Cyclooxygenase 2 gene polymorphisms and chronic periodontitis in a North Indian population: a pilot study

Anika Daing1,†, Sarvendra Vikram Singh2,†, Charanjeet Singh Samb1, Mohammad Akhlaq Khan1, Srikanta Kumar Rath2,*

1Department of Periodontics, Faculty of Dental Sciences, Chhatrapati Shahuji Maharaj Medical University, Lucknow, India
2Genotoxicity Laboratory, Toxicology Division, CSIR-Central Drug Research Institute, Lucknow, India

Purpose: Cyclooxygenase (COX) enzyme catalyzes the production of prostaglandins, which are important mediators of tissue destruction in periodontitis. Single nucleotide polymorphisms of COX2 enzyme have been associated with increasing susceptibility to inflammatory diseases. The present study evaluates the association of two single nucleotide polymorphisms in COX2 gene (-1195G>A and 8473C>T) with chronic periodontitis in North Indians.

Methods: Both SNPs and their haplotypes were used to explore the associations between COX2 polymorphisms and chronic periodontitis in 56 patients and 60 controls. Genotyping was done by polymerase chain reaction followed by restriction fragment length polymorphism. Chi-square test and logistic regression analysis were performed for association analysis.

Results: By the individual genotype analysis, mutant genotypes (GA and AA) of COX2 -1195 showed more than a two fold risk (odds ratio [OR]>2) and COX2 8473 (TC and CC) showed a reduced risk for the disease, but the findings were not statistically significant. Haplotype analysis showed that the frequency of the haplotype AT was higher in the case group and a significant association was found for haplotype AT (OR, 1.79; 95% confidence interval, 1.03 to 3.11; P=0.0370) indicating an association between the AT haplotype of COX2 gene SNPs and chronic periodontitis.

Conclusions: Individual genotypes of both the SNPs were not associated while haplotype AT was found to be associated with chronic periodontitis in North Indians.

Keywords: Chronic periodontitis, Cyclooxygenase 2, Single nucleotide polymorphism.

INTRODUCTION

Chronic periodontitis is an inflammatory disease of the tooth supporting tissues and the alveolar bone. While presence of pathogenic bacteria is essential for the initiation of this disease, environmental and genetic factors are instrumental in modifying the disease process [1]. Human genetic variants that occur most frequently in the form of single nucleotide polymorphisms (SNPs) contribute to individual variations in susceptibility to chronic periodontitis and its severity [2]. SNPs in the genes of certain inflammatory mediators and enzymes have shown strong association with chronic periodontitis in previous studies [3-10].

Cyclooxygenase (COX), also known as prostaglandin endoperoxide synthetase (PTGS) converts arachidonic acid to prostaglandins (PGs) and plays a pivotal role in the inflammatory process [11]. The two isoforms of COX designated as COX1 and COX2 are encoded on distinct genes but essentially catalyse the same reaction. COX1 is constitutively expressed in many tissues and COX2 is inducible in inflammatory con-
ditions including periodontitis. Release of proinflammatory cytokines (interleukin [IL]-1, IL-6, tumor necrosis factor-α), growth factors, and lipopolysaccharides in periodontitis activate a variety of cells of periodontal tissues to produce COX2-mediated prostaglandin E2 (PGE2) [12-14]. COX2 expression and the abundance of its enzymatic product PGE2 play key roles in influencing tissue destruction in periodontitis [15,16]. Currently, there are very few reports on the role of COX2 gene polymorphism in chronic periodontitis [13,17,18]. The present study aimed to evaluate the association of two common SNPs, -1195G>A (rs689466) and 8473T>C (rs5275) in the COX2 gene, with chronic periodontitis in a North Indian population as the COX2 gene polymorphism might play a role in modifying the risk of the disease.

MATERIALS AND METHODS

Selection of subjects and study design

One hundred sixteen subjects were recruited from the outpatient wing of the Department of Periodontics, Faculty of Dental Sciences, C.S.M.M.U, Lucknow, Uttar Pradesh, India for the present case-control study. All the subjects who participated in the study were from North Indian subpopulations, belonging to the state of Uttar Pradesh. Written informed consent was obtained from each subject as per human ethics guidelines. The study protocol was reviewed and approved by the institution’s ethical review board (CDRI/IEC/CBM/07-07-10). The medical and dental histories of the subjects were recorded by means of a questionnaire.

A complete periodontal examination was carried out by a single examiner using a UNC-15 probe. Clinical periodontal parameters including probing pocket depth, clinical attachment level, plaque index [19], calculus index [20], and gingival index [21] were recorded. The diagnosis of the subjects was established based on the clinical criteria proposed by the 1999 International Workshop for Classification of Periodontal Diseases and Conditions [22]. A radiographic analysis was performed using full mouth intraoral periapical radiographs to assess the bone level and confirm the clinical diagnosis. The subjects were categorized into two groups: a generalized moderate to severe chronic periodontitis group (n=56), which consisted of subjects exhibiting clinical attachment loss ≥3 mm at >30% of the sites; and the control group (n=60), which consisted of subjects showing no signs of periodontitis as determined by absence of clinical attachment loss and no sites with a probing pocket depth ≥3 mm. Exclusion criteria included the following: systemic diseases like diabetes, hepatitis, immunosuppressive disorders, and bleeding disorders; history of any disease known to severely compromise immune function; current pregnancy or lactation; smoking tobacco or using smokeless tobacco; and currently taking anti-inflammatory drugs or having taken them in the previous 3 months.

Blood sample collection and DNA isolation

Two mL blood was collected from each subject in ethylenediaminetetraacetic acid tubes by venipuncture at the antecubital fossa. The tubes were maintained in ice and transported to the Genotoxicity Laboratory, CSIR-Central Drug Research Institute for isolation of genomic DNA and SNP analysis. DNA was isolated from the blood samples using a kit (Gene Elute Blood Genomic DNA kit, NA2010, Sigma-Aldrich Co., St. Louis, MO, USA) following the manufacturer’s protocol, quantified using a spectrophotometer (Genequant, Biochrom Ltd., Cambridge, UK) and stored at -20°C until further processing.

Selection of SNPs

Two SNPs, rs689466, G>A (chromosome 1, promoter region) and rs5275, T>C (chromosome 1, 3’UTR), in the prostaglandin endoperoxidase synthetase 2 (PTGS2 or COX2) gene were selected for the study based on the available literature.

Polymerase chain reaction (PCR)

A gradient PCR reaction was performed initially for standardization of DNA amplification conditions and optimization of the annealing temperature for the primer sets. Subsequently, PCR reactions were performed using the optimized annealing temperatures. All PCR reactions were performed in 200 μL transparent PCR tubes on a Peltier-based thermal cycler (MJ Research PTC-100 Thermal Cycler, Watertown, MA, USA) using reagents from Fermentas Life Sciences (Fermentas Life Sciences, Burlington, ON, Canada) in a total reaction volume of 20 μL containing nearly 100 ng genomic DNA, 1.5 U Taq polymerase in 1× PCR buffer, 1.5 mM MgCl2, 10 pmol of each primer and 10 mM dNTPs. The primers used are shown in Table 1 [23,24]. Thermal cycling conditions were as follows: initial denaturation step at 95°C for 7 minutes, 31 cycles of PCR consisting of denaturation at 94°C for 30 seconds, annealing at 61°C and 49°C (COX2-1195 and COX2-8473, respectively) for 30 seconds and extension at 72°C for 30 seconds, followed by a final extension step at 72°C for 7 minutes. The reaction was held at 4°C. The PCR products were visualized by electrophoresis on 1.2% agarose gel. For gel electrophoresis, 5 μL of the amplified product was mixed with 1 μL of 6× gel loading buffer (analytical grade water containing 30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol) and resolved on 1.2% agarose gel in TAE buffer at 80 volts for 2 hours. 50 bp DNA markers (Gene Ruler, SM0371, Fermentas Inc., a subsidiary of Thermo Fisher Scientific Inc., Rockford,
Anika Daing et al.

IL, USA) were run with the amplified products as a reference. PCR products were assessed based upon the presence of a 273 base pair (bp) product for COX2 -1195 and a 177 bp product for COX2 8473.

Restriction fragment length polymorphism
COX2 -1195G>A

The 10 µL PCR product was mixed with 2 µL buffer and was digested with 0.5 µL of PvuII enzyme. The reaction mixture was incubated at 37°C overnight in a water bath. The resulting digestion products of 220+53 bp (allele G) and 273 bp (allele A) were visualized using 2% agarose gel electrophoresis with ethidium bromide staining (Table 1).

COX2 8473T>C

The 10 µL PCR product was digested with 0.5 µL of BcII enzyme. The reaction mixture was incubated at 50°C overnight in a water bath. The resulting digestion products of 177 bp (T allele) and 156+21 bp (C allele) were visualized using 2.5% agarose gel electrophoresis with ethidium bromide staining (Table 1).

Statistical analysis

For the present study, statistical analysis of data was performed using the SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism ver. 5 (GraphPad Software Inc., La Jolla, CA, USA). Hardy-Weinberg equilibrium was tested to compare the observed and expected genotype frequencies in the control group. The distribution of genotype and allele frequency in the chronic periodontitis and control groups were compared using an independent t-test and chi-squared test, respectively. SHEsis software was used for the linkage disequilibrium (LD) index (D’ and r²) and haplotype analysis between genetic markers [25,26]. Haplotypes were constructed in the following order: COX2-1195GA/COX2 8473TC. All tests used for the analysis were two sided and a probability value <0.05 was considered statistically significant.

RESULTS

The mean age (±SD) was 43.30 (±11.8) years for chronic periodontitis patients and 41.35 (±11.68) years for control subjects (P=0.373). No statistically significant differences were present in age or gender distribution between the study groups (Table 2). Genotype and allelic distribution of COX2 -1195 and COX2 8473 polymorphisms in the control and chronic periodontitis groups are shown in Table 3. In case of COX2 -1195 SNP, distribution of mutant allele A and genotypes containing allele A (GA+AA) were higher in the chronic periodontitis group (58% and 82.1%, respectively) than in the control group (45.8% and 66.7%). The results show that an OR of more than two was found for the mutant genotypes when compared with the wild type (GA/GG: OR, 2.16; P=0.106 and AA/GG: OR, 2.53; P=0.073). For the COX2 8473 SNP, the prevalence of C (mutant) allele carriers was higher in the controls (35%) than in the diseased subjects (26.8%). The results show that an OR of more than two was found for the mutant genotypes when compared with the wild type (GA/GG: OR, 2.16; P=0.106 and AA/GG: OR, 2.53; P=0.073). For the COX2 8473 SNP, the prevalence of C (mutant) allele carriers was higher in the controls (35%) than in the diseased subjects (26.8%). When the genotypes were compared for association, an OR of less than one was found for mutant genotypes (TC/TT: OR, 0.617; P=0.247 and CC/TT: OR, 0.59; P=0.349); however, neither of these two SNPs was found to be significantly associated with chronic periodontitis (P>0.05) prior to or after adjustment for age and gender.

Haplotype analysis

To evaluate the extent of LD, D’ and r² between pairs of polymorphisms were calculated. It was found that neither of the SNPs were in LD (D’-0.134 and r²-0.008). When both the SNPs were assessed for haplotype analysis, four potential haplotypes were constructed: COX2-1195GA/COX2 8473TC. All tests used for the analysis were two sided and a probability value <0.05 was considered statistically significant.

Table 1. Polymerase chain reaction primers, restriction enzymes and lengths of fragments generated upon restriction digestion.

| SNPs         | Primers                                  | Restriction enzyme | Allele | Fragment size (bp) | Reference       |
|--------------|------------------------------------------|--------------------|--------|--------------------|-----------------|
| COX2 -1195GA | FP: 5’CCCTGAGCACTACCCATGAT-3’             | PvuII              | G      | 220+53             | Zhang et al. [23] (2005) |
|              | RP: 5’GCCCTTCATAGGAGATACTGG-3’            |                    | A      | 273                |                 |
| COX2 8473TC  | FP: 5’GAATTATAAAGTACCTTATGAT-3’           | BcII               | T      | 177                | Sanak et al. [24] (2005) |
|              | RP: 5’CTTTACAGGTGATTCTTACC-3’             |                    | C      | 156+21             |                 |

SNPs: single nucleotide polymorphisms, FP: forward primer, RP: reverse primer.

Table 2. Characteristics of control and chronic periodontitis groups.

| Characteristic | Controls (n = 60) | ChP (n = 56) | Pvalue |
|---------------|------------------|--------------|--------|
| Age (year)    | 41.35±11.686     | 43.30±11.818 | 0.373  |
| Gender        |                  |              |        |
| Female        | 24 (40)          | 26 (46.4)    | 0.4848 |
| Male          | 36 (60)          | 30 (53.6)    |        |

Values are presented as mean±SD or number (%). ChP: chronic periodontitis group.

1 Differences between groups was analyzed by an independent t-test.
1 Differences between groups were analyzed by chi-square test (P<0.5).
Table 3. Distribution of genotype and allele of COX2 gene polymorphism in control and chronic periodontitis groups.

| SNP       | Controls (n=60) | Chronic periodontitis (n=56) | P-value | OR (95% CI, P-value) | ORa (95% CI, P-value) |
|-----------|----------------|-----------------------------|---------|----------------------|-----------------------|
| COX2 -1195G>A |                |                             |         |                      |                       |
| Genotypes |                |                             |         |                      |                       |
| GG (WW)   | 20 (33.3)      | 10 (17.9)                   | 0.1535  | 2.16 (0.85-5.50, 0.106) | 2.05 (0.79-5.3, 0.139) |
| GA (WM)   | 25 (41.7)      | 27 (48.2)                   |         |                      |                       |
| AA (MM)   | 15 (25.0)      | 19 (33.9)                   |         |                      |                       |
| Alleles   |                |                             |         |                      |                       |
| G (W)     | 65 (54.2)      | 47 (42.0)                   | 0.0630  | 1.63 (0.97-2.75)      |                       |
| A (M)     | 55 (45.8)      | 65 (58.0)                   |         |                      |                       |
| COX2 8473T>C |                |                             |         |                      |                       |
| Genotypes |                |                             |         |                      |                       |
| TT (WW)   | 28 (46.7)      | 33 (58.9)                   | 0.4166  | 0.617 (0.27-1.39, 0.247) | 0.601 (0.26-1.39, 0.233) |
| TC (MM)   | 22 (36.7)      | 16 (28.6)                   |         |                      |                       |
| CC (MM)   | 10 (16.6)      | 7 (12.5)                    | 0.59 (0.20-1.76, 0.349) | 0.587 (0.193-1.785, 0.348) |
| Alleles   |                |                             |         |                      |                       |
| T (W)     | 78 (65)        | 82 (73.2)                   | 0.1766  | 0.68 (0.39-1.91)      |                       |
| C (M)     | 42 (35)        | 30 (26.8)                   |         |                      |                       |

Values are presented as number (%).

P < 0.05 is considered to be significant.

SNPs: single nucleotide polymorphisms, OR: odds ratio, CI: confidence interval, W: wild, M: mutant.

aAge and gender adjusted odds ratio.

Table 4. Distribution of COX2 haplotype frequencies and odds ratios among chronic periodontitis and control groups.

| Haplotype | Controls (n=120) | Chronic periodontitis (n=112) | OR (95% CI, P-value) |
|-----------|------------------|-----------------------------|---------------------|
| AC        | 0.1855 (22)      | 0.1782 (20)                 | 0.9518 (0.9881-1.8562) | 0.8848 |
| AT        | 0.2728 (33)      | 0.4022 (45)                 | 1.7933 (10.0332-3.1125) | 0.0370 |
| GC        | 0.1645 (20)      | 0.0897 (10)                 | 0.5004 (0.2230-1.1228) | 0.0888 |
| GT        | 0.3772 (45)      | 0.3300 (37)                 | 0.6131 (0.4739-1.3951) | 0.4524 |

Haplotypes were constructed in the following order: COX2 -1195GA/COX2 8473TC using SHEsis software.

In inflamed periodontal tissues, activity of proinflammatory molecules leads to the production of COX2 mediated PGE2, which plays an important role in tissue destruction and bone resorption. COX2 is the crucial enzyme involved in conversion of arachidonic acid to PGs. COX2 dependent PGE2 acts on osteoblasts to increase the expression of receptor activation of NF-kB ligand on their surfaces and thus enhances osteoclastogenesis [37]. Polymorphisms within the COX2 gene therefore influence the inflammatory disease processes as suggested by previous studies [24, 28]. This report explored the probable association of two SNPs in the COX2 gene with chronic periodontitis in North Indians.

All of the subjects inducted into this study were recruited strictly from the North Indian subpopulation of Uttar Pradesh to maintain homogeneity within the ethnic group constituting mostly the Caucasian morphological subtype of the Indo-European linguistic group [29]. Smokers and smokeless tobacco users were excluded from the study as tobacco is a major risk factor in the development of periodontal diseases and previous studies have found that genetic association of chronic periodontitis was more evident when smokers were excluded [4-6, 30].

The human COX2 gene mapped to chromosome 1q25.2-q25.3 is about 8.3 kbp in size and consists of 10 exons [31]. Few SNPs in this gene have been studied for their effect in altering its expression or function although many SNPs have been identified. This study deals with COX2 -1195G>A, located in the promoter region and 8473T>C, located in the 3'UTR region. No association was found between COX2 -1195G>A haplotypes were formed, as presented in Table 4. There was a predominance of haplotype 'AT' in the chronic periodontitis group and haplotype 'GT' in the control group. Haplotype 'AT' was at significantly higher risk for chronic periodontitis (OR, 1.79; 95% CI, 1.03 to 3.11; P = 0.0370), indicating that the A allele at the -1195 locus increases the risk.

**DISCUSSION**

In inflamed periodontal tissues, activity of proinflammatory molecules leads to the production of COX2 mediated PGE2, which plays an important role in tissue destruction and bone resorption. COX2 is the crucial enzyme involved in conversion of arachidonic acid to PGs. COX2 dependent PGE2 acts on osteoblasts to increase the expression of receptor activation of NF-kB ligand on their surfaces and thus enhances osteoclastogenesis [37]. Polymorphisms within the COX2 gene therefore influence the inflammatory disease processes as suggested by previous studies [24, 28]. This report explored the probable association of two SNPs in the COX2 gene with chronic periodontitis in North Indians.
and chronic periodontitis (Table 3), even after adjustment of age and gender, indicating that age and gender were not additive to the risk of this SNP. Similar results were observed by Schaefer et al. [17] in Europeans. However, in a Chinese population, allele A was significantly associated with the risk of chronic periodontitis (OR, 1.46) [9]. A similar trend with a higher frequency of allele A in a chronic periodontitis group (58%) than in a control group (45.8%) was observed in the present study, nearing statistical significance (OR, 1.62; \(P = 0.0630\)).

The exact mechanism by which the variant alleles affect the COX2 gene function remains unclear. However, a few possible mechanisms have been suggested in previous studies. As -1195G>A is in the 5’ flanking region of the COX2 gene and many putative transcription factor binding sites exist in the promoter region of the COX2 gene [32], it is likely that variant alleles could influence the gene function by interfering with the specific binding between the transcription factors and the promoter sequences. A previous report indicated that the A allele of SNP COX2 -1195 resulted in a heightened gene expression and thus an increased enzymatic activity conferring an increased disease susceptibility to the host [33]. The same may be true in the present study as an increased prevalence of the A allele was observed in the chronic periodontitis group.

An association could not be observed for the allelic and genotypic distribution of COX2 8473T>C with chronic periodontitis in the current study (Table 3). A higher carriage of allele C was observed in the control group (35%) than in the chronic periodontitis group (26.8%), similar to the findings of Xie et al. [9] in a Chinese population where the 8473C allele was associated with a decreased risk for chronic periodontitis. The possible explanatory mechanism is that adenine-uracil rich motifs are present in the mRNA of the 3’UTR region of the COX2 gene, which are involved in regulation of COX2 production by acting as a message instability determinant and a translation inhibitory element [34,35]. The 8473T>C polymorphism changes these motifs to cause degradation of COX2 transcripts and thus a differential COX2 expression.

Haplotype analysis is thought to be more potent than analysing individual SNPs for detecting associations between genotype and phenotype. A haplotype model is better at detecting LD than are individual SNP markers [36,37]. Statistically, low D’ and r² values predict that neither of the SNPs were in LD and the two loci tend to be inherited in a random manner for the present endeavour. Further haplotype analysis revealed that the -1195A/8473T haplotype was significantly associated with increased risk (OR, 1.79) for chronic periodontitis (Table 4), while the -1195G/8473C haplotype showed a reduced risk (OR, 0.501). These findings are consistent with a report from a Chinese population [9] where the AT haplotype was a strong predictor for chronic periodontitis.

The present study adds to the understanding of the role of genetic variants in the development of chronic periodontitis. New insights into the COX2 genetic polymorphism provide an altered paradigm of periodontal disease that emphasizes the variation of the host response. The small sample size is a limitation of the current study but randomness needed to be taken into account. In order to determine the exact direct involvement of the COX2 gene in periodontitis, studies that simultaneously examine the distribution and dynamics of genetic variants at many loci on the COX2 gene are warranted. Studies evaluating local and circulating levels of COX2 mRNA and protein could be undertaken to further support the findings.

The results of this pilot study suggest that SNPs COX2 -1195G>A and COX2 8473T>C are not individually associated with chronic periodontitis; however, haplotype AT significantly increased the risk of chronic periodontitis in a North Indian population. Further studies with a larger sample size and more elaborate study designs are needed for validation of the above findings.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

SVS is a recipient of a fellowship from the Council of Scientific and Industrial Research (CSIR), India. This work was supported by the CSIR, network project NWP0034. It bears communication number 170/2011/SKR of the CDRI.

REFERENCES

1. McDevitt MJ, Wang HY, Knobelman C, Newman MG, di Giovine FS, Timms J, et al. Interleukin-1 genetic association with periodontitis in clinical practice. J Periodontol 2000;71:156-63.
2. Schork NJ, Fallin D, Lanchbury JS. Single nucleotide polymorphisms and the future of genetic epidemiology. Clin Genet 2000;58:230-64.
3. Yoshie H, Kobayashi T, Tai H, Galicia JC. The role of genetic polymorphisms in periodontitis. Periodontol 2000 2007;43:102-32.
4. Kornman KS, Crane A, Wang HY, di Giovine FS, Newman MG, Pirk FW, et al. The interleukin-1 genotype as a severity factor in adult periodontal disease. J Clin Periodontol 1997;24:72-7.
5. Agrawal AA, Kapley A, Yeltiwar RK, Purohit HJ. Assessment of single nucleotide polymorphism at IL-1A+4845 and IL-1B+3954 as genetic susceptibility test for chronic periodontitis in Maharashtrian ethnicity. J Periodontol 2006;77:1515-21.

6. Shete AR, Joseph R, Vijayan NN, Srinivas L, Banerjee M. Association of single nucleotide gene polymorphism at interleukin-ibeta +3954, -511, and -31 in chronic periodontitis and aggressive periodontitis in Dravidian ethnicity. J Periodontol 2010;81:62-9.

7. Engebretson SP, Lamster IB, Herrera-Abreu M, Celenti RS, Timms JM, Chaudhary AG, et al. The influence of interleukin gene polymorphism on expression of interleukin-ibeta and tumor necrosis factor-alpha in periodontal tissue and gingival crevicular fluid. J Periodontol 1999;70:567-73.

8. Guan ZM, Liu JJ, Ma X, Wu DH, Yu J, Huang GQ. Relationship between interleukin-6 gene-572C/G polymorphism and chronic periodontitis. Zhonghua Kou Qiang Yi Xue Za Zhi 2008;43:410-3.

9. Xie CJ, Xiao LM, Fan WH, Xuan DY, Zhang JC. Common single nucleotide polymorphisms in cyclooxygenase-2 and risk of severe chronic periodontitis in a Chinese population. J Clin Periodontol 2009;36:198-203.

10. Astolfi CM, Shinohara AL, da Silva RA, Santos MC, Line SR, de Souza AP. Genetic polymorphisms in the MMP-1 and MMP-3 gene may contribute to chronic periodontitis in a Brazilian population. J Clin Periodontol 2006;33:699-703.

11. Noguchi K, Ishikawa I. The roles of cyclooxygenase-2 and prostaglandin E2 in periodontal disease. Periodontol 2000 2000;2007;43:85-101.

12. Offenbacher S, Salvi GE. Induction of prostaglandin release from macrophages by bacterial endotoxin. Clin Infect Dis 1999;28:505-13.

13. Xu Q, Ji YS, Schmedtje JF Jr. Spi increases expression of cyclooxygenase-2 in hypoxic vascular endothelium. Implications for the mechanisms of aortic aneurysm and heart failure. J Biol Chem 2000;275:24583-9.

14. Miyauchi M, Hiraoka M, Oka H, Sato S, Kudo Y, Ogawa I, et al. Immuno-localization of COX-1 and COX-2 in the rat molar periodontal tissue after topical application of lipopolysaccharide. Arch Oral Biol 2004;49:739-46.

15. Offenbacher S, Heasman PA, Collins JG. Modulation of host PGF2 secretion as a determinant of periodontal disease expression. J Periodontol 1993;64(Suppl):432-44.

16. Yen CA, Damoulis PD, Stark PC, Hibberd PL, Singh M, Papas AS. The effect of a selective cyclooxygenase-2 inhibitor (cecloxib) on chronic periodontitis. J Periodontol 2008;79:104-13.
32. Papafili A, Hill MR, Brull DJ, McAnulty RJ, Marshall RP, Humphries SE, et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. Arterioscler Thromb Vasc Biol 2002;22:1631-6.

33. Guo Y, Zhang X, Tan W, Miao X, Sun T, Zhao D, et al. Platelet 12-lipoxygenase Arg261Gln polymorphism: functional characterization and association with risk of esophageal squamous cell carcinoma in combination with COX-2 polymorphisms. Pharmacogenet Genomics 2007;17:197-205.

34. Dixon DA, Kaplan CD, McIntyre TM, Zimmerman GA, Prescott SM. Post-transcriptional control of cyclooxygenase-2 gene expression. The role of the 3’-untranslated region. J Biol Chem 2000;275:11750-7.

35. Cok SJ, Morrison AR. The 3’-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. J Biol Chem 2001;276:23179-85.

36. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225-9.

37. International HapMap Consortium, Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449:851-61.