MMP-13 Polymorphism as a Risk Factor in Implant Loss
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**Purpose:** To investigate whether MMP-13 g.-77 A > G (rs2252070) gene polymorphism is associated with early implant loss. **Materials and Methods:** Two hundred nonsmoking volunteers in good oral health, > 18 years of age, and found to be periodontally healthy by clinical examination were matched by age, sex, and implant position and separated into two groups: control group (100 patients with one or more healthy implants for a minimum of 1 year) and test group (100 patients who had suffered early implant loss, considered when implants presented mobility and/or pain before or during abutment connection, requiring their removal). Genomic DNA from saliva was genotyped by PCR-RFLP. Statistical analysis of the results was done using Mann-Whitney U and chi-square tests, with a significance level of 5%. **Results:** A significant difference in the presence of the different alleles and genotype was found between groups for the MMP-13 g.-77 A > G (rs2252070) gene polymorphism ($P = .0161$, OR 95% = 0.57 [0.37 to 0.89]; $P = .007$, OR 95% = 0.44 [0.25 to 0.78]). The A allele increased susceptibility to early implant loss and appeared to be a genetic risk factor. **Conclusion:** The findings suggest that MMP-13 g.-77 A > G (rs2252070) polymorphism may contribute to early implant loss. *Int J Oral Maxillofac Implants* 2019;34:768–771. doi: 10.11607/jomi.7057

**Keywords:** implant loss, metalloproteinase, polymorphism, risk factor

One major challenge of modern implant dentistry is to comprehend the implant loss that tends to cluster into subsets of individuals. Despite the high clinical success rate and an implant permanence of more than 95% in the first 10 years, some patients present failures during the osseointegration process, culminating in implant loss.¹,² In the majority, early failure occurs, due to unsuccessful osseointegration, indicating impaired bone healing.

Early implant loss is a multifactorial condition depending on several extrinsic and intrinsic risk factors, such as age, sex, smoking, medical pre-conditions, bone quality, implant characteristics, and position.³,⁴ However, some losses occur without a clinically recognized mechanism or causes. These findings suggest a crucial impact of genetic influence on osseointegration.

Nowadays, some research is answering questions concerning the role of genetics in implant loss. In this context, single nucleotide polymorphisms (SNPs), the most common form of DNA variation, have been investigated in recent years. Some SNPs in cytokines and matrix metalloproteinases (MMPs) gene already were associated with implant loss and peri-implantitis,⁵⁻¹² which explains some of the inter-individual risk factors.

MMPs are an important class of endopeptidase enzymes secreted by local cells to degrade all substrates of the extracellular matrix and its components. Among this family of enzymes, the subgroup of collagenases (MMP-1, MMP-8, and MMP-13) plays a crucial role in tissue remodeling. MMP-13, or collagenase-3, is capable of degrading aggrecan, gelatin, and all collagen molecules, especially fibrillar collagens,¹³ an essential component in the formation of new bone matrix and in the integration of the implant surface to the surrounding bone.

Although the sequences of the 3.2 billion bases of human DNA are more than 99.9% identical, sequence variations (polymorphisms) contribute to biologic variation and affect how humans develop diseases. Some genetic polymorphisms (SNPs) may affect gene expression levels and protein production or functions;
consequently, they influence osteogenesis and inflammatory responses. Some SNPs in cytokines and lipid mediator genes were associated with implant loss and peri-implantitis, explaining some of the interindividual risk factors.

The MMP-13 gene has a functional SNP characterized by the exchange of the A to G in -77 positions on the gene, which has already been related to various pathologies, including different cancers,14 aneurysms,15 disc degeneration,16 tendinopathy,17 dental agenesis,18 and caries.19

Considering that MMP-13 is one of the important factors in the bone remodeling process and studies in this area are scarce, the present study investigated the contribution of MMP-13 g. -77 A > G (rs2252070) SNP to evaluate the risk for early implant loss.

**MATERIALS AND METHODS**

**Study Population**

This was a case-control study; the Ethical Committee in Research CEP/SD-PB CAAE: 54921616.0.0000.0102 approved the study protocol. This study followed the guidelines of the Helsinki Declaration.

Two hundred nonsmoker volunteers were recruited in three pools of dental clinics from the South and Southeast of Brazil (Dental Clinics of the Faculty of Dentistry of Piracicaba, São Paulo; Latin American Institute for Dental Research, Paraná; and Institute of Postgraduate and Research in Dentistry, Santa Catarina). The implant loss rate (early and later) of these centers was less than 3%. Patients who had suffered early implant loss, agreed to participate in the study, and did not have any of the exclusion criteria were included. The control group was composed of volunteers without implant loss, matched by age, sex, and implant number and position selected from the same dental clinics, presenting for a minimum of 1 year of implant function. The three clinics used 90% Neodent implants and 10% other brands, according to clinical indication and cost.

All volunteers were in good general and oral health, nonsmoking, > 18 years of age, and found to be periodontally healthy by clinical examination. Patients with diabetes, osteoporosis, HIV infection, hepatitis, immunosuppressive chemotherapy, or severely compromised immune function were excluded. Participants who had postsurgical infection were also excluded.

Participants were divided into a control group, 100 patients with one or more healthy implants for a minimum period of 1 year; and a test group, 100 patients who had suffered one or more early implant losses. Healthy implants were considered by evaluating implant immobility, health of peri-implant tissues (including the extent of bone loss, pocket depth, and bleeding on probing), function, and comfort of the patient. Diagnostic criteria used for the assessment of implant loss were presenting mobility and/or pain before or during the abutment connection, requiring removal. All patients with early implant loss in the study period who agreed to participate and did not have any of the exclusion criteria were selected.

**Genotyping**

DNA was extracted from epithelial buccal cells,20 and the concentration (ng/μL) was quantified by optical density 260/280 nm ratio greater than 1.9.

The MMP-13 g.-77 A > G (rs2252070) polymorphism had previously been identified with minor allele frequency greater than 0.3 in the database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/).

PCR-RFLP assay determined the MMP-13 genotype, with approximately 200 ng of DNA, 200 nmol of each PCR primer (forward 5’-GATACGTTCTTACAGAGGC-3’ and reverse 5’-GACAAATCATCTTCTCATCACC-3’), and 1 unit of Go Taq Green PCR Master Mix (Promega). Amplification was carried out with 35 cycles at 94°C, 50°C, and 72°C for 1 minute. One unit of BsrI (BseNI) enzyme digested 10 μL of PCR products at 65°C overnight. These products were electrophoresed on a 2% agarose gel at 20 mA and stained by GelRed (Biotium).

**Statistical Analysis**

The Mann-Whitney U test was used to determine any significant differences between age, sex, and implant position of both groups. Both groups showed similar mean ages, as follows: mean 49 ± 10.1 years (range: 18 to 76 years) for the control group and 49.2 ± 11.2 years (range: 20 to 80 years) for the test group (P = .08). Each group had 65% women, 61% maxillary implants, and a mean 4.6 implants by volunteers. In both groups, 90% used Neodent implants.

Chi-squared test, with P < .05, was used to differentiate frequencies of polymorphism between both groups. Hardy-Weinberg equilibrium was used as a PHASE software (http://stephenslab.uchicago.edu/phase/download.html).

**RESULTS**

Genotype distribution was in Hardy-Weinberg equilibrium. In the present study, MMP-13 rs2252070 A/G polymorphism had significant differences in the frequencies of alleles and genotypes between groups (Table 1). In the test group, the A allele and A/A genotype were found in 76% and 56%, while in the control

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group, the same allele and genotype were found in 64.5% and 36% (P = .0161; OR 95% 0.57 [0.37 to 0.89] and P = .007; OR 95% 0.44 [0.25 to 0.78], respectively).

**DISCUSSION**

Bone remodeling in implant osseointegration is a dynamic process that depends on the correct balance between resorption and deposition of bone, which must be firmly combined quantitatively, in time and in space.\(^2^1\) It should be stressed that this continuous process of bone remodeling ensures long-term implant functionality. Bone turnover processes are regulated by various humoral factors, including a significant role of MMPs.

MMPs interfere in local bone metabolism and are involved in the destruction of connective tissues. The recent literature has shown that MMPs are present in peri-implant sulcular fluid and levels, and molecular forms of MMPs can play a pathologic role in bone loss.\(^2^2^–2^6\) In addition, increased inflammation in combination with the production of MMPs leads to the destruction of periodontal tissue.\(^2^5\) Specific increased MMP-13 activity in gingival fluid was associated with progressive periodontal disease,\(^2^7\) supporting this MMP role in alveolar bone loss.

The present study evaluated a SNP in a heterogeneous and miscegenational Brazilian population, which have significantly overlapping genotypes. Thus, the results reported here should be tested in other populations.

A strong association of MMP-13 g.-77 A > G (rs2252070) SNP with early implant loss was identified in this case-control cross-sectional study. Patients bearing A/A genotypes or A allele appear more probable to have implant loss. The transcriptional activity of A allele was found to be two times higher than the G in MMP-13 g.-77 A > G (rs2252070) SNP.\(^2^8\) Thus, it is considered that this allele potentially alternates the protein expression and influences the process of implant loss, making the extracellular matrix degradation more intense, with excessive collagen fiber breakdown. It might impair the tissue remodeling process and also deregulate signaling pathways and bone cell action.

In previous studies, the present authors also showed association between polymorphism in other collagenases (MMP-1 and MMP-8) and early implant loss.\(^6^–8\) Therefore, it is reasonable to postulate that MMP polymorphisms, mainly in collagenase genes, may play a crucial role in early implant loss.

An important point in validating the association between polymorphism and pathologic processes is a restricted methodologic and study design. The present study observed a number of volunteers with reliable estimated statistical power, despite the exclusion of smokers. In addition, other risk factors including age, periodontal status, medically compromised, and implant position were excluded or matched. This makes the results more robust and showed that MMP-13 g.-77 A > G (rs2252070) polymorphism alone is a risk factor for early implant loss. Interestingly, this same SNP was not associated with peri-implantitis.\(^2^9\)

Nevertheless, nonrestricted criteria for inclusion of volunteers, for example, smoker participants, would possibly mask the genetic influence.

Since that intensity of the inflammation surrounding implants is an important pathophysiologic factor in osseointegration,\(^3^0\) it seems important to investigate SNPs in different regions of MMP gene or other genes involved with periodontal inflammatory mediators, which may be an act synergistically in combination in osseointegration.

The genetic identification of individuals at higher risk of implant loss can contribute to strategies of modulation of the genetic markers and ensure appropriate and individual implant treatment.

<p>| Allele and Genotype Frequencies of Genes MMP-13 g.-77 A &gt; G (rs2252070) in Control and Test Groups |
|-------------------------------------------------|-------|-------|----------|-------|</p>
<table>
<thead>
<tr>
<th><strong>Gene/SNPs</strong></th>
<th><strong>Control group</strong></th>
<th><strong>Test group</strong></th>
<th><strong>P value</strong></th>
<th><strong>OR (95% CI)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-13 (rs2252070)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td>n = 200</td>
<td>n = 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>64.5% (129)</td>
<td>76% (152)</td>
<td>.0161</td>
<td>0.57 (0.37–0.89)</td>
</tr>
<tr>
<td>G</td>
<td>35.5% (71)</td>
<td>24% (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Genotype</strong></td>
<td>n = 100</td>
<td>n = 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>36% (36)</td>
<td>56% (56)</td>
<td>.007</td>
<td>0.44 (0.25–0.78)</td>
</tr>
<tr>
<td>A/G</td>
<td>57% (57)</td>
<td>40% (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>07% (7)</td>
<td>04% (4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed in percentage, with the number of participants (n) in parentheses.
CONCLUSIONS

MMP-13 g.-77 A > G (rs2252070) polymorphism might increase implant loss susceptibility, indicating that this polymorphism could be a potential diagnostic and prognostic factor for early implant loss.

ACKNOWLEDGMENTS

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REFERENCES