Bone Morphogenetic Protein-4 Gene Polymorphism and Early Marginal Bone Loss Around Endosseous Implants

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Purpose: *At stage II surgery during dental implant treatment, early marginal bone loss around the implant occasionally occurs despite a lack of apparent causal events, and the etiology of this bone loss is unclear. This study was designed to investigate whether the bone morphogenetic protein-4 (BMP-4) genetic polymorphism is associated with early marginal bone loss around implants.* ***Materials and Methods:*** *The BMP-4 polymorphism was detected by restriction fragment length analysis using HphI digestion after polymerase chain reaction. A total of 262 implants were placed in 41 patients, and early marginal bone loss was observed in 25 of the 109 maxillary implants and 14 of the 153 mandibular implants.* ***Results:*** *In the mandible, the patients with the BMP-4 AV genotype had a significantly higher rate of occurrence of marginal bone loss than those with the BMP-4 VV genotype (P = .012). According to multiple logistic regression analyses, the odds ratio of the AV versus the VV BMP-4 genotype was 8.106 between patients with and those without bone loss in the mandible (95% CI = 1.30 to 50.51; P = .025).* ***Discussion:*** *These results suggest that the BMP-4 genetic polymorphism influences early marginal bone loss around implants.* ***Conclusion:*** *While perhaps premature in recommendation, genetic screening before implant surgery may prove to be a very useful aid to consider the risk of implant treatment.* (Int J Oral Maxillofac Implants 2003;18:500–504)

Key words: *bone morphogenetic protein, endosseous dental implants, gene, marginal bone loss, polymorphism*

Dental implant surgery can proceed in 2 stages, and it is generally accepted that bone loss does not occur at stage II surgery provided that determining events, such as infection or oral exposure of the cover screws, do not take place. However, early marginal bone loss around implants occasionally does occur in the absence of any apparent determining events. Since the implants do not receive mechanical loading during the healing period, the risk of further bone loss after prosthetic treatment is considerable.

Nosaka and colleagues[1] examined the relationship between early marginal bone loss around implants and calcitonin receptor (CTR) genetic polymorphism and showed that patients with the TC genotype had a likelihood of early marginal bone loss in the mandible that was 20 times greater than for patients with the CC genotype. This was the first investigation of early marginal bone loss around implants from a genetic perspective. Since many factors control bone formation and resorption, it is desirable that other genetic polymorphisms concerning bone metabolism be investigated.

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The remodeling of bone usually occurs adjacent to the implant during the healing period and continues as long as the implants remain in situ.[2,3] Bone morphogenetic protein (BMP) plays an important role in bone remodeling,[4,5] and a new polymorphism has recently been identified within the human BMP-4 gene.[6] Thus, it is conceivable that the BMP-4 gene polymorphism could influence the propensity for early marginal bone loss.

The purpose of the present study was to investigate whether the polymorphism of the BMP-4 gene was associated with early marginal bone loss around endosseous dental implants at stage II surgery.

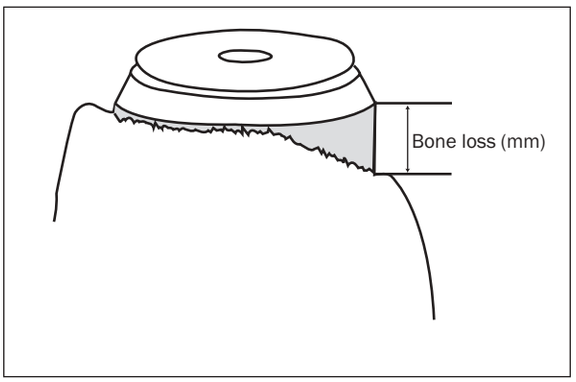

Fig 1 Measurement of early marginal bone loss around implant.

MATERIALS AND METHODS

Subjects

The study population consisted of 41 unrelated, systemically healthy Japanese patients (16 men and 25 women; mean age 54.8 ± 9.4 years; range, 29 to 74 years) who underwent implant treatment for edentulousness of the maxilla and/or mandible at the same private dental clinic. The clinical treatment was undertaken between 1999 and 2001. The same oral surgeon placed all of the blasted implants (Astra Tech, Mölndal, Sweden), and all the prosthetic treatment was performed by 1 prosthodontist. Information about current systemic disorders, medical and dental histories, smoking status, and menstruation status was recorded. Nonsmokers were patients who had never smoked. Patients were also considered nonsmokers if they had ceased smoking more than 1 year before implant treatment. Current smokers were those who had been smoking or had ceased smoking less than 1 year before implant treatment.

Bone quality was determined according to the classification described by Lekholm and Zarb.[7] Fifty unrelated Japanese who were in good health and did not suffer from oral disease served as a control population (23 men and 27 women; mean age 50.9 ± 11.9 years; range, 30 to 70 years). Patients demonstrating early marginal bone loss around implants were designated as patients with bone loss. The occurrence of bone loss was defined as the number of implants with bone loss divided by the total number of implants placed and was expressed as a percentage. Written and oral informed consent was obtained from all subjects in accordance with the Helsinki Declaration of 1975 (revised in 1983).

Surgical Procedures

Implant placement was carried out under local anesthesia in an aseptic environment. All implants were placed until the rough surface was submerged, following the commonly accepted 2-stage surgical protocol. Cover screws were placed, and the mucoperiosteal flaps were repositioned and sutured with vertical mattress sutures, which remained in place for 10 days. Previously fabricated temporary removable prostheses were lined with tissue conditioner and were placed no sooner than 2 weeks after the operation so as not to disrupt healing.

After an adequate healing period of 4.5 months (mandible) and 6.9 months (maxilla), implant sites were identified, and the cover screws were exposed by a continuous incision in the alveolar crest. The stability of marginal bone around the implants was investigated directly by a single examiner who was blinded to the results of genotype, smoking status, menstruation status, and bone quality. The early marginal bone levels were measured from the top of the implant to the first point of bone-implant contact with a vernier micrometer (Fig 1).

DNA Extraction and Genotyping

DNA was extracted from peripheral leukocytes using a DNA extraction kit (Qiagen, Valencia, CA). The BMP-4 genetic polymorphism was examined 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 24 ng of genomic DNA; 0.5 units of Taq polymerase (Applied Biosystems, Foster City, CA); and oligonucleotide primers as follows: forward: 5'-GCTATCTCTTGACTCTTCCATC-3' and reverse: 5'-CATAGTTTGGCTGCTTCTCC-3'. For optimal amplification, the Mg^{2+} concentration of the reaction buffer was adjusted to 1.5 mmol/L. The PCR was performed using 38 cycles consisting of the following steps: denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 30 seconds. Following amplification, 2 μL

Table 1 Distribution of BMP-4 Genotypes in Patients and Controls

	n	Genotype (%) AV	VV	*P* value
Patients				
Maxilla	21	7 (33.3)	14 (66.7)	.531*
Mandible	36	11 (30.6)	25 (69.4)	.642†
Total	41	13 (31.7)	28 (68.3)	.549‡
Controls	50	13 (26.0)	37 (74.0)	

*Controls vs patients with maxillary implant treatment.
†Controls vs patients with mandibular implant treatment.
‡Controls vs total patients.

Table 2 Distribution of Implants

	Bone loss Yes	No	Total	Occurrence of bone loss (%)
Maxilla	25	84	109	22.9
Mandible	14	139	153	9.2
Total	39	223	262	14.9

of PCR product was digested with 5 units of *Hph*I restriction endonuclease at 37°C for 3 hours, yielding 172 + 232 bp fragments (allele V) and a single 404 bp fragment (allele A). The digested product was visualized after electrophoresis on a 3% agarose gel by ethidium bromide staining.

Statistical Analysis

Differences in the prevalence of sex, smokers, postmenopausal women, bone quality, and BMP-4 genotypes between controls, patients with bone loss, and patients without bone loss were tested using the chi-square test or the Fisher exact test using standard statistical software (SAS Institute, Cary, NC). The difference in age between groups was tested using the Student *t* test for unpaired data. Multiple logistic regression analyses were performed on smokers, postmenopausal women, bone quality, and the BMP-4 genotype. Odds ratios (OR) were calculated as indices of associations between the variables and early marginal bone loss, and the *P* value and 95% confidence intervals (95% CI) were calculated for each OR. Differences were considered to be statistically significant at a level of $P < .05$.

RESULTS

The distribution of BMP-4 genotypes in patients and controls is summarized in Table 1. There was no significant difference in the distribution of BMP-4 genotypes between patients and controls.

No complications, such as infection or oral exposure of cover screws, were observed in any of the patients during the healing period. All implants were stable, and osseointegration had been achieved physically and radiographically. The healing abutments were connected uneventfully at stage II surgery. On average, a healing period of 6.9 months (SD 1.0 months; range, 6.0 to 10.3 months) was required for maxillary implants and 4.5 months (SD 1.2 months; range, 2.4 to 6.0 months) for mandibular implants.

A total of 262 implants were placed in the patients. One hundred nine implants were placed in maxillae and 153 implants were placed in mandibles. Of the 109 maxillary implants placed, early marginal bone loss (mean 3.0 ± 1.5 mm; range, 1 to 6 mm) was observed in 25 implants. Of the 153 mandibular implants placed, early marginal bone loss (mean 1.5 ± 0.5 mm; range, 1 to 2 mm) was observed in 14 implants. The occurrence of bone loss was 22.9% in the maxilla, 9.2% in the mandible, and 14.9% overall (Table 2).

Table 3 shows the distribution of age, sex, smokers, postmenopausal women, bone quality, and the BMP-4 genotypes in patients with and without bone loss. A significant difference in the distribution of BMP-4 genotypes was found between patients with and without bone loss in the mandible ($P = .012$). In the maxilla, there was no significant difference in the distribution of BMP-4 genotypes between patients with and without bone loss ($P = .642$). The BMP-4 AA genotype was not observed in any of the subjects studied. There were no significant differences in the distribution of age, sex, smokers, postmenopausal women, and bone quality in the maxilla and mandible.

Table 4 shows the OR of the well-known risk factors bone loss, smokers, postmenopausal women, and bone quality, and the BMP-4 genotype following multiple logistic regression analysis. In the mandible, the OR of the AV versus VV BMP-4 genotypes was 8.106 between patients with and those without bone loss; this was statistically significant ($P = .025$). The differences between the other risk factors and the BMP-4 genotype in the maxilla were not significant.

Table 3 Characteristics of BMP-4 Genotypes of Patients With or Without Bone Loss

Variable	Maxilla			Mandible		
	Patients with bone loss (n = 12)	Patients without bone loss (n = 9)	*P* value	Patients with bone loss (n = 9)	Patients without bone loss (n = 27)	*P* value
Age (y)*	53.7 ± 13.4	57.0 ± 4.5	.485	55.7 ± 11.5	55.1 ± 9.1	.883
Sex (M/F)	2/10	5/4	.159	3/6	11/16	> .999
Smokers (yes/no)	3/9	2/7	> .999	2/7	12/15	.432
Postmenopausal women (yes/no)†	6/4	4/0	.251	5/1	10/6	.616
Bone quality (Type 2/Type 3)	8/4	4/5	.396	8/1	18/9	.392
BMP-4 genotypes (AV/VV)	5/7	2/7	.642	6/3	5/22	.012

*Findings given as mean ± SD.
†Female patients only.

Table 4 Multiple Logistic Regression Analyses for Bone Loss

Variable	Maxilla			Mandible		
	Odds ratio	95% CI	*P* value	Odds ratio	95% CI	*P* value
Smokers (yes/no)	2.990	0.18–49.61	.445	0.405	0.05–3.30	.398
Postmenopausal women (yes/no+male)	2.202	0.22–22.36	.504	2.316	0.31–17.14	.411
Bone quality (Type 3/Type 2)	0.323	0.04–2.54	.283	0.203	0.01–2.98	.245
BMP-4 genotypes (AV/VV)	2.187	0.28–17.28	.458	8.106	1.30–50.51	.025

DISCUSSION

This study is apparently the first analysis of BMP-4 genetic polymorphism in a case-control study of patients with early marginal bone loss around dental implants. Since there have been no previous reports on the distribution of BMP-4 genetic polymorphisms among Japanese subjects, a distribution of BMP-4 genotypes was investigated by comparing a population without any medical or oral diseases and a population currently undergoing implant treatment. As shown in Table 1, no significant difference was found in the distribution of BMP-4 genetic polymorphism in patients versus controls. Thus, the patients selected for implant treatment in the present study were not atypical with regard to the distribution of the BMP-4 polymorphism and were therefore suitable for this genetic study.

Smoking,[8,9] bone quality,[10,11] and osteoporosis[12] have thus far been implicated as possible risk factors for bone loss around implants. However, there was no significant difference in these risk factors between patients with and without marginal bone loss, as indicated in Table 3. Furthermore, no complications, such as infection or oral exposure of the cover screw, were detected in any of the patients. This indicated that the early marginal bone loss could not be ascribed easily to any of the known risk factors and supported the contention that an individual constitutional factor could be associated with the early marginal bone loss.

As shown in Table 3, the prevalence of the BMP-4 AV genotype among patients with early marginal bone loss was found to be significantly higher than in those without bone loss in the mandible. Multiple logistic regression analyses that took the known risk factors into account indicated the same tendency. However, this study had a small sample size, so the statistical analysis was reflected in a wide 95% CI, unfortunately. Thus, further study is needed to confirm any correlation between the BMP-4 gene polymorphism and early marginal bone loss around implants.

Why is the BMP-4 AV genotype associated with more early marginal bone loss than the VV genotype? During implant treatment, bone formation

usually occurs adjacent to the implants, and remodeling of bone has already started during the healing period.[2,3] BMP-4 is generally considered to be one of the proteins implicated in bone remodeling,[4,5] and the concentration of BMP-4 is known to be increased during the healing period following a bone fracture.[13] In this study, the polymorphism (T to C) was located in exon 4[14] and resulted in an amino acid change from Val to Ala. The base change in BMP-4 could result in a phenotypic change. Thus, it is conceivable that 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.

Long-term follow-up examinations have revealed that implants in the maxilla generally have a lower success rate than those in the mandible,[15] and as shown in Table 2, the incidence of early marginal bone loss was higher in the maxilla than in the mandible. This finding has been attributed to anatomic differences in the amount of cancellous bone in the maxilla and mandible, rather than to genetic risk factors.

CONCLUSION

Nosaka and colleagues[1] reported that the CTR genetic polymorphism might be a risk factor for early marginal bone loss in the mandible. In this study, the BMP-4 genetic polymorphism has also been identified as a possible risk factor for early marginal bone loss. Once early marginal bone loss has occurred, bone loss after prosthetic treatment would increase, leading eventually to a failure of implant treatment. Obtaining more information on the risk of bone loss at the preoperative stage may help to increase the success rate of implant treatment, and identification of the genetic risk factors associated with early marginal bone loss is a promising new strategy that may lead to a desired improvement in success rates.

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