Osseointegrated Implant Failure Associated with MMP-1 Promotor Polymorphisms (-1607 and -519)

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Purpose: *Two polymorphisms in the promoter region of human MMP-1 gene, an insertion of a guanine at position -1607 and A-519G substitution, have been shown to increase the transcriptional activity of these matrix metalloproteinases (MMPs). The objective of this study was to investigate the possible relationship between these polymorphisms and early implant failure.* ***Materials and Methods:*** *A sample of 104 nonsmokers was divided into 2 groups: a test group comprising 44 patients with 1 or more early failed implants and a control group consisting of 60 individuals with 1 or more healthy implants. Genomic DNA from oral mucosa was amplified by polymerase chain reaction and analyzed by restriction endonucleases. The significance of the differences in observed frequencies of polymorphisms were assessed by* χ^2 *tests.* ***Results:*** *The G-1607GG polymorphism with the genotype G/G was observed at a frequency of 62% in the control group, while in the test group this genotype was noted in 34% of the individuals (P = .011). The allele G was found at a frequency of 75% in control group and 61.66% in the test group (P = .05). No significant differences were seen in the genotypes and allele frequencies in the A-519G polymorphism among the groups (P = .064 and P = .124, respectively). The distribution of the haplotypes arranged as alleles and genotypes showed a significant difference between control and test groups (P = .031 and P = .002, respectively).* ***Conclusion:*** *On the basis of this study of 60 patients who experienced no implant failure and 44 patients who experienced implant failure, the results suggest that G-1607GG polymorphism in MMP-1 gene is associated with early implant failure, while A-519G polymorphism in MMP-1 gene does not show a significant relationship with implant loss. This study also suggests that haplotypes G-1607GG and A-519G of MMP-1 may be associated with the osseointegration process.* Int J Oral Maxillofac Implants 2008;23:653–658

Key words: *implant failure, MMP-1, polymorphism*

The clinical evidence of osseointegration effectiveness revolutionized the implantology, making implant replacement of missing natural teeth a viable alternative for the treatment of different edentulous situations. Despite the long-term success shown by longitudinal multicenter studies,[1,2] there is an inevitable, albeit small, risk of failure. Implant losses can be divided into early losses (ie, osseointegration is not achieved) and late losses (ie, osseointegration is lost after a period of function).[3] Implant loss can be attributed to biological, microbiological, and biomechanical factors, but the causes and mechanisms of early implant failure are still obscure. The cluster phenomenon, multiple implant failures in the same subject, supports the evidence that individual characteristics play an important role in the early failure process. However, little is known about the influence of genetic susceptibility on osseointegration.

With respect to gene polymorphisms, individuals may exhibit variations within the range considered biologically normal.[4] Polymorphisms in metalloproteases genes have been associated with several pathologies.[5–9]

Matrix metalloproteinases (MMPs) represent the major class of enzymes responsible for extracellular matrix metabolism.[10] They are metal-dependent proteolytic enzymes that contribute to the degradation and removal of collagen from damaged tissue. MMPs are secreted by inflammatory cells in response to stimuli such as lipopolysaccharide and cytokines.[11] Previous studies have also shown that proteinases (collagenases, gelatinases, elastases) are present in peri-implant sulcular fluid[12–15] and can play a pathologic role in peri-implant bone loss.[16]

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Table 1 Baseline Clinical Parameters of the Subject Population (n = 104)

	Control group (n = 60)	Test group (n = 44)
Mean age/range	46/21–71	45.6/18–73
Gender		
Female (%)	54.83	53.06
Male (%)	45.17	46.94
Maxillary implants (%)	43.48	38.92
Maxillary failure (%)	–	21.70
Mandibular implants (%)	56.52	61.98
Mandibular failure (%)	–	78.30
Mean time of successful implants/ range of failure after surgery	2.5/9–72 mo	5.5/1–12 mo**

*Mean time of failure after dental implant placement.

Fibroblast-type collagenase (MMP-1) is the interstitial collagenase expressed most widely among tissues and therefore plays a prominent role in collagen degradation.[17] MMP-1 degrades types I, II, III, and IX, which are the most abundant protein components of extracellular matrix.[9,18] Interstitial collagenase allows osteoblasts to initiate bone resorption by generating collagen fragments that activate osteoclasts.[19] Normally, expression of MMP-1 is low, but it is readily induced by phorbol esters, growth factors, and inflammatory cytokines.[20] Overexpression of MMP-1 is associated with several pathologic conditions.[21]

Insertion of a guanine at position -1607 of human MMP-1 gene creates the 2G allele, which has been shown to increase transcriptional activity.[22] The presence of this allele has been associated with the development of ovarian cancer,[5] endometrial carcinomas,[23] changes in bone mineral density,[17] the premature rupture of fetal membranes,[8] and chronic severe periodontitis.[9] Another polymorphic site in MMP-1, A-519G substitution has been reported.[24] Jurajda et al[24] observed linkage between these 2 polymorphisms. The A allele at -519 was more often found with the 2G allele at -1607.[24]

The finding of genetic markers related with early implant failure could allow the identification of individuals susceptible to implant loss. The purpose of this study was to investigate a possible relationship between 2 polymorphisms in the promoter of MMP-1 gene (G-1607GG and A-519G) and early failure of osseointegrated oral implants.

MATERIALS AND METHODS

Subject Selection

This retrospective study was carried out with the approval of the UNICAMP Ethics Committee (protocol 006/02), and informed consent was obtained from all subjects. A sample of 104 nonsmoking subjects (46 samples from a previous study[9] older than 18 years of age were recruited for study from the patient pool at the Dental Clinics of the Faculty of Dentistry of Piracicaba, University of Campinas (UNICAMP), São Paulo, Brazil. The subjects were randomly recruited. All subjects were in good general and oral health and did not have any of the following exclusion criteria: a history of diabetes or osteoporosis, hepatitis or HIV infection, immunosuppressive chemotherapy, or a history of any disease known to severely compromise immune function. Patients with early loading or regenerative surgery, such as bone grafting, and those who had had postsurgical complications, such as infection, were also excluded. All patients studied had been treated with implant systems that followed Brånemark 2-stage protocol: Biomet/3i (Palm Beach Gardens, FL) and Conexão Implant System and Prosthesis (São Paulo, SP, Brazil). No significant differences were observed in male-female ratio or maxilla-mandible implant ratio between the control and test groups. The baseline clinical parameters for the subject population are presented in Table 1. Subjects were divided into two groups:

- Control group: 60 patients with 1 or more healthy implants. These patients had at least 1 implant that had been implanted for a minimum of 9 months.
- Test group: 44 patients who had suffered 1 or more early implant failures. The implants were considered early losses if they presented mobility and/or pain before or during abutment connection and needed to be removed.

Sampling

The sampling of epithelial buccal cells was performed as described by Trevilatto and Line.[25] Briefly, 104 individuals undertook a mouthrinse containing 5 mL 3% glucose for 2 minutes. Following the mouthrinse, a sterile wood spatula was used to scrape oral mucosa. The tip of the spatula was then dipped into the retained mouthrinse solution. Buccal epithelial cells were pelleted by centrifugation at 2000 rpm for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended in 500 µL of extraction buffer (10 mmol/L Tris-HCl (pH 7.8), 5 mol/L EDTA, 0.5% SDS). The samples were then frozen at –20°C until used for DNA extraction.

DNA Extraction

After being defrosted, samples were incubated overnight with 100 ng/mL proteinase K (Sigma Chemical, St Louis, MO) at 52°C with agitation. DNA was then purified by sequential phenol/chloroform extraction

Table 2 Primer Sequences, PCR Conditions and Restriction Enzymes Used for Genotype for MMP-1 Polymorphisms

Gene	Primer sequences (5'-3')	Annealing temperature	Restriction endonulcease
MMP-1 (-1607)	F:TCGTGAGAATGTCTTCCCATT R:TCTTGGATTGATTTGAGATAAGTGAAATC	55°C	XmnI
MMP-1 (-519)	F: CATGGTGCTATCGCAATAGGGT R: TGCTACAGGTTTCTCCACACAC	45°C	KpnI

and salt/ethanol precipitation. DNA was estimated by measurements of optical density (OD) 260/280.

Polymerase Chain Reaction and Restriction Endonucleases Digestion

Polymerase chain reactions (PCRs) were carried out in a total volume of 50 µL, containing 500 ng genomic DNA; 10 mmol/L Tris-HCl (pH 8.3); 50 mmol/L KC; 1.5 mmol/L $MgCl_2$; 1 µL of each primer; 200 mmol/L each dATP, dCTP, dGTP, and dTTP; and 4 units of Taq DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden). The PCR products were then digested with 1 unit of the appropriate enzyme at 37°C overnight. The primer sequences, PCR conditions, and restriction enzymes are detailed in Table 2.

Gel Electrophoresis

The entire digest was mixed with 3 µL of loading buffer and electrophoresed on a 10% vertical nondenaturing polyacrylamide gel at 20 mA. The gel was silver staining by DNA Silver Staining Kit (Amersham Pharmacia Biotech, Uppasala, Sweden).

Statistical Analysis

The significance of the differences in observed frequencies of polymorphism in both groups was assessed by the χ^2 test. $P < .05$ was considered statistically significant. Linkage combination was assessed by the ARLEQUIN program (Schneider et al, 2000). The Clump program (Sham and Curtis) was used to assess differences in haplotype frequencies between the control and test groups.

RESULTS

Two mismatches were introduced in the reverse primer of the MMP-1 gene G-1607GG position annealed to the proximity of the polymorphism,[18] creating a recognition sequence (5′-GAANNNNTTC-3′) for the restriction endonuclease *XmnI* when the DNA template contains 1G (but not 2G) at the polymorphism site. Thus, *XmnI* digests the 1G allele, creating 2 fragments of 89bp and 29bp. In A-519G position, the *KpnI* enzyme digestion cleaves the PCR products in 2 fragments of 176bp and 24bp when the polymorphism site contains allele A (but not G).

There was a significant difference in the presence of the different genotype between the control group and test group for the G-1607GG position ($P = .011$). In the control group, the GG/GG genotype was observed with a frequency of 62%, while in the test group (patients with early failure of dental implants) 55% were heterozygous (G/GG genotype). However, the most frequent allele was G in both groups: 75% in the control group and 62% in the test group ($P = .05$). No significant differences were observed in the genotypes and alleles to the A-519G polymorphism between the 2 groups ($P = .064$ and $P = .124$, respectively). The genotype A/G was observed in 50% of the control group, whereas this same genotype was found in 68% of the test group. The allele A was found in 62% of the control group and 50% of the test group. The frequencies of different alleles and genotypes of the MMP-1 gene are shown in Table 3.

The distribution of the haplotypes arranged as alleles and genotypes showed a significant difference between the control and test groups ($P = .031$ and $P = .002$, respectively). The most frequent haplotype allele in both groups was AG, but in the control group only 12.5% of the patients were GGG, while in the test group 28.8% were GGG. The haplotype genotype AG/GG was observed in 36.7% of individuals in the control group, while in the test group 35.6% were AG/GGG (Table 4).

DISCUSSION

Implant losses can arbitrarily be divided into early losses (ie, when osseointegration fails to occur) and late losses (ie, when the achieved osseointegration is lost after a period of function).[26] One way to discriminate between early and late losses is to include all failures occurring before prosthesis placement in the early group and those occurring after functional loading in the late group, if implants are not immediately loaded. Implant loss can be attributed to bio-

Table 3 Distribution of the MMP-1 Allele and Genotype in the Control and Test Group

	Control Group		Test Group		
MMP-1	n	%	n	%	*P*
-1607 Allele	120		88		(qui-quadrado)
G	90	75.00	54	61.36	.050
GG	30	25.00	34	38.63	
Genotype	60		44		
G/G	37	61.66	15	34.06	(qui-quadrado)
GG/GG	07	11.66	05	08.33	.011
G/GG	16	26.66	24	54.54	
-519 Allele	120		88		
A	74	61.66	44	50.00	
G	46	38.33	44	50.00	.124
Genotype	60		44		
A/A	22	36.66	07	15.90	χ^2
G/G	08	13.33	07	15.90	.064
A/G	30	50.00	30	68.18	

Table 4 Frequency of the Haplotype of the MMP-1 Gene in the Control and Test Group

	Control Group		Test Group		
Haplotype	n	%	n	%	*P*
A-519C/G-1607GG Allele					
AG	59	49.2	35	38.9	(Clump)
AGG	15	12.5	09	10.0	.031
GG	31	25.8	20	22.2	
GGG	15	12.5	26	28.9	
Genotype					
AG/GGG	07	11.7	16	35.6	(Clump)
AG/AGG	08	13.3	04	08.8	.002
AG/GG	22	36.7	10	22.2	
AG/AG	11	18.3	03	06.7	
AGG/GGG	01	01.7	04	08.8	
AGG/AGG	03	05.0	00	00.0	
GG/GGG	01	01.7	06	13.3	
GG/GG	04	06.7	02	04.4	
GGG/GGG	03	05.0	00	00.0	

logical, microbiological, or biomechanical factors, but the cause and mechanism of the early implant failure are still obscure. The cluster phenomenon, multiple implant failures in the same subject, supports the evidence that individual characteristics play an important role in the early failure process.[27–29] However, little is known about the influence of genetic susceptibility on osseointegration.

An abnormal immune response involving different cell types such as macrophages, polymorphonuclear neutrophils, T and B lymphocytes, endothelial cells, fibroblasts, keratinocytes, osteoclasts, and osteoblasts can destroy peri-implant tissues.[30–32] If activated, these cells can synthesize and release cytokines[33,34] and lipid mediators,[35] which mediate both the inflammatory and the osteolytic processes. Overall, collagenase is likely to cause increased proteolytic tissue destruction in periprosthetic tissue.[36] Since elevated levels of these mediators are present in diseased implant sites, their analysis may provide an effective monitoring of the disease around dental implants. Genetic polymorphisms probably influence the osseointegration process through the accumulated effect of multiple polymorphisms. To understand the importance of polymorphisms of each allele, it is important to analyze the relative contribution of each polymorphism to the disease phenotype.[37]

Recent studies have failed to present genetic association in implant loss.[38–41] Wilson and Nunn[38] assessed the impact of composite genotype status to IL-1 on implant retention among patients who were smokers or nonsmokers. The authors concluded that no increased risks for implant failure could be attributed to polymorphisms IL-1 genotype-positive. Nevertheless, the presence of smokers in the study group would possibly mask the genetic influence, since it is known that smoking is a strong risk factor for early implant failure—smokers had a 3% greater chance of losing an implant compared to nonsmokers.[37] Feloutzis et al[41] demonstrated that IL-1 composite genotype alone does not appear to influence the risk for peri-implant bone loss, but the risk was significantly higher when the IL-1 genotype was associated with smoking.

A previous report showed that the G-1607GG polymorphism in MMP-1 gene is strongly associated with the early implant failure in nonsmokers.[27] The MMP-1 promoter polymorphisms G-1607GG was also associated with increased susceptibility to chronic periodontitis in Brazilian patients.[9] In this study, the GG/GG phenotype in -1607 polymorphism of MMP-1 gene was associated with early implant failure in nonsmokers. The G/G genotype was observed in 62% of the control group, while it was found in 34% in the test group. Patients bearing this genotype are more likely to attain successful implant osseointegration. The present study also showed that A-519G polymorphism of MMP-1 gene is not associated with the early implant failure. One possible explanation could be the size of the sample. In fact, the small *P* value found in the genotype analysis ($P = .064$) can be attributed to the limited sample size. Unfortunately, early implant failure is not a frequent event, and when smokers are ruled out, the study population is substantially reduced. Early implant failure associated strict exclusion criteria may indicate that these patients have a genetic predisposition. The present

work has the largest sample of implant-loss patients studied so far. Furthermore, the rate of implant loss in the dental service where the patients were recruited is less than 5%. Previous studies used from 39 to 90 patients but also included both smokers and nonsmokers,[38–41] whereas the analysis presented here was performed in 104 nonsmoking subjects. Moreover, the effect of these promoter polymorphisms could be buffered by polymorphisms present in the coding region of this gene or by polymorphisms in other genes that participate in the complex network of interactions mediating the inflammatory process at the periodontal region.

In this study, haplotypes arranged as alleles and genotypes were associated with implant loss. It suggests that a haplotype combination of polymorphisms in MMP-1 gene can influence the osseointegration process. A negative association with chronic periodontitis was observed when the Brazilian population was analyzed for the same haplotype combination.[42] The data suggest a contribution of MMP-1 gene variation to interindividual variability in osseointegration.

An increasing number of studies have shown that a disease phenotype can be associated with a haplotype made up of polymorphisms that are not individually associated with the phenotype.[43–45] The present study showed that risk of implant loss was associated with haplotypes derived from the G-1607GG and A-519G polymorphisms, although there was no association between the osseointegration and A-519G polymorphism individually.

It appears that there are at least 2 reasons that might explain why a phenotype can be associated with a haplotype but not with the individual polymorphisms that make up the haplotype. First, a functional effect on gene expression can be dependent on the interaction between 2 or more polymorphisms[46]; second, haplotypes generally have a higher probability than individual polymorphisms of showing useful linkage disequilibrium with an unknown causal variant.[47] However, a complete explanation awaits analyses to characterize the nuclear proteins involved and their interactions.

Although no association was found between A-519G polymorphism and early implant loss, the role of MMP-1 in osseointegration is confirmed by haplotype combination associated with failure implant in nonsmokers and by the involvement of this cytokine in the inflammatory reaction and bone resorption, which are important in the genesis of implant failure. The results suggest that the MMP-1 gene can have a modifying effect on the osseointegration process and that patients carrying higher MMP-1 expression haplotypes are more likely to experience implant failure because of excessive degradation of fibrillar collagens.

CONCLUSION

On the basis of this study of 60 patients who experienced no implant failure and 44 patients who experienced implant failure, these results indicate that the polymorphism G-1607GG in the promoter of the MMP-1 gene could be a risk factor for early implant failure. This polymorphism could be used as a genetic marker for unsuccessful implants. Although the findings may suggest that A-519G polymorphism maker is not a predictor of the pathologic or clinical consequences of osseointegration, studies with larger sample size are needed to confirm this finding. This study suggests that haplotype G-1607GG and A-519G of MMP-1 may be associated with the osseointegration process. However, more studies are needed to investigate this finding.

The finding of genetic markers related with early implant failure could be of clinical value for precise and early identification of individuals at high risk to implant loss. It could lead to a more strict selection of patients and in the future, individual therapeutics could be developed, thereby increasing the implant success rates.

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