Association of bone morphogenetic protein-4 gene polymorphism with periodontitis in a Taiwanese population

Shin-Chie Wu a,†, Earl Fu a,†, Hsien-Chung Chiu a, Fu-Gong Lin b, E-Chin Shen c, Cheng-Yang Chiang a *

a Department of Periodontology, School of Dentistry, National Defense Medical Center and Tri-Service General Hospital, Taipei, Taiwan, ROC
b School of Public Health, National Defense Medical Center, Taipei, Taiwan, ROC
c Dental Department, Buddhist Tzu Chi General Hospital, Sindian, Taipei County, Taiwan, ROC

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Abstract  Background/purpose: Bone morphogenetic protein-4 (BMP-4) plays an important role during embryonic development of tooth and bone. Studies have found that the BMP-4 polymorphism rs17563 (T>C) influenced bone loss around implants; however, its impact on periodontitis has never been determined. Association of the polymorphism with periodontitis was evaluated.

Materials and methods: Two hundred Taiwanese were grouped into aggressive periodontitis (AgP), chronic periodontitis (CP), and healthy controls (HC) according to a clinical examination. BMP-4 polymorphism was evaluated by polymerase chain reaction—restriction fragment length polymorphism. Distributions of the polymorphism among the groups were compared.

Results: In the AgP, CP, and HC groups, no significant differences of genotype and allele distributions among homozgotes (CC or TT) and heterozygotes (C/T), between TT and CT+CC, or between the alleles of T and C was found. Using logistic regression, there was no significantly different distribution between each disease group (AgP, CP, or AgP+CP) and HC, although their odds ratios increased in the genotypes of CC, CT, and CC+CT if compared with that of TT, and in the allele of C when compared with the allele T. A higher C allele frequency, but without significance (P = 0.066), was observed in CP than that in HC.

KEYWORDS
bone morphogenetic protein 4; genetic polymorphism; periodontitis

* Corresponding author. School of Dentistry, National Defense Medical Center, P.O. Box 90048-507, Taipei, Taiwan, ROC.
E-mail address: dentalab@tpts5.seed.net.tw (C.-Y. Chiang).
† These authors contributed equally to this work.

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Introduction

Periodontitis is an inflammatory disease characterized by immune cell infiltration into the gingival tissues, leading to connective tissue destruction, attachment loss, and alveolar bone resorption. Pathogenic microflora and various environmental risk factors and genetics are also involved in the pathogenesis of the disease. A plethora of genetic factors, ranging from single gene defects to more subtle combinations of single nucleotide polymorphisms, have been suggested as predisposing to aggressive periodontitis (AgP). Single nucleotide polymorphisms within candidate genes may be causally related to the changes in protein expression, structure, and function. These, in turn, may lead to variations in phenotypic expression.

Bone morphogenetic proteins (BMPs)-2 to BMP-7 are members of the transforming growth factor superfamily. They have been linked to morphogenesis and bone cell differentiation. In mammalian embryonic development, BMP-4 is important in cell proliferation, mesoderm formation, neural patterning, tooth formation, skeletal development, bone marrow formation, and limb morphogenesis. Increased BMP-4 expression has been shown to be associated with fracture repair and fibrodysplasia ossificans progressiva, a devastating genetic disorder of ectopic bone formation. The BMP-4 gene is located on chromosome 14q22 and contains several polymorphic sites. In a recent study, the BMP-4 polymorphism rs17563(T>C) has been identified as a possible risk factor for implant early marginal bone loss around endosseous implants. However, detailed information regarding the association of rs17563 with chronic periodontitis is still lacking. This case-control study, therefore, was designed to evaluate the association of rs17563 with periodontitis in a Taiwanese population.

Materials and methods

The participants in this study were all Han Chinese. The study took place at the Department of Periodontology of the Tri-service General Hospital via questionnaire and review of history. All of the individuals were free from systemic diseases, such as diabetes mellitus, human immunodeficiency virus infection, and immunological disorders. The diseases diagnosed in the study participants were defined according to the classification proposed at the International Workshop for the Classification of Periodontal Diseases and Condition in 1999. In brief, the diagnoses [aggressive periodontitis (AgP), chronic periodontitis (CP), and periodontally healthy controls (HC)] were made based on the clinical examinations (probing depth and attachment loss) and radiographic patterns of alveolar bone destruction. The criteria for CP included: (1) age ≥35 years; (2) attachment loss ≥5 mm on more than one tooth; and (3) more than three sites of probing depth >6 mm on more than one tooth distributed in each quadrant. The criteria for AgP were: (1) the onset of periodontal disease occurred at <35 years of age; (2) at least eight teeth with AL ≥5 mm, PD >6 mm; and (3) at least three affected teeth were not first molars or incisors. The HC controls were selected if there was no evidence of attachment loss and probing depth at more than one site >3 mm.

The smoking status of participants was classified as nonsmoker or current smoker. Individuals who had never smoked or had quit smoking for at least 6 months were recorded as nonsmokers. The betel nut chewing status of participants was recorded. The study protocol was approved by the Institutional Review Board of Tri-service General Hospital, and written informed consent was obtained from each participant.

Sample collection, DNA extraction, and genotyping

Peripheral blood (10 mL in heparin) was collected from each study participant. DNA was extracted from peripheral leukocytes using a DNA extraction kit (QiAmp DNA Mini Kit; Qiagen GmbH, Hilden, Germany). The genotype for the BMP-4 rs17563 was determined by the polymerase chain reaction (PCR)–restriction fragment length polymorphism method. BMP-4 PCR was carried out in a total volume of 25 μL containing 2 μL genomic DNA; and 0.5 units of Taq polymerase (Applied Biosystems, Foster City, CA, USA). The oligonucleotide primers are as follows: forward: 5'-GC TACCTTGACTCTTCCATC-3' and reverse: 5'-CATGTGTGG GTCGCTTCTCC-3'. The PCR was performed using 38 cycles consisting of the following steps: denaturation at 95°C for 30 seconds, annealing at 62°C for 30 seconds, and extension at 72°C for 30 seconds. Following the amplification, 4 μL of PCR product was digested with 5 units of HphI restriction endonuclease at 37°C for 3 hours, yielding 172 + 232 bp fragments (T) and a single 404 bp fragment (C; Fig. 1). The digested product was visualized after electrophoresis on a 3% agarose gel by ethidium bromide staining.

Statistical analysis

Statistical analysis was carried out using the program SPSS 15.0 (SPSS 15.0, SPSS Inc., Chicago, IL, USA). A P-value of <0.05 was taken to be significant. A Chi-square test was used to examine the differences among the groups of HC, AgP, and CP in demographic characters, including sex, age, smoking status, betel nut chewing, and drinking habits. Using the Chi-square test, the BMP-4 genotypes and allele frequencies among the patient groups were also examined.

Conclusion: The BMP-4 polymorphism may not be correlated with periodontitis. However, there is a trend that patients with chronic periodontitis may have a high C allele frequency in BMP-4 compared to healthy controls.
To examine the distributions of genotypes and alleles in the periodontitis groups (AgP, CP, and AgP+CP) versus those in the HC group, a logistic regression with the adjustment variables of sex, age, smoking status, betel nut chewing, and drinking, was selected and used.

Results

In this study, 200 individuals were included, 24 in AgP, 137 in CP, and 39 in HC groups. The distributions of age, sex, and the habits of smoking, betel nut chewing, and drinking are summarized in Table 1. The CP was significantly older than the AgP and HC groups.

The distribution of genotypes in the three groups of AgP, CP, and HC were shown in Table 2. No statistical difference was observed in the distribution of homozygotes (TT and CC) and heterozygotes (CT) among the three disease groups (P > 0.05; Table 2). There were also no differences in the distribution between the genotypes of TT and CT+CC, as well as between the alleles for T and C among all three types of periodontal patterns (Table 2).

When the genotype and allele distributions were compared between AgP patients and the healthy controls, no statistical differences were observed (Table 3). After adjustment by sex, smoking, betel nut chewing, and drinking, the results still showed no difference. However, the increased odds ratio and adjusted odds ratio in the genotypes of CC, CT, and CC+CT, as well as in the alleles of C, were observed when compared with genotype of TT and allele of C. Similar findings were observed in the distributions of genotypes and alleles in the patients with CP, and with AgP+CP, if compared with those in the healthy controls (Tables 4 and 5). Moreover, a higher incidence of the C allele was observed in the individuals with CP than that in the healthy control, but no statistical significance (P = 0.066) was obtained (Table 4).

Discussion

BMP-4 is important in cell proliferation and mesoderm formation, neural patterning, tooth formation, skeletal development, bone marrow formation, and limb morphogenesis.8

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### Table 1: Comparison of the demographic characteristics of patients with aggressive periodontitis (AgP) and chronic periodontitis (CP), and in healthy controls (HC).

<table>
<thead>
<tr>
<th></th>
<th>AgP (n = 24)</th>
<th>CP (n = 137)</th>
<th>HC (n = 39)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years ± SD)</td>
<td>35.08 ± 6.37</td>
<td>52.82 ± 8.16</td>
<td>35.23 ± 13.64</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (50.0)</td>
<td>80 (58.4)</td>
<td>26 (66.7)</td>
<td>0.412</td>
</tr>
<tr>
<td>Female</td>
<td>12 (50.0)</td>
<td>57 (41.6)</td>
<td>13 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (16.7)</td>
<td>38 (27.7)</td>
<td>5 (12.8)</td>
<td>0.107</td>
</tr>
<tr>
<td>No</td>
<td>20 (83.3)</td>
<td>99 (72.3)</td>
<td>34 (87.2)</td>
<td></td>
</tr>
<tr>
<td>Betel nut chewing, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0.0)</td>
<td>14 (10.2)</td>
<td>1 (2.6)</td>
<td>0.092</td>
</tr>
<tr>
<td>No</td>
<td>24 (100.0)</td>
<td>123 (98.8)</td>
<td>38 (97.4)</td>
<td></td>
</tr>
<tr>
<td>Drinking, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (20.8)</td>
<td>34 (24.8)</td>
<td>12 (30.8)</td>
<td>0.644</td>
</tr>
<tr>
<td>No</td>
<td>19 (79.2)</td>
<td>103 (75.2)</td>
<td>27 (69.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference at P < 0.05, by Chi-square test.
BMP-4 rs17563 T/C is located in exon 4 and results in an amino acid position 152 change from valine (V) to alanine (A). Some studies have investigated the relationship between the BMP-4 polymorphisms and human diseases. A correlation of the polymorphisms with the nonsyndromic cleft lip has been observed, but other studies found no correlation with osteoporosis or with nephropathy in Type 1 diabetes mellitus.

This study is the first analysis of BMP-4 genetic polymorphism in patients with periodontitis. In a study by Shimpuku et al, in a Japanese population, the BMP-4 polymorphism was suggested to be a possible risk factor for early marginal bone loss of mandibular dental implantation. In both studies (one for patients with periodontitis and one for individuals receiving dental implant placement), the same genotypes were tested. The polymorphism T to C, described in the present study, was located in exon 4, which resulted in an amino acid change from V to A that was described in the study for evaluating the bone loss around dental implant. Allele distributions of BMP-4 rs17563 for T and C in this study were similar to those in the Japanese study. The prevalence of C allele in the Taiwanese population reported in the current study, however, was lower than that in Italian individuals reviewed in a previous study. A significant difference in the distribution of BMP-4 genotypes was found between patients with and without bone loss in the mandible. The patients with the BMP-4 AV genotype may be deficient in the function and/or production of BMP-4 when compared to those with the VV genotype. This could cause an imbalance in bone remodeling that leads to early marginal bone loss around implants. However, it must be noted when reviewing the data that the differences of the BMP-4 genotype between individuals with and without bone loss in the maxilla was not significant. Moreover, the etiology of the edentulous area was not mentioned in the Japanese study, which might be quite important for investigating the bone loss around the implants. Nevertheless, the periodontitis and peri-implantitis are the process of tissue destruction around the tooth and the inserted dental implant, respectively, caused by specific bacteria. The tooth- or implant-tissue interface, the osseointegration or periodontal ligament insertion, and the examining population selections (Japanese vs. Taiwanese), are all influencing results in these two studies. In our study, the mean age of CP group was 52.82 years, which was similar to the implant study (mean, 54.8; range, 29–74 years). A higher distribution of the C allele frequency tends to be found in patients with CP than those in the healthy controls (P = 0.066, Table 4). The finding might indicate that individuals with a higher distribution of the C allele frequency in BMP-4 genotype might have a tendency to be a risk for chronic periodontitis.

Moreover, the criteria for grouping periodontitis used in this study were based on the Classification of Periodontal Diseases and Condition in 1999 at the International Workshop. However, these criteria are clinical measurements, while certain characteristics of AgP, such as the family aggregation or the amounts of microbial deposits, are not described in detail. In this study, the mean probing depth was 4.3 mm in AgP, 3.9 mm in CP, and 2.6 mm in HC groups.
and the attachment loss was 4.8 mm in AgP, 4.6 mm in CP, and 3.1 mm in HC groups. Both parameters were significantly greater in AgP and CP groups than those in HC group. Nevertheless, this is the first report to evaluate whether the BMP-4 rs17563 polymorphism is associated with the development of periodontitis. Our results showed that there is no statistically different distribution in the genotypes or in the alleles of the BMP-4 polymorphism in the groups with two different types of periodontal diseases. Therefore, the BMP-4 polymorphism may not be correlated with the chronic and aggressive periodontitis. However, there is a trend that C allele can be observed more commonly in the patients with chronic periodontitis than that in healthy controls (P = 0.066).

Acknowledgments

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References


Table 5  The genotype and allele frequencies of bone morphogenetic protein-4 (BMP-4) polymorphisms in all patient groups [aggressive periodontitis (AgP) and chronic periodontitis (CP)] and in healthy controls (HC).

<table>
<thead>
<tr>
<th>BMP-4 genotype</th>
<th>AgP (n = 161) (%)</th>
<th>HC (n = 39) (%)</th>
<th>χ² test P</th>
<th>Crude OR (95% CI)</th>
<th>P</th>
<th>Adjusted OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>76 (47.2)</td>
<td>24 (61.5)</td>
<td>0.265</td>
<td>1.017 (1.171)</td>
<td>1</td>
<td>0.009</td>
</tr>
<tr>
<td>CT</td>
<td>65 (40.4)</td>
<td>12 (30.8)</td>
<td>1.711</td>
<td>0.794–3.687</td>
<td>2.215 (0.870–5.644)</td>
<td>0.291</td>
</tr>
<tr>
<td>CC</td>
<td>20 (12.4)</td>
<td>3 (7.7)</td>
<td>2.105</td>
<td>0.575–7.705</td>
<td>0.261</td>
<td>0.241 (0.502–10.010)</td>
</tr>
<tr>
<td>TT</td>
<td>76 (47.2)</td>
<td>24 (61.5)</td>
<td>0.108</td>
<td>1.011 (1.119)</td>
<td>1</td>
<td>0.072</td>
</tr>
<tr>
<td>CT+CC</td>
<td>85 (52.8)</td>
<td>15 (38.5)</td>
<td>1.789</td>
<td>0.875–3.660</td>
<td>0.221</td>
<td>0.932–5.291</td>
</tr>
<tr>
<td>T allele</td>
<td>217 (67.4)</td>
<td>60 (76.9)</td>
<td>1.020</td>
<td>1.004 (1.231)</td>
<td>1</td>
<td>0.092</td>
</tr>
<tr>
<td>C allele</td>
<td>105 (32.6)</td>
<td>18 (23.1)</td>
<td>1.613</td>
<td>0.907–2.869</td>
<td>1.774</td>
<td>0.910–3.457</td>
</tr>
</tbody>
</table>

CI = confidence interval; OR = odds ratio.

* After adjustment for sex, smoking, betel nut chewing, and drinking by logistic regression analysis.