J Clin Periodontol 2008; 35: 1–8 doi: 10.1111/j.1600-051X.2007.01167.x

Journal of Clinical Periodontology

Cyclooxygenase-2 Gene $^{-765}$ single nucleotide polymorphism as a protective factor against periodontitis in Taiwanese

Ya-Ping Ho[1,2], Ying-Chu Lin[3], Yi-Hsin Yang[4,5], Kun-Yen Ho[1,2], Yi-Min Wu[1,2] and Chi-Cheng Tsai[2,3]

[1]Faculty of Dentistry, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [2]Department of Periodontology, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan; [3]Graduate Institute of Dental Sciences, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [4]Graduate Institute of Oral Health Sciences, College of Dental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [5]Statistical Analysis Laboratory, Department of Clinical Research, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung, Taiwan

Ho Y-P, Lin Y-C, Yang Y-H, Ho K-Y, Wu Y-M, Tsai C-C. Cyclooxygenase-2 Gene $^{-765}$ single nucleotide polymorphism as a protective factor against periodontitis in Taiwanese. J Clin Periodontol 2008; 35: 1–8. doi: 10.1111/j.1600-051X.2007.01167.x.

Abstract

Aim: Prostaglandin E_2 (PGE_2) is considered to be an important mediator of tissue destruction in periodontitis. The cyclooxygenase (COX) catalyses the production of PGs. COX-2, which is induced in an inflammatory response, is responsible for PGs synthesis at sites of inflammation. A single nucleotide polymorphism of COX-2 $^{-765}$ has been shown to alter the expression of the COX-2 gene. The purpose of the present study was to evaluate the association of the COX-2 $^{-765}$ polymorphism and susceptibility to periodontitis in Taiwanese.

Material and Methods: Eighty-five cases of aggressive periodontitis (AgP), 343 cases of chronic periodontitis (CP) and 153 cases of healthy controls (HC) were recruited for the study. Genotypes of COX-2 $^{-765}$ were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The distribution of genotypes among groups was compared by logistic regression analyses. The risk for periodontitis associated with genotypes was calculated as the odds ratio (OR).

Results: The prevalence of the GC and CC genotypes was significantly lower in AgP (5%) and in CP (29%) compared with the HC (42%). The ORs for carriage of the -765C allele (GC+CC *versus* GG) in AgP and CP were 0.068 (95% CI = 0.020–0.173, $p < 0.0001$) and 0.571 (95% CI = 0.385–0.849, $p = 0.006$), respectively. After adjustment for age, gender and smoking status, the OR was 0.071 (95% CI = 0.017–0.219) and 0.552 (95% CI = 0.367–0.829) for AgP and CP, respectively.

Conclusions: The results of the study suggest that the -765G to C polymorphism of the COX-2 gene is associated with a decreased risk for periodontitis in Taiwanese, especially in AgP. However, the biological meaning needs further investigation.

Key words: COX-2; genetic polymorphism; risk of periodontitis; Taiwanese

Accepted for publication 23 October 2007

Periodontitis, one of the most common infectious disorders in human beings, is an inflammatory disease of the supporting tissue of teeth. Prostaglandins (PGs) are potent biochemical mediators of inflammatory response in periodontitis (Offenbacher et al. 1993). A large body of evidence supports the concept that prostaglandin E_2 (PGE_2) mediates much of the tissue destruction occurring in periodontitis. The levels of PGE_2 in gingival crevicular fluid (GCF) have been shown to be elevated in patients with periodontal disease compared with periodontally healthy patients (Tsai et al. 1998, Biyikoğlu et al. 2006). In addition, the concentration of PGE_2 in GCF is increased in periodontitis sites demonstrating attachment loss. Hence, PGE_2 is considered to be a diagnostic marker for future attachment loss (Offenbacher et al. 1986). It was reported that the total amount of PGE_2 was significantly higher in sites of periodontitis when specific periodontal pathogens presented and was also positively associated with clinical

Conflict of interest and source of funding statement

The authors declare that they have no conflict of interests.

This study was supported by a research grant from the National Science Council, Taiwan (NSC 93-2314-B-037-070, NSC 94-2314-B-037-010).

parameters of inflammation and disease severity (Airila-Månsson et al. 2006).

PGE_2 is also a well-known mediator involved in the regulation of osteoclast differentiation (Fujita et al. 2003). Cyclooxygenase-2 (COX-2)-dependent PGE_2 synthesis by osteoblasts is considered to play an important role in bone resorption associated with inflammatory disease (Dubois et al. 1998). In the process of osteoclast differentiation from haematopoietic stem cells, the receptor activator of NF-κB ligand (RANKL) is essential and crucial (Hofbauer et al. 2000). PGE_2 acts on osteoblasts to increase the expression of RANKL on their surfaces. Therefore, PGE_2 enhances the osteoclastogenesis.

PG synthesis is regulated by two successive metabolic processes (Marnett et al. 1999): the release of arachidonic acid from phospholipids that are found in the plasma membrane of most cells by the action of phospholipase A2, and then the conversion of arachidonic acid to prostanoids by the effect of the COX. COX is also called PG-endoperoxide synthase (PTGS). There are two isoforms of COX, designated as COX-1 and COX-2 (Smith et al. 1996). These isoforms are encoded on distinct genes but catalyse essentially the same reaction. COX-1 is a housekeeping enzyme that is constitutively expressed in many tissues. On the other hand, COX-2 is generally undetectable under physiologic conditions, but it is inducible by inflammation (Dubois et al. 1998). It is believed that COX-2 is responsible for PGs synthesis at sites of inflammation. Elevated levels of COX-2 are detected in a variety of inflammatory conditions, including periodontitis (Morton & Dongari-Bagtzoglou 2001, Zhang et al. 2003). Besides this, the COX-2 gene is an immediate-early gene: COX-2 can be induced rapidly. A variety of cells in periodontal tissue, such as fibroblasts, cementoblasts and osteoblasts in periodontal ligament, epithelial cells, gingival fibroblasts, endothelial cells, macrophages, monocytes and neutrophils, produce PGE_2 regulated by COX-2 after activation with pro-inflammatory cytokines, including interleukin-1 (IL-1), IL-6, tumour necrosis factor-α (TNF-α) and growth factors, lipopolysaccharide (LPS), tumour promoters or PGE_2 itself (Tai et al. 1997, Offenbacher & Salvi 1999, Yucel-Lindberg et al. 1999, Xu et al. 2000, Miyauchi et al. 2004). As mentioned above, COX-2-dependent-PGE_2 synthesis is considered to be an important mediator of tissue destruction in periodontitis.

The aetiology of periodontal disease is bacterial plaque. However, the bacterial factor cannot fully explain the extent of periodontal destruction. A genetic contribution to periodontitis has been suggested in previous studies (Kornman et al. 1997, Soga et al. 2003, Trevilatto et al. 2003, Loos et al. 2005). It is reasonable to hypothesize that a genetic polymorphism in the COX-2 gene that results in alteration of the function, activity and/or expression of this enzyme consequently has an impact on PGs synthesis and may modulate the inflammatory response, thus modifying the risk of periodontal diseases. In this study, we evaluated the impact of the COX-2^{-765} genetic polymorphism on the risk of aggressive periodontitis (AgP) and CP.

Material and Methods

Study population

A case–control study was designed. The recruitment of study subjects took place at the Department of Periodontology of the Kaohsiung Medical University Hospital between January 2004 and May 2006. All subjects who participated in this study were of Han ethnicity and were free from systemic diseases that correlate with the destructive periodontal disease, such as diabetes mellitus, immunosuppression, HIV infection or polymorphonuclear and/or monocyte defects. Subjects who had taken antibiotics in the previous 3 months, who were pregnant, having current lactation or needed antibiotic prophylaxis before periodontal treatment were also excluded from this study.

The study subjects were diagnosed as AgP, CP and periodontally healthy controls (HC) on the basis of clinical examinations (probing depth and attachment loss) and radiographic patterns of alveolar bone destruction. The diagnostic criteria for AgP and CP were defined in accordance with the classification agreed at the World Workshop for Periodontics and The American Academy of Periodontology (1999). Briefly, subjects more than 35 years of age, with attachment loss equal to or >5 mm at more than one tooth, with more than three sites of probing depth >6 mm and lesions distributed at more than two teeth in each quadrant were diagnosed with CP. Subjects who had more than eight teeth with attachment loss >5 mm and probing depth >6 mm, and at least three affected teeth that were not first molars or incisors were diagnosed with AgP. The age at diagnosis was below 35 years in most AgP patients. Subjects with no evidence of attachment loss at more than one site or pocket depth >3 mm were diagnosed as periodontally HC. The HC recruited were older than 35 years, which may have helped to avoid misclassification. We also recorded the smoking status as non-smoker, current smoker or former smoker. Subjects who still had the smoking habit or had quit smoking within the last 6 months before enrolling in this study were designated as current smokers. Former smokers were subjects who had quit smoking for at least 6 months.

The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University, and a written informed consent was obtained from each subject.

Sample collection and DNA extraction

Twenty millilitres of heparin anti-coagulated peripheral blood was collected from each study subject. DNA was extracted from the peripheral blood leucocytes by standard phenol/chloroform extraction techniques and precipitation with ethanol (Blin & Stafford 1976). The DNA concentration was determined by ultraviolet (UV) spectrophotometry.

Analysis of the COX-2 genotype

The genotype for the COX-2 at position −765 from the transcription start site was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Papafili et al. 2002). A 306 bp fragment of the COX-2 gene was generated using a forward primer (5′-CCGCTTCCTTT GTCCATCAG-3′) and a reverse primer (5′-GGCTGTATATCTGCTCTATATGC-3′) in a thermocycler (Applied Biosystems, Foster city, CA, USA). The reaction conditions and cycling parameters were as follows: 100 ng genomic DNA was used for PCR amplification in a reaction mixture containing 1 × reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1.25 U Taq polymerase (JMR Holdings, London, UK) and 1.0 μM of each primer. The PCR cycle conditions were 94°C for 5 min., 35 cycles of 94°C for 30 s, 62°C for 20 s and 72°C for 50 s, followed by a final

extension at 72°C for 5 min. After cooling the samples, 10 μl of the PCR product was digested with *Aci*I (New England Biolabs, Ipswich, MA, USA; 5 U/sample, 37°C, 3 h). The digested fragments were electrophoretically separated on 3% agarose gel (Cambrex, Rockland, ME, USA) containing ethidium bromide and visualized under UV light to determine the genotypes. When the nucleotide at position −765 of the COX-2 gene was G, the PCR product could be digested by *Aci*I into two fragments of 188 and 118 bp, whereas a 306-bp fragment presented if the nucleotide was C (Fig. 1).

Samples that did not yeild a definitive result in the first round of genotyping were re-submitted to up to two additional rounds of genotyping. If reliable results were still not obtained after this procedure, the results were discarded. For quality control purpose, almost one-fifth of the samples, which were randomly selected, were genotyped a second time. There were no discrepancies between the two results.

Statistical methods

Comparisons of descriptive statistics among the three groups were expressed as mean (± SD) and within-group proportions. The χ^2-test and ANOVA test were used to evaluate the statistical significance of differences among the three groups. A p-value <0.05 was considered to indicate a statistically significant difference. The risk association of genotypes and periodontal diseases was computed by the simple logistic regression and expressed as the odds ratio (OR) with a 95% confidence interval (CI). To increase statistical power for the less frequent variant, the rare homozygotes were combined with the heterozygotes assuming a dominant effect. To control for possible confounding effects, age, gender and smoking status were used as independent variables in a multiple logistic regression for adjustment. The statistical analysis was conducted by JMP software (SAS Institute, Cary, NC, USA).

Results

There were 85 AgP patients (AgP), 343 CP patients and 153 periodontally HC in this study. The characteristics of the study subjects and the distribution of the COX-2 genotype and allelic frequencies are presented in Table 1. The gender distribution was similar for these three groups. The mean age of the AgP patients was significantly lower than that of the CP patients and the HC. The subjects with AgP or CP were more likely to be current or former smokers than the HC. The Genotype frequency of COX-2 did not deviate from Hardy–Weinberg equilibrium ($p>0.05$). The prevalence of the −765C allele homozygote was very low (2.2%) in our study population.

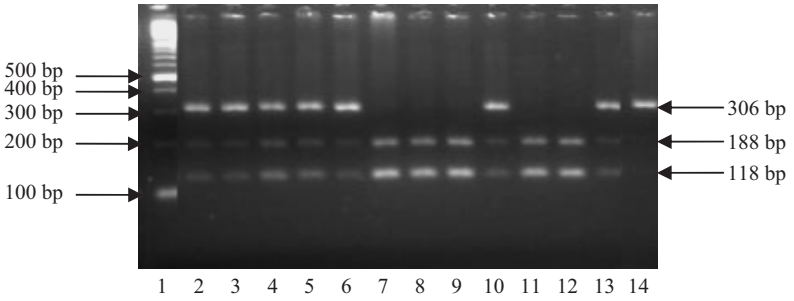

*Fig. 1. Aci*I restriction patterns of the various COX-2^{-765} genotypes. Three restriction fragments were present at 306, 188 and 118 bp in lanes 2–6, 10, 13 representing GC heterozygous subjects. The GG genotype (lanes 7–9, 11 and 12) had the two bands at 188 and 118 bp. The CC genotype (lane 14) had only the 306 bp band.

Table 1. Characteristics of patients and distribution of COX-2 genotype among patients with aggressive periodontitis, chronic periodontitis and healthy controls

	Aggressive periodontitis ($n=85$)	Chronic periodontitis ($n=343$)	Healthy control ($n=153$)	p-value*
Gender				
F	36 (42)	156 (45)	82 (54)	0.1369
M	49 (58)	187 (55)	71 (46)	
Age				
	37.46 (±7.02)	52.73 (±8.08)	51.56 (±10.00)	<0.0001
Smoking				
None	61 (72)	245 (72)	129 (84)	0.0013
Current	12 (14)	73 (22)	11 (7)	
Former	12 (14)	21 (6)	13 (9)	
Genotype				
CC	1 (1)	8 (2)	4 (3)	<0.0001
GC	3 (4)	93 (27)	60 (39)	
GG	81 (95)	242 (71)	89 (58)	
Allele				
C	5 (3)	109 (16)	68 (22)	<0.0001
G	165 (97)	577 (84)	238 (78)	

*Comparisons performed by χ^2-test or ANOVA.
Values represent numbers (%) of subjects or mean ± SD.

AgP

There was a significant difference in the distribution of the COX-2 genotypes and allelic frequencies between AgP patients and HC ($p<0.0001$). The GC and CC genotypes were present in 5% of the AgP patients, which was significantly lower ($p<0.0001$) than in HC (42%). The G allele was overexpressed in AgP patients. The crude OR for carriage of the −765C allele (the GC and CC genotypes combined compared with the GG genotype) to be associated with AgP was 0.068 (95% CI = 0.020–0.173, $p<0.0001$) (Table 2). After adjustment for age, gender and smoking status, the OR was 0.071 (95% CI = 0.017–0.219, $p<0.0001$).

CP

The COX-2 genotype distribution of CP was significantly different from that of HC ($p=0.024$). The prevalence of the GC and CC genotypes was significantly lower among CP than among HC (29% *versus* 42%, Fisher's exact p-value = 0.0075). The crude OR for carriage of the −765C allele (the GC and CC genotypes combined compared with the GG genotype) was 0.571 (95% CI = 0.385–0.849, $p=0.006$) for CP (Table 2). After adjustment for age, gender and smoking status, the OR was 0.552 (95% CI = 0.367–0.829, $p=0.004$)

Table 2. COX-2 genotype frequencies and odds ratio (OR) for periodontitis patients

Genotype	GG	GC/CC	95% CI	*p*-value
Healthy control				
n (%)	89 (58)	64 (42)		
Aggressive periodontitis				
n (%)	81 (95)	4 (5)		
Crude OR	1	0.068	(0.020, 0.173)	<0.0001
Adjusted OR*	1	0.071	(0.017, 0.219)	<0.0001
Chronic periodontitis				
n (%)	242 (71)	101 (29)		
Crude OR	1	0.571	(0.385, 0.849)	0.006
Adjusted OR*	1	0.552	(0.367, 0.829)	0.004

*Adjusted for gender, age, and smoking status for logistic regression analysis.
CI, confidence interval.

Discussion

COX-2 is a critical enzyme involved in the production of PGs, which play an important role in the tissue destruction of periodontal diseases. We hypothesized that a polymorphism in the COX-2 gene might influence PGs production in inflamed cells, thus affecting susceptibility to periodontitis. In this study, subjects with AgP or CP carried the COX-2 −765C allele significantly less frequently than the periodontally HC. Homozygotes as well as heterozygotes for the −765C allele were associated with a significantly lower risk for AgP and CP (OR is 0.068 and 0.571, respectively; $p<0.01$). This indicated that the carriage of the COX-2 −765C allele was protective for the development of periodontitis, especially for AgP, in this study population. After adjustment for gender, age and smoking status, the association between this genetic variant and risk of AgP and CP was still highly significant (adjusted OR is 0.071 and 0.552, respectively; $p<0.01$). These adjustments give further credibility to the findings that the COX-2^{-765} polymorphism is associated with the susceptibility to AgP and CP.

Previous studies have evaluated the association between polymorphisms in candidate genes and AgP in different ethnic backgrounds (Table 3). The results are inconsistent. For instance, IL-$1B^{+3954}$ was reported to be associated with risks of AgP in Caucasians and Chileans, but was not associated with AgP in Hispanic, Chinese, Greek and Japanese individuals. Based on the literature, AgP was associated with polymorphisms of IL-$1B^{+3954}$, TLR4(299), FcγRIIa-H131 and NADPH genes in Caucasians; IL-6R, FcαRI324, IL6ST, PTGDS, COL4A1, COL1A1, KRT23 and IL-1RN(VNTR) genes in Japanese; and MMP-1^{-1607}, IL-$1A^{+4845}$, IL-$1B^{-511}$ and ER-α in Chinese. The results of the present study showed the COX-2^{-765} polymorphism to be associated with AgP in Taiwanese. There are some major differences between the present study and those reviewed in Table 3. The mean ages of the HC matched those of AgP patients in most of the reviewed studies. In the present study, the controls were significantly older than the AgP patients. These controls should be considered better controls: they were old enough to show expression of periodontitis if they would have been susceptible. In addition, COX-2 gene expression is age related (Kim et al. 2000), a special and unique character among the candidate genes studied.

The human COX-2 gene, mapped to chromosome 1q25.2-q25.3, is about 8.3 kbp in size and consists of 10 exons (Kosaka et al. 1994). Many single nucleotide polymorphisms (SNPs) have been identified in the COX-2 gene. So far, few of these have been studied regarding their effect in altering the expression or the function of COX-2 (Fritsche et al. 2001). Variation at position −765 upstream of the transcription start site (−765G>C; a G residue was replaced by C) has been shown to alter the expression of the COX-2 gene. In other words, the COX-2^{-765} polymorphism is functional. The −765C allele revealed a significantly (30%) lower promoter activity compared with the −765G allele (Papafili et al. 2002). Cipollone et al. (2004) reported that the COX-2 expression and activity in peripheral blood monocytes and carotid plaque macrophages were COX-2 genotype dependent. The macrophages from patients carrying the −765C allele had a lower COX-2 expression. After stimulation with LPS, the COX-2, MMP-2, MMP-9 and PGE_2 productions were significantly enhanced in monocytes isolated from patients carrying the −765GG genotype compared with monocytes from patients carrying the −765GC and CC genotypes. Hill et al. (2006) examined the effect of the −765 G>C genotype on PGE_2 synthesis in lung fibroblast cell lines. The PGE_2 production by GC cell lines was very low and it was not enhanced by the stimulation of transforming growth factor-β1 (TGF-β1). By comparison, the PGE_2 production by GG cell lines was much higher and its synthesis was enhanced up to 14-fold when the culture media contained TGF-β1. According to the results of those studies, the −765C allele reduces the COX-2 gene expression and consequently the inflammatory response. Some possible mechanisms for this phenomenon are provided. The sequence of the 1.69-kb region of nucleotides preceding the transcription-start site, which is the 5′-flanking region of human COX-2 gene involved in the regulation of gene transcription, contains several transcription factor-binding sites. The −765G>C is located within a putative Sp1-binding site in the promoter of the COX-2 gene. Sp1 is a transcription factor binding to the multiple GC-boxes and it serves to activate gene transcription (Suske 1999). In addition to the Sp1, other DNA-binding elements including the Sp3 and the early growth response (Egr)-1 transcription factor could also bind to the Sp1-binding site (Hill et al. 2006). Sp1 and Sp3 recognize the classical Sp1-binding site with identical affinity and are co-expressed in the same cells. Sp3 is a bi-functional regulator shown to act as a transcriptional activator and as a repressor of Sp1-mediated activation (Suske 1999). In vivo studies using various cell types reported that the expression of Egr-1 regulated the expression of cytokine-induced PGE synthase, hence regulating the PGE_2 production (Arikawa et al. 2004, Moon et al. 2005). The −765G>C polymorphism could change the transcription factors binding to this region. This postulation was proven by Hill et al. (2006) by electrophoretic mobility shift assays (EMSA). Sp1 and Sp3 could bind to the region containing −765G. However, Sp1 and Sp3 binding was disrupted and the Egr-1 binding was increased in the −765C allele. It seems that Egr-1 could displace

Table 3. Studies for association of aggressive periodontitis and single nucleotide polymorphisms (SNP) in different ethnic groups

Authors	Ethnic group	Subjects	SNP	*p*-value	OR	95% CI
Atilla et al. (2006)	Turkey	43 GAgP, 40 H	TGF-β1(+915)	NS		
Berdeli et al. (2006)	Turkey	48 GAgP, 173 H	eNOS Glu298Asp	NS		
Brett et al. (2005)	Caucasian	51 AgP, 100 H	IL-1A(−889)	NS		
			IL-1B(−511)	NS	3.6	1.4–9.7
			IL-1B(+3954)	0.012		
			IL-6(−174)	NS		
			IL-10(−627)	NS		
			IL-10(−1082)	NS		
			VDR(1056)	NS		
			TLR-4(−299)	NS		
			TLR-4(−399)	NS		
			TNF-α(−308)	NS		
Cao et al. (2005)	Chinese	40 GAgP, 52 H	MMP-1(−1607) Allele 2	0.007	2.286	1.243–4.205
			Genotype	0.004	3.684	1.505–9.018
Galicia et al. (2006)	Japanese	43 AgP, 140H	IL-6R(+48892)	0.04	Not available	
			IL-6R(−183)	NS		
Gonzales et al. (2002)	German	18 AgP, 21 H	IL-10(−597)	NS		
			IL-10(−824)	NS		
Gonzales et al. (2003)	Caucasian	28 AgP, 33 H	IL-1α (+4845)	NS		
	Central American Hispanics	16 AgP, 14 H	IL-1β (+3954)	NS		
Gonzales et al. (2004)	Japanese	31 AgP, 30 H	IL-4	NS		
	Caucasian	30 AgP, 33 H		NS		
Itagaki et al. (2004)	Japanese	37 GAgP, 142 H	MMP-1(−1607)	NS		
			MMP-3(−1171)	NS		
James et al. (2007)	Caucasian (UK)	73 AgP, 123 H	TLR4(299)		0.30	0.10–0.91
			CD14(−159)	NS		
			CD14(−1359)	NS		
Jordan et al. (2005)	African-American	46 AgP, 78H	Lactoferrin T11A	0.0007	2.564	1.475–4.459
	Caucasian	77 AgP, 131 H		NS		
Kaneko et al. (2004)	Japanese	46 AgP, 80 H	FcαRI 324	0.014	2.54	1.20–5.38
Li et al. (2004)	Chinese	122 GAgP, 95 H	IL-1A(+4845)	0.039	5.58	1.09–28.68
			IL-1B(−511)	0.048	3.16	1.01–9.89
			IL-1B(+3954)	NS		
Loos et al. (2003)	Caucasian (Northern European)	12 AgP, 61 H	FcγRIIIa-V158	NS		
			FcγRIIa-H131 allele	0.013	3.68	1.29–10.5
			Genotype	0.026	9.07	1.29–63.56
			FcγRIIIb	NS		
Nibali et al. (2006)	Caucasian (UK)	224 AgP, 231 H	FcγRIIIa, FcγRIIb	NS		
			FcγRIIIb	NS		
			FPR 301,546,568	NS		
			FcγRIIa	NS		
			FcαR	NS		
			NADPH	0.003	1.87	1.27–2.83
Quappe et al. (2004)	Chilean	36 AgP, 75 H	IL-1A(−889)	NS		
			IL-1B(+3954)	0.03	2.86	1.06–7.71
Sakellari et al. (2006)	Greek	46 AgP, 90 H	IL-1A (+4845), IL-1B (+3954), IL-1RN (VNTRs), TNFA (−308), COL1A1, SP1	NS		
Soedarsono et al. (2006)	Japanese	99 AgP, 89 H	RNAK, RANKL, OPG	NS		
Suzuki et al. (2004)	Japanese	134 AgP, 125 H	310 SNPs in 125 genes	AgP associated with IL6ST, PTGDS, COL4A1, COL1A1, KRT23		
Tai et al. (2002)	Japanese	47 AgP, 97 H	IL-1α(+4845)	NS		
			IL-1β(−511)	NS		
			IL-1β(+3954)	NS		
			IL-1 RN(VNTR)	0.007	3.40	1.24–9.52
Zhang et al. (2004)	Han Chinese	90 AgP, 91 H	ER-α	NS		

OR, odds ratio; 95% CI, 95% confidence interval; GAgP, generalized aggressive periodontitis; H, healthy controls; NS, not significant; TGF-β1, transforming growth factor-beta 1; eNOS, endothelial nitric oxide synthase; IL, interleukin; VDR, vitamin D receptor; TLR4, toll-like receptor 4; TNF, tumor necrosis factor; MMP, matrix metalloproteinase; IL-6R, interleukin-6 receptor; CD14, cluster differentiation 14; FcαR, immunoglobulin A Fc receptor; FcγR, Fc-gamma receptor; FPR, formyl peptide receptor; NADPH, NADPH oxidase $P22^{phox}$ (CYBA gene); IL-1RN(VNTRs), interleukin-1 receptor antagonist, a variable number of tandem repeats; COL1A1, collagen type I, α1; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; OPG, osteoprotegrin; IL6ST, interleukin 6 signal transducer; PTGDS, prostaglandin D2 synthase; COL4A1, collagen type IV, α1; KRT 23, keratin 23; ER-α, estrogen receptor-α.

Sp1, resulting in transcription repression. In sum, it is postulated that the −765C allele disrupts the Sp1 binding to the promoter of the COX-2 gene, resulting in transcription repression subsequently. COX-2 gene expression is lower and consequently the COX-2-dependent PGE_2 production is reduced. The GC and CC genotypes were less responsive upon stimulation of LPS, resulting in less PGE_2 production compared with the GG genotype. This might relate to the protective effect of the −765C allele for periodontitis.

The results of Szczeklik et al. (2004) study were in contrast to those mentioned above. The PGE_2 and PGD_2 productions by peripheral monocytes were significantly higher in the CC homozygotes compared with the GG homozygotes. They implicated that the C allele had a functional consequence, resulting in increased biosynthesis of PGs by monocytes. Egr-1 regulates gene transcription either positively or negatively, because there are both transactivation and repression domains in the Egr-1 gene (Gashler et al. 1993, Tan et al. 2003). This might help explain why the results of these studies for the function of this genetic variant were conflicting.

The precise mechanism by which the −765G>C polymorphism of COX-2 gene may affect the susceptibility of periodontitis is unclear. COX-2 is classically categorized as a pro-inflammatory enzyme. Nevertheless, COX-2 may have anti-inflammatory properties revealed from the carrageenin-induced pleurisy model (Gilroy et al. 1999). Prostanoids can act both to promote and inhibit inflammation (Tilley et al. 2001). The inflammatory response is associated with the level and the profile of prostanoids produced during inflammation and is determined by the nature and the activation state of the cells present in the inflammatory lesion. It is unclear whether the expressions of Sp1, Sp3 and Egr-1 depend on the cell/tissue type or not. Further investigations are needed to understand the interaction between this genetic variant and the related regulatory factors and to clarify the role of this genetic variant in the pathogenesis of periodontitis.

The association of functional outcome in the −765G>C polymorphism and a variety of diseases has been evaluated. The results are disparate. Some studies reported that the −765C allele may be protective in cardiovascular diseases (myocardial infarction, stroke and outcome of coronary artery bypass surgery), accompanied by decreased plasma C-reactive protein levels (Papafili et al. 2002, Cipollone et al. 2004). Other studies, however, revealed that the −765C allele led to susceptibility in type 2 diabetes (Konheim & Wolford 2003), bronchial asthma of females (Szczeklik et al. 2004) and sarcoidosis (Hill et al. 2006). The fact that the COX-2^{-765} genetic polymorphism seems to be protective or is associated with susceptibility in some diseases is consistent with COX-2 having pro-, anti-inflammatory and anti-fibrotic functions. Genetic linkage analysis performed at localized AgP (LAP) families showed that LAP was linked to chromosome 1q25, covering the COX-2 gene, but no nucleotide variation in the COX-2 gene was identified in these LAP patients (Li et al. 2004). According to the results of our study, it is likely that the −765C allele is protective for periodontitis. As far as we know, the result of this study is the first to link COX-2 genetic polymorphism strongly to periodontitis.

As in any case–control study, there are limitations, such as the periodontal diagnostic categories. Further, only one polymorphism of the COX-2 gene was evaluated by the present study. In the promoter region of the COX-2 gene, there are several putative transcription-factor-binding sites, including nuclear factor-IL-6 (NF-IL-6) and NF-κB. We did not evaluate the other variants of this gene and the effect of the haplotype combinations. In addition, the effects of −765G>C on the in vitro and in vivo expression of COX-2 and the prostanoids are conflicting and unknown, respectively. We did not carry out biological studies on the effects of the COX-2 polymorphism on the production, activity and/or function of PGE_2 upon stimulation in periodontal tissues. Therefore, the interpretation of the results of the present study in relation to the COX-2 genotype and susceptibility to periodontitis should be cautious. To establish the overall association between this genetic variant and periodontitis, further studies among larger populations and populations of other ethnicities are needed because of the relatively low frequency of the CC genotype. Further studies are also necessary to investigate the extent to which COX-2 genetic polymorphism alters or modifies the expression or function of this enzyme induced in resident cells of periodontal tissue, such as macrophages and fibroblasts. Then, the importance of the COX-2^{-765} polymorphism can be confirmed and the definite link between the COX-2 genotype and the phenotypes of periodontitis can be made.

Periodontitis is multifactorial in nature, involving interactions between the gene, environment and lifestyle. New insights into the PGs production affected by the COX-2 genetic polymorphism provide an altered paradigm of periodontal disease that emphasizes the variation of host response, rather than the bacterial aetiology. In addition, it also provides a possible explanation for host susceptibility to periodontal disease.

In summary, we have identified a common promoter variant in the COX-2 gene, −765G>C, which is significantly inversely associated with the risks of both AgP and CP. The presence of the GC or the CC genotype in COX-2^{-765} may have implications for a decreased risk of periodontitis in Taiwanese, especially for AgP. Studies in periodontitis patients in other ethnic populations are needed to confirm the significance of the findings of this study.

References

Airila-Månsson, S., Söder, B., Kari, K. & Meurman, J. H. (2006) Influence of combinations of bacteria on the levels of prostaglandin E_2, interleukin-1β, and granulocyte elastase in gingival crevicular fluid and on the severity of periodontal disease. *Journal of Periodontology* **77**, 1025–1031.

Arikawa, T., Omura, K. & Morita, I. (2004) Regulation of bone morphogenetic protein-2 expression by endogenous prostaglandin E_2 in human mesenchymal stem cells. *Journal of Cellular Physiology* **200**, 400–406.

Atilla, G., Emingil, G., Kose, T. & Berdeli, A. (2006) TGF-beta 1 gene polymorphisms in periodontal diseases. *Clinical Biochemistry* **39**, 929–934.

Berdeli, A., Gurkan, A., Emingil, G., Atilla, G. & Kose, T. (2006) Endothelial nitric oxide synthase Glu298Asp gene polymorphism in periodontal diseases. *Journal of Periodontology* **77**, 1348–1354.

Biyikoğlu, B., Buduneli, N., Kardesler, L., Aksu, K., öder, G. & Kütükçüler, N. (2006) Evaluation of t-PA, PAI-2, IL-1β and PGE_2 in gingival crevicular fluid of rheumatoid arthritis patients with periodontal disease. *Journal of Clinical Periodontology* **33**, 605–611.

Blin, N. & Stafford, D. W. (1976) A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Research* **3**, 2303–2308.

Brett, P. M., Zygogianni, P., Griffiths, G. S., Tomaz, M., Parkar, M., D'Aiuto, F. & Tonetti,

M. (2005) Functional gene polymorphisms in aggressive and chronic periodontitis. *Journal of Dental Research* **84**, 1149–1153.

Cao, Z., Li, C., Jin, L. & Corbet, E. F. (2005) Association of matrix metalloproteinase-1 promoter polymorphism with generalized aggressive periodontitis in a Chinese population. *Journal of Periodontal Research* **40**, 427–431.

Cipollone, F., Toniato, E., Martinotti, S., Fazia, M., Iezzi, A., Cuccurullo, C., Pini, B., Ursi, S., Vitullo, G., Averna, M., Arca, M., Montali, A., Campagna, F., Ucchino, S., Spigonardo, F., Taddei, S., Virdis, A., Ciabattoni, G., Notarbartolo, A., Cuccurullo, F. & Mezzetti, A. (2004) A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. *The Journal of the American Medical Association* **291**, 2221–2228.

Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B. A. & Lipsky, P. E. (1998) Cyclooxygenase in biology and disease. *FASEB Journal* **12**, 1063–1073.

Fritsche, E., Baek, S. J., King, L. M., Zeldin, D. C., Eling, T. E. & Bell, D. A. (2001) Functional characterization of cyclooxygenase-2 polymorphisms. *The Journal of Pharmacology and Experimental Therapeutics* **299**, 468–476.

Fujita, D., Yamashita, N., Iita, S., Amano, H., Yamada, S. & Sakamoto, K. (2003) Prostaglandin E_2 induced the differentiation of osteoclasts in mouse osteoblast-depleted bone marrow cells. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **68**, 351–358.

Galicia, J. C., Tai, H., Komatsu, Y., Shimada, Y., Ikezawa, I. & Yoshie, H. (2006) Interleukin-6 receptor gene polymorphisms and periodontitis in a non-smoking Japanese population. *Journal of Clinical Periodontology* **33**, 704–709.

Gashler, A. L., Swaminathan, S. & Sukhatme, V. P. (1993) A novel repression module, an extensive activation domain, and a bipartite nuclear localization signal defined in the immediate-early transcription factor Egr-1. *Molecular and Cellular Biology* **13**, 4556–4571.

Gilroy, D. W., Colville-Nash, P. R., Willis, D., Chivers, J., Paul-Clark, M. J. & Willoughby, D. A. (1999) Inducible cyclooxygenase may have anti-inflammatory properties. *Nature Medicine* **5**, 698–701.

Gonzales, J. R., Kobayashi, T., Michel, J., Mann, M., Yoshie, H. & Meyle, J. (2004) Interleukin-4 gene polymorphisms in Japanese and Caucasian patients with aggressive periodontitis. *Journal of Clinical Periodontology* **31**, 384–389.

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.

Gonzales, J. R., Michel, J., Rodriguez, E. L., Herrmann, J. M., Bodeker, R. H. & Meyle, J. (2003) Comparison of interleukin-1 genotypes in two populations with aggressive periodontitis. *European Journal of Oral Science* **111**, 395–399.

Hill, M. R., Papafili, A., Booth, H., Lawson, P., Hubner, M., Beynon, H., Read, C., Lindahl, G., Marshall, R. P., McAnulty, R. J. & Laurent, G. J. (2006) Functional prostaglandin-endoperoxide synthase 2 polymorphism predicts poor outcome in sarcoidosis. *American Journal of Respiratory and Critical Care Medicine* **174**, 915–922.

Hofbauer, L. C., Khosla, S., Dunstan, C. R., Lacey, D. L., Boyle, W. J. & Riggs, B. L. (2000) The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *Journal of Bone and Mineral Research* **15**, 2–12.

Itagaki, M., Kubota, T., Tai, H., Shimada, Y., Morozumi, T. & Yamazaki, K. (2004) Matrix metalloproteinase-1 and -3 gene promoter polymorphisms in Japanese patients with periodontitis. *Journal of Clinical Periodontology* **31**, 764–769.

James, J. A., Poulton, K. V., Haworth, S. E., Payne, D., McKay, I. J., Clarke, F. M., Hughes, F. J. & Linden, G. J. (2007) Polymorphisms of TLR4 but not CD14 are associated with a decreased risk of aggressive periodontitis. *Journal of Clinical Periodontology* **34**, 111–117.

Jordan, W. J., Eskdale, J., Lennon, G. P., Pestoff, R., Wu, L., Fine, D. H. & Gallagher, G. (2005) A non-conservative, coding single-nucleotide polymorphism in the N-terminal region of lactoferrin is associated with aggressive periodontitis in an African–American, but not a Caucasian population. *Genes Immunology* **6**, 632–635.

Kaneko, S., Kobayashi, T., Yamamoto, K., Jansen, M. D., van de Winkel, J. G. & Yoshie, H. (2004) A novel polymorphism of FcalphaRI (CD89) associated with aggressive periodontitis. *Tissue Antigens* **63**, 572–577.

Kim, H. J., Kim, K. W., Yu, B. P. & Chung, H. Y. (2000) The effect of age on cyclooxygenase-2 gene expression: NF-κB activation and IκBα degradation. *Free Radical Biology and Medicine* **28**, 683–692.

Konheim, Y. L. & Wolford, J. K. (2003) Association of a promoter variant in the inducible cyclooxygenase-2 gene (PTGS2) with type 2 diabetes mellitus in Pima Indians. *Human Genetics* **113**, 377–381.

Kornman, K. S., Crane, A., Wang, H.-Y., di Giovine, F. S., Newman, M. G., Pirk, F. W., Wilson, T. G. Jr., Higginbottom, F. L. & Duff, G. W. (1997) The interleukin-1 genotype as a severity factor in adult periodontal disease. *Journal of Clinical Periodontology* **24**, 72–77.

Kosaka, T., Miyata, A., Ihara, H., Hara, S., Sugimoto, T., Takeda, O., Takahashi, E. & Tanabe, T. (1994) Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. *European Journal of Biochemistry* **221**, 889–897.

Li, Q. Y., Zhao, H. S., Meng, H. X., Zhang, L., Xu, L., Chen, Z. B., Shi, D., Feng, X. H. & Zhu, X. L. (2004) Association analysis between interleukin-1 family polymorphisms and generalized aggressive periodontitis in a Chinese population. *Journal of Periodontology* **75**, 1627–1635.

Li, Y., Xu, L., Hasturk, H., Kantarci, A., DePalma, S. R. & Van Dyke, T. E. (2004) Localized aggressive periodontitis is linked to human chromosome 1q25. *Human Genetics* **114**, 291–297.

Loos, B. G., John, R. P. & Laine, M. L. (2005) Identification of genetic risk factors for periodontitis and possible mechanisms of action. *Journal of Clinical Periodontology* **32** (Suppl. 6), 159–179.

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

Marnett, L. J., Rowlinson, S. W., Goodwin, D. C., Kalgutkar, A. S. & Lanzo, C. A. (1999) Arachidonic acid oxygenation by COX-1 and COX-2. *Journal of Biological Chemistry* **274**, 22903–22906.

Miyauchi, M., Hiraoka, M., Oka, H., Sato, S., Kudo, Y., Ogawa, I., Noguchi, K., Ishikawa, I. & Takata, T. (2004) Immunolocalization of COX-1 and COX-2 in the rat molar periodontal tissue after topical application of lipopolysaccharide. *Archives of Oral Biology* **49**, 739–746.

Moon, Y., Glasgow, W. C. & Eling, T. E. (2005) Curcumin suppresses interleukin-1β mediated microsomal prostaglandin E synthase 1 by altering early growth response gene 1 and other signaling pathways. *The Journal of Pharmacology and Experimental Therapeutics* **315**, 788–795.

Morton, R. S. & Dongari-Bagtzoglou, A. I. (2001) Cyclooxygenase-2 is upregulated in inflamed gingival tissues. *Journal of Periodontology* **72**, 461–469.

Nibali, L., Parkar, M., Brett, P., Knight, J., Tonetti, M. S. & Griffiths, G. S. (2006) NADPH oxidase (CYBA) and FcγR polymorphisms as risk factors for aggressive periodontitis A case-control association study. *Journal of Clinical Periodontology* **33**, 529–539.

Offenbacher, S., Heasman, P. A. & Collins, J. G. (1993) Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. *Journal of Periodontolology* **64**, 432–444.

Offenbacher, S., Odle, B. M. & van Dyke, T. E. (1986) The use of crevicular fluid prostaglandin E_2 levels as a predictor of periodontal attachment loss. *Journal of Periodontal Research* **21**, 101–112.

Offenbacher, S. & Salvi, G. E. (1999) Induction of prostaglandin release from macrophages by bacterial endotoxin. *Clinical Infectious Diseases* **28**, 505–513.

Papafili, A., Hill, M. R., Brull, D. J., McAnulty, R. J., Marshall, R. P., Humphries, S. E. & Laurent, G. J. (2002) Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arteriosclerosis, Thrombosis, and Vascular Biology* **22**, 1631–1636.

Quappe, L., Jara, L. & Lopez, N. J. (2004) Association of interleukin-1 polymorphisms with aggressive periodontitis. *Journal of Periodontology* **75**, 1509–1515.

Sakellari, D., Katsares, V., Georgiadou, M., Kouvatsi, A., Arsenakis, M. & Konstantinidis, A. (2006) No correlation of five gene polymorphisms with periodontal conditions in a Greek population. *Journal of Clinical Periodontology* **33**, 765–770.

Smith, W. L., Garavito, R. M. & DeWitt, D. L. (1996) Prostaglandin endoperoxide H synthases (cyclooxygenase)-1 and -2. *Journal of Biological Chemistry* **271**, 33157–33160.

Soedarsono, N., Rabello, D., Kamei, H., Fuma, D., Ishihara, Y., Suzuki, M., Noguchi, T., Sakaki, Y., Yamaguchi, A. & Kojima, T. (2006) Evaluation of RANK/RANKL/OPG gene polymorphisms in aggressive periodontitis. *Journal of Periodontal Research* **41**, 397–404.

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

Suske, G. (1999) The Sp-family of transcription factors. *Gene* **238**, 291–300.

Suzuki, A., Ji, G., Numabe, Y., Muramatsu, M., Gomi, K., Kanazashi, M., Ogata, Y., Shimizu, E., Shibukawa, Y., Ito, T., Sugaya, A., Arai, T., Yamada, S., Deguchi, S. & Kamoi, K. (2004) Single nucleotide polymorphisms associated with aggressive periodontitis and severe chronic periodontitis in Japanese. *Biochemical and Biophysical Research Communications* **317**, 887–892.

Szczeklik, W., Sanak, M. & Szczeklik, A. (2004) Functional effects and gender association of COX-2 gene polymorphism $G_{-765}C$ in bronchial asthma. *Journal of Allergy and Clinical Immunology* **114**, 248–253.

Tai, H., Endo, M., Shimada, Y., Gou, E., Orima, K., Kobayashi, T., Yamazaki, K. & Yoshie, H. (2002) Association of interleukin-1 receptor antagonist gene polymorphisms with early onset periodontitis in Japanese. *Journal of Clinical Periodontology* **29**, 882–888.

Tai, H., Miyaura, C., Pilbeam, C. C., Tamura, T., Ohsugi, Y., Koishihara, Y., Kubodera, N., Kawaguchi, H., Raisz, L. G. & Suda, T. (1997) Transcriptional induction of cyclooxygenase-2 in osteoblasts is involved in interleukin-6-induced osteoclast formation. *Endocrinology* **138**, 2372–2379.

Tan, L., Peng, H., Osaki, M., Choy, B. K., Auron, P. E., Sandell, L. J. & Goldring, M. B. (2003) Egr-1 mediates transcriptional repression of COL2A1 promoter activity by interleukin-1 β. *The Journal of Biological Chemistry* **278**, 17688–17700.

Tilley, S. L., Coffman, T. M. & Koller, B. H. (2001) Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *The Journal of Clinical Investigation* **108**, 15–23.

Trevilatto, P. C., Scarel-Caminaga, R. M., de Brito, R. B. Jr., de Souza, A. P. & Line, S. R. P. (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.

Tsai, C. C., Hong, Y. C., Chen, C. C. & Wu, Y. M. (1998) Measurement of prostaglandin E_2 and leukotriene B_4 in the gingival crevicular fluid. *Journal of Dentistry* **26**, 97–103.

Xu, Q., Ji, Y. S. & Schmedtje, J. F. Jr. (2000) Sp1 increases expression of cyclooxygenase-2 in hypoxic vascular endothelium. Implications for the mechanisms of aortic aneurysm and heart failure. *Journal of Biological Chemistry* **275**, 24583–24589.

Yucel-Lindberg, T., Ahola, H., Carlstedt-Duke, J. & Modéer, T. (1999) Involvement of tyrosine kinases on cyclooxygenase expression and prostaglandin E_2 production in human gingival fibroblasts stimulated with interleukin-1β and epidermal growth factor. *Biochemical and Biophysical Research Communications* **257**, 528–532.

Zhang, F., Engebretson, S. P., Morton, R. S., Cavanaugh, P. F. Jr., Subbaramaiah, K. & Dannenberg, A. J. (2003) The overexpression of cyclooxygenase-2 in chronic periodontitis. *Journal of American Dental Association* **134**, 861–867.

Zhang, L., Meng, H., Zhao, H., Li, Q., Xu, L., Chen, Z., Shi, D. & Feng, X. (2004) Estrogen receptor-alpha gene polymorphisms in patients with periodontitis. *Journal of Periodontal Research* **39**, 362–366.

Address:
Chi-Cheng Tsai
Graduate Institute of Dental Sciences
College of Dental Medicine
Kaohsiung Medical University
No. 100 Shih-Chuan
1st Road, Kaohsiung 807
Taiwan
E-mail: chchts@kmu.edu.tw

Clinical Relevance

Scientific rationale for the study: Periodontitis is multifactorial in nature, involving bacterial, genetic and environmental factors. COX-2 catalyses the synthesis of PGE_2, a potent biomediator in periodontal tissue destruction, at sites of inflammation. COX-2 genetic polymorphism might modulate the inflammatory response. To identify the genetic contribution of COX-2 in periodontitis, we analysed the association of $COX\text{-}2^{-765}$ SNP and the risk for periodontitis.

Principal findings: The rare −765C allele of the COX-2 gene was associated with decreased susceptibility to AgP and CP.

Practical implications: COX-2 −765C allele might be a protective factor against periodontitis in Taiwanese.