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Bone Morphogenetic Protein-4 rs17563 T/C Gene Polymorphism with Radix Entomolaris of Mandibular First Molars in the Taiwanese Population

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Background: Bone morphogenetic protein-4 (BMP-4) plays an important role during the embryonic development of tooth and bone. Studies have shown around 20% to 35% of Taiwanese exhibit an extra distolingual root, radix entomolaris, in the first mandibular molar. However, the association of the polymorphisms with the mandibular first molar exhibiting a distolingual root has never been evaluated. **Methods:** Two hundred Taiwanese were grouped using two different characteristics: subjects with and without distolingual roots. BMP-4 polymorphism was evaluated by polymerase chain reaction-restriction fragment length polymorphism, and compared among the groups for each characteristic. **Results:** Twenty four subjects showed the presence and 92 the absence of the root. The distributions of age, gender, and the habits of smoking, betel nut chewing, and drinking were similar in these two groups. No differences in genotype and allele distributions between the two subject groups were shown. This was regardless of the homo- and hetero-zygosity of C/T, between TT and CT+CC, and between the alleles of T and C. Comparing the distribution of T or TT in the two subject groups, the odds ratio and the adjusted odds ratio in the genotypes and the allele of C were further confirmed with non-difference. **Conclusions:** The BMP-4 polymorphism may not be correlated with the presence of distolingual roots.

Key words: bone morphogenetic protein, genetic polymorphism, mandibular molar, radix entomolaris

INTRODUCTION

The presence of an extra distolingual root on the mandibular first molar, also called the radix entomolaris, is uncommon in Caucasians[1,2]; however, recent studies have shown around 20% to 35 % of Taiwanese possess that extra root.[3-9] The presence of the extra root is not only a challenge for endodontic treatment, but also a risk factor for local periodontal destruction.[1,5]

Bone morphogenetic proteins (BMPs)-2 to -7 are members of the transforming growth factor superfamily. They have been linked to morphogenesis and bone cell differentiation.[10] 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.[11] Increased BMP-4 expression has been shown to be associated with fracture repair[12] and fibrodysplasia ossificans progressiva, a devastating genetic disorder of ectopic bone formation.[13]

The BMP-4 gene is located on chromosome 14q22-23 and contains several polymorphic sites. BMP-4 has been shown to repress the expression of Barx-1 (barH1 homologue in vertebrates) gene, affecting the development of mandibular molars.[14] Moreover, BMP-4 polymorphism rs17563 (T->C) has been identified as a possible risk factor for implant of early marginal bone loss around endosseous implants.[15] The presence of radix entomolaris may contribute to localized periodontal destruction because a greater probing depth and attachment loss was observed at the disto-lingual sites of molars with the roots when compared with those without the root.[5] Whether the BMP4 rs17563 polymorphism is associated with the development of distolingual root is not known. The present case-control study was designed to evaluate the association of BMP-4 polymorphism rs17563 with the presence of the distolingual root on the mandibular first molar in a Taiwanese population.

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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 using a questionnaire and review of history. All subjects were free from systemic diseases, such as diabetes mellitus, human immune - deficiency virus infection, and immunological disorders. All subjects received periapical radiographs to identify any distolingual roots on either mandibular first molar. The smoking statuses of participants were a non-smoker or current smoker. Subjects 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 for participation in the study was obtained from each subject.

Sample collection, DNA extraction and Genotyping

Ten milliliters of heparin-anti-coagulated peripheral blood was collected from each study subject. DNA was extracted from peripheral leukocytes using a DNA extraction kit (QIAamp DNA Mini Kit®, Qiagen GmbH, Germany).

The genotype for the BMP-4 rs17563 was determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) 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'-GCTATCTCTTGACTCTTCCATC-3' and reverse: 5'-CATAGTTTGGCTGCTTCTCC-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) (Figure 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, USA). *P*-Values of <0.05 were considered significant. A chi-square test was used to examine the differences between the two groups in terms of demographic characteristics, including gender, age, smoking status, betel nut chewing, and drinking habits. Using the chi-square test, the BMP-4 genotypes and allele frequencies among the subject groups were examined. To examine the distributions of genotypes and alleles in the subjects with the distolingual root versus those in the non-distolingual root group, a logistic regression with the adjustment variables of gender, age, smoking status, betel nut chewing, and drinking, was selected and used.

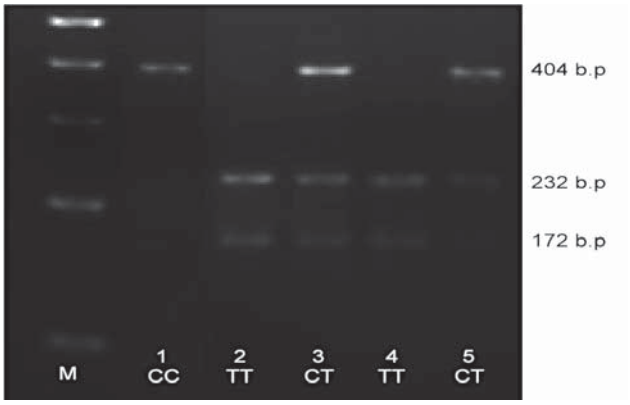

Fig. 1 Genotypes of the BMP-4 rs17563 determined by the polymerase chain reaction-restriction fragment length polymorphism. Single band 404 bp was CC genotype (line 1), the two bands at 232 and 172 bp were TT genotype (line 2 and 4), and the three bands at 404, 232, 172 bp (line 3 and 5) were representing CT heterozygous. (M: marker)

RESULTS

In this study, 200 subjects were included, 24 had the root(s) and 92 did not, while 83 subjects were excluded due to missing teeth or difficulty in identifying the presence or absence of the root. The distributions of age, and habits of smoking, betel nut chewing, and alcohol drinking did not differ between the two subject groups (Table 1). For gender, however, males had more distal lingual roots than females (p=0.032).

The distributions of genotypes in the two subject groups are shown in Table 2. No statistical difference in the distributions of homozygosity (TT and CC) and heterozygosity (CT) was observed (Table 2). Comparison of the distribution of the genotype of TT or allele of T in the two subject groups, the crude odds ratio and the adjusted odds ratio in the distribution of the genotypes of CT, CC and CT+CC, or those of the allele of C, showed no further differences.

Table 1 Comparison of the demographic characteristics of patients with and without the root (a: number of the patients; b: percentage of the patients; *: significant difference at $p < 0.05$, by chi-square test)

	The distribution of distolingual root		
	With root (n=25)	Without root (n=92)	*P* value
Age(years±SD)	51.24±12.49	48.46±11.21	0.674
Gender			0.032*
Male	20[a] (80.0%[b])	52 (56.5%)	
Female	5 (20.0%)	40 (46.5%)	
Smoking			0.556
Smokers	8 (32.0%)	24(26.1%)	
Nonsmokers	17 (68.0%)	68 (73.9%)	
Betel nut chewing			0.190
Yes	0 (0.0%)	6 (6.5%)	
No	25 (100.0%)	86 (93.5%)	
Drinking			0.466
Yes	5 (20.0%)	25 (27.2%)	
No	20 (80.0%)	67 (72.8%)	

Table 2 The genotype and allele frequencies of BMP-4 polymorphisms in the patients exhibiting, and not exhibiting, the distolingual root (DL) in mandibular first molar. (a: number of the patients; b: percentage of the patients; OR: odds ratio; CI: confidence interval; #: adjusted by logistic regression analysis)

Genotypes	With root (N=25)	Without root (N=92)	*p value*	Crude OR (95% CI)	*p value*	Adjusted OR# (95% CI)	*p value*
TT	11[a] (44.0%[b])	46 (50.0%)	0.460	1		1	
CT	13 (52.0%)	37 (40.2%)		1.469 (0.590-3.658)	0.408	1.281 (0.507-3.233)	0.601
CC	1 (4.0%)	9 (9.8%)		0.465 (0.053-4.062)	0.488	0.433 (0.049-3.847)	0.452
TT	11 (44.0%)	46 (50.0%)	0.595	1		1	
CT+CC	14 (56.0%)	46 (50.0%)		1.273 (0.523-3.097)	0.595	1.122 (0.455-2.768)	0.802
T allele	35 (70.0%)	129 (70.1%)	0.988	1		1	
C allele	15 (30.0%)	55 (29.9%)		1.005 (0.508-1.989)	0.988	0.932 (0.468-1.859)	0.842

DISCUSSION

BMP-4 is important in cell proliferation and mesoderm formation, neural patterning, tooth formation, skeletal development, bone marrow formation, and limb morphogenesis.[11] BMP-4 rs17563 T/C is located in exon 4[16] and can result in an amino acid position 152 change from Valine to Alanine. A few 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[17], but other studies found no correlation between those with osteoporosis nor with nephropathy in Type 1 diabetes mellitus.[18,19]

Recently, the BMP-4 polymorphism was suggested to be a possible risk factor for early marginal bone loss of mandibular dental implantation.[15] In the present study, however, no association between the polymorphism and the presence of the distolingual root in mandibular first molars was observed. Studies have shown smoking and betel nut chewing may significantly influence the progression of periodontal disease.[20-22] Therefore, the demographic characteristics of patients with and without the root were also compared in this study, with our data showing no significant differences between the two subject groups. Although the exact reasons for the different results is uncertain, the root morphology and peri-implant tissue, as well as the selections of the examined population (Japanese vs Taiwanese), differed in these studies.

There were many genes which may regulate tooth development, such as fibroblast growth factor, Pax (paired box homeotic gene), Msx (Msh-like genes in vertebrates), Dlx (distaless homologue in vertebrates), Barx (barH1 homologue in vertebrates), and etc. The homeobox genes Msx-1, Msx-2, Dlx-2, and Barx-1, which express in mandibular ectomesenchyme in mice, are found to be associated with differing tooth types. The incisors are regulated by Msx-1,and Msx-2, and the molars are controlled by Barx-1 and Dlx-2.[14] The expression of Barx-1 is localized to the proximal ectomesenchyme (molar), and BMP-4 is found to repress the expression of Barx-1 in the distal ectoderm.[23] Therefore, BMP-4 might play important roles in the molar root formation. As to the limitations of the study, however, no significant differences were found in genotype or allele distribution for the BMP-4 polymorphism in the subjects with and without the distolingual roots. Further detailed examination, including increasing the observed subjects, is needed.

CONCLUSION

This is the first report to evaluate whether the BMP4

rs17563 polymorphism is associated with the presence of the distolingual root in mandibular first molars. Our results showed no statistically different distributions in the genotypes and in the alleles of BMP-4 polymorphism in the subjects with or without the root. Therefore, we suggest the BMP-4 polymorphism may not be correlated with the presence of the distolingual root.

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DISCLOSURE

All authors declare that this study has no conflict of interest.

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