, W. L., Suzuki, T., Yoshie, H., van de Winkel, J. G. & Hara, K. (1997) Relevance of immunoglobulin G Fc receptor polymorphism to recurrence of adult periodontitis in Japanese patients. *Infection and Immunity* **65**, 3556–3560.

Kocher, T., Sawaf, H., Fanghanel, J., Timm, R. & Meisel, P. (2002) Association between bone loss in periodontal disease and polymorphism of *N*-acetyltransferase (NAT2).

Journal of Clinical Periodontology **29**, 21–27.

Kornman, K. S. & de Giovine, F. S. (1998) Genetic variations in cytokine expression: a risk factor for severity of adult periodontitis. *Annals of Periodontology* **3**, 327–338.

Laine, M. L., Farre, M. A., Crusius, J. B., van Winkelhoff, A. J. & Pena, A. S. (2000) The mouthwash: a non-invasive sampling method to study cytokine gene polymorphisms. *Journal of Periodontology* **71**, 1315–1318.

Laine, M. L., Farre, M. A., Gonzalez, G., van Dijk, L. J., Ham, A. J., Winkel, E. G., Crusius, J. B., Vandenbroucke, J. P., van Winkelhoff, A. J. & Pena, A. S. (2001) Polymorphisms of the interleukin-1 gene family, oral microbial pathogens, and smoking in adult periodontitis. *Journal of Dental Research* **80**, 1695–1699.

Loos, B. G., Leppers-Van de Straat, F. G., Van de Winkel, J. G. & Van der Velden, U. (2003) Fcgamma receptor polymorphisms in relation to periodontitis. *Journal of Clinical Periodontology* **30**, 595–602.

Meisel, P., Siegemund, A., Grimm, R., Herrmann, F. H., John, U., Schiwahn, C. & Kocher, T. (2003) The interleukin-1 polymorphism, smoking, and the risk of periodontal disease in the population-based SHIP study. *Journal of Dental Research* **82**, 189–193.

Meisel, P., Timm, R., Sawaf, H., Fanghanel, J., Siegmund, W. & Kocher, T. (2000) Polymorphism of the *N*-acetyltransferase (NAT2), smoking and the potential risk of periodontal disease. *Archives of Toxicology* **74**, 343–348.

Nakachi, K., Imai, K., Hayashi, S. & Kawajiri, K. (1993) Polymorphisms of the CYP1A1 and glutathione S-transferase genes associated with susceptibility to lung cancer in relation to cigarette dose in a Japanese population. *Cancer Research* **53**, 2994–2999.

Offenbacher, S. (1996) Periodontal diseases: pathogenesis. *Annals of Periodontology* **1**, 821–878.

Park, J. Y., Muscat, J. E., Kaur, T., Schantz, S. P., Stern, J. C., Richie, J. P. Jr. & Lazarus, P. (2000) Comparison of GSTM polymorphisms and risk for oral cancer between African-Americans and Caucasians. *Pharmacogenetics* **10**, 123–131.

Park, J. Y., Muscat, J. E., Ren, Q., Schantz, S. P., Harwick, R. D., Stern, J. C., Pike, V., Richie, J. P. Jr. & Lazarus, P. (1997) CYP1A1 and GSTM1 polymorphisms and oral cancer risk. *Cancer Epidemiology, Biomarkers & Prevention* **6**, 791–797.

Parkhill, J. M., Hennig, B. J., Chapple, I. L., Heasman, P. A. & Taylor, J. J. (2000) Association of interleukin-1 gene polymorphisms with early-onset periodontitis. *Journal of Clinical Periodontology* **27**, 682–689.

Persson, I., Johansson, I., Bergling, H., Dahl, M. L., Seidegard, J., Rylander, R., Rannug, A., Hogberg, J. & Sundberg, M. I. (1993) Genetic polymorphism of cytochrome P4502E1 in a Swedish population. Relationship to incidence of lung cancer. *FEBS Letters* **319**, 207–211.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the USA* **74**, 5463–5467.

Sato, M., Sato, T., Izumo, T. & Amagasa, T. (1999) Genetic polymorphism of drug-metabolizing enzymes and susceptibility to oral cancer. *Carcinogenesis* **20**, 1927–1931.

Sato, M., Sato, T., Izumo, T. & Amagasa, T. (2000) Genetically high susceptibility to oral squamous cell carcinoma in terms of combined genotyping of CYP1A1 and GSTM1 genes. *Oral Oncology* **36**, 267–271.

Scarel-Caminaga, R. M., Trevilatto, P. C., Souza, A. P., Brito, R. B. & Line, S. R. (2002) Investigation of an IL-2 polymorphism in patients with different levels of chronic periodontitis. *Journal of Clinical Periodontology* **29**, 587–591.

Scarel-Caminaga, R. M., Trevilatto, P. C., Souza, A. P., Brito, R. B. Jr. & Line, S. R. (2003) Investigation of IL4 gene polymorphism in individuals with different levels of chronic periodontitis in a Brazilian population. *Journal of Clinical Periodontology* **30**, 341–345.

Schenkein, H. A., Gunsolley, J. C., Koertge, T. C., Schenkein, J. G. & Tew, J. G. (1995) Smoking and its effects on early-onset periodontitis. *Journal of American Dental Association* **126**, 1107–1113.

Sikdar, N., Mahmud, S. A., Paul, R. R. & Roy, B. (2003) Polymorphism in CYP1A1 and CYP2E1 genes and susceptibility to leukoplakia in Indian tobacco users. *Cancer Letters* **195**, 33–42.

Soga, Y., Nishimura, F., Ohyama, H., Maeda, H., Takashiba, S. & Murayama, Y. (2003) Tumor necrosis factor-alpha gene (TNF-alpha) −1031/–863, –857 single-nucleotide polymorphisms (SNPs) are associated with severe adult periodontitis in Japanese. *Journal of Clinical Periodontology* **30**, 524–531.

Sun, J. L., Meng, H. X., Cao, C. F., Tachi, Y., Shinohara, M., Ueda, M., Imai, H. & Ohura, K. (2002) Relationship between vitamin D receptor gene polymorphism and periodontitis. *Journal of Periodontal Research* **37**, 263–267.

Tachi, Y., Shimpuku, H., Nosaka, Y., Kawamura, T., Shinohara, M., Ueda, M., Imai, H. & Ohura, K. (2003) Vitamin D receptor gene polymorphism is associated with chronic periodontitis. *Life Science* **73**, 3313–3321.

Tanimoto, K., Hayashi, S. I., Yoshiga, K. & Ichikawa, T. (1999) Polymorphisms of the CYP1A1 and GSTM1 gene involved in oral squamous cell carcinoma in association with a cigarette dose. *Oral Oncology* **35**, 191–196.

Trevilatto, P. C., Scarel-Caminaga, R. M., de Brito, R. B. Jr., de Souza, A. P. & Line, S. R. (2003) Polymorphism at position -174 of IL-6 gene is associated with susceptibility to chronic periodontitis in a Caucasian Brazilian population. *Journal of Clinical Periodontology* **30**, 438–442.

Walker, S. J., Van Dyke, T. E., Rich, S., Kornman, K. S., di Giovine, F. S. & Hart, T. C. (2000) Genetic polymorphisms of the IL-1alpha and IL-1beta genes in African-American LJP patients and an African-American control population. *Journal of Periodontology* **71**, 723–728.

Yamazaki, K., Tabeta, K., Nakajima, T., Ohsawa, Y., Ueki, K., Itoh, H. & Yoshie, H. (2001) Interleukin-10 gene promoter polymorphism in Japanese patients with adult and early-onset periodontitis. *Journal of Clinical Periodontology* **28**, 828–832.

Yoshihara, A., Sugita, N., Yamamoto, K., Kobayashi, T., Miyazaki, H. & Yoshi, H. (2001) Analysis of vitamin D and Fcgamma receptor polymorphisms in Japanese patients with generalized early-onset periodontitis. *Journal of Dental Research* **80**, 2051–2054.

Address:
K. K. Park
Department of Oral Biology
Yonsei University College of Dentistry
134 Shinchon-Dong
Seodaemoon-Ku
Seoul 120-752
South Korea
Fax: (+82) 2 364 7113
E-mail: biochelab@yumc.yonsei.ac.kr