Genetic polymorphisms of the Fc γR genes in periodontally healthy Korean population

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I. INTRODUCTION

Over the years, numerous risk factors have been proposed for periodontal disease such as microorganism, smoking, anatomic factors, systemic diseases, genetics.¹ After completion of the human genome, the genetic factor has been emphasized as a risk factor of periodontal disease.

Most of the study related with periodontitis and polymorphism is on the proinflammatory cytokine gene such as Interleukine-1(IL-1), TNF-α.²³ And the polymorphisms of the receptors for the constant part of IgG (Fc γR) also have been studied.⁴⁵

Receptors for the constant part (Fc) of immunoglobulins (especially IgG) is important in the immunologic defense system. Polymorphonuclear neutrophils (PMNs), macrophages, monocytes play a major role in the innate defense system against bacterial infection in humans. These cells may move toward a site of infection and recognize, bind and phagocytose the bacteria. Fc receptor of immunoglobulins play a crucial role in this step.

Functional biallelic polymorphisms have been identified for 3 Fc γR subclasses: Fc γRIa, Fc γRIIa, and Fc γRIIb. Fc γRIa has either an arginine (Fc γRIa-R131) or histidine (Fc γRIa-H131) at amino acid position 131 in the second extracellular Ig-like domain, Macrophages/monocytes and natural killer (NK) cells express Fc γRIIIa, which bear a valine (V)-phenylalanine (F) polymorphism at amino acid position 158 in the second extracellular domain. The PMN-specific Fc γRIIIb bears the NA1-NA2 polymorphism caused by 4 amino acid substitutions within the first extracellular Ig-like domain.⁶⁸

Polymorphisms of the Fc γR have been shown to influence PMN phagocytic function.⁹ Studies on the

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these polymorphisms suggest that there would be the interindividual differences in the efficacy of Fc γR mediated effector functions depend on Fc γR polymorphisms and react differently at the bacterial infection such as periodontitis.

The genetic polymorphism of periodontitis-associated gene such as IL-1 have been known to influence by race and ethnicity. Previous reports suggested a strong effect of ethnicity on Fc γRIIA and Fc γRIIB genotype frequencies. Japanese and white Dutch donors, for example, differ significantly in genotype distributions of Fc γRIIA and Fc γRIIB.

In this study, the genotype distribution of Fc γRIIA and Fc γRIIB, and Fc γRIIB was studied by PCR method in periodontally healthy Korean population.

II. MATERIALS AND METHODS

1. Subjects and clinical assessments

Sixty five systemically healthy subjects were included in this study. Subjects (49 males and 16 females; age 19-39 years (mean age: 24.90± 3.04 years), who showed neither attachment loss nor probing depth greater than 4mm at more than one sites. All subjects were Korean and none had a history or current manifestation of systemic disease. The study population consists of dentists and dental assistants who works at the Seoul National University Dental Hospital and senior students of College of Dentistry, Seoul National University. The study was approved by the Institution Review Board at Seoul National University Hospital and written informed consent was obtained from all subjects.

Clinical parameters including probing depth, clinical attachment level, bleeding on probing, gingival index were assessed. Probing depth and clinical attachment level was recorded using the Florida Probe (Florida Probe Co., Gainesville, Fl, USA) in 6 sites of the tooth.

2. Isolation of genomic DNA

Genomic DNA was obtained from peripheral blood by using a DNA extraction kit (Puregen, Gentra System, Minneapolis, MN) according to the manufacturer's instructions. The genotyping procedure was performed according to the previously described method by Kobayashi et al.

1) Fc γRIIA-R-H131 genotyping

Genotypes of the Fc γRIIA-R-H131 were determined by polymerase chain reaction (PCR) using PCR premixture (Bioneer, Korea). First, 100ng of genomic DNA was added to PCR premixture with each of primer P65 (5'-CAA GCC TCT GGT CAA GGT C-3') and P52 (5'-GAA GAG CTG CCC ATG CTG-3'), and 1U Ampli Taq gold DNA polymerase.

First PCR procedure was done as follows: 1 cycle at 95°C for 5 minutes, 55°C for 5 minutes, and 72°C for 5 minutes. This was followed by 35 cycles at 95°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes, ending with an extension step at 72°C for 10 minutes. This first PCR product was divided and one half of the first PCR product was subsequently reamplified using the combination of the common antisense primer P13 (5'-CTA GCA GCT CAC CAC TCC TC-3') located on intron 4 and the nt 507 G-specific primer P5G (5'-GAA AAT CCC AGA AAT TTT TCC G-3') or A-specific primer P4A (5'-GAA AAT CCC AGA AAT TTT TCC A-3'). Conditions for the second PCR were as follows: 95°C for 5 minutes followed by 30 cycles of 95°C for 15 seconds, 62°C for 30 seconds, and 72°C for 30 seconds, with an extension step at 72°C for 10 minutes. The PCR products were determined by electrophoresis on a 3% agarose gel stained with ethidium bromide.
reactions yielded a 278 bp product for both Fc γRIIa alleles.

2) Fc γRIIa-158V-F genotyping

Fc γRIIa-158V-F genotyping also used a allele-specific PCR procedure. Three different primers were used for the detection of one base transition (nt 559-G/T). Following primer was used as common Fc γRIIa-specific forward primer: 5'-TCA CAT ATT TAC AGA ATG GCA ATG G-3'; nt 449-473. The nt 559G-specific reverse primer (5'-TCT CTG AAG ACA CAT TTC TAC TCC CTA G-3'; nt 586-559) was used for finding Fc γRIIa-158V allele, and The 559T-specific reverse primer (5'-TCT CTG AAG ACA CAT TTC TAC TCC CTA A-3'; nt 586-559) was used for finding Fc γRIIa-158F allele. PCR was performed with PCR premixture (Bioneer, Korea) and 100 ng genomic DNA, 200 nM of each primer in a 50 µl reaction volume. PCR conditions were as follows: 1 cycle at 95°C for 9 minutes, 37 cycles at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 20 seconds, with a final extension at 72°C for 10 minutes. The PCR products were determined by electrophoresis on a 3% agarose gel stained with ethidium bromide. PCR product with 138 bp can be found at the electrophoresis.

3) Fc γRIIb-NA1-NA2 genotyping

A PCR using allele-specific primers was used for Fc γRIIbNA1-NA2 genotyping.

For genotyping for NA1, 100ng of genomic DNA was added to PCR premixture (Bioneer, Korea) with the sense primer (5'-CAG TGG TTT CAC AAT GTG AA-3'; nt 208-227) and the antisense primer (5'-CAT GGA CTT CTA GCT GCA CCG-3'; nt 329-349) PCR conditions were as follows: 1 cycle at 95°C for 9 minutes, 35 cycles at 95°C for 30 seconds, 63°C for 30 seconds, and 72°C for 30 seconds, ending at 72°C for 10 minutes.

For genotyping for NA2, 2 different primers were used; NA2 sense primer (5'-CTC AAT GGT ACA GCG TGC TT-3'; nt 128-147) and NA2 antisense primer (5'-CTG TAC TCT GCA CTG TCG TT-3'; nt 277-296). The amplification protocol was as follows: 1 cycle at 95°C for 9 minutes, 35 cycles at 95°C for 30 seconds, 64°C for 15 seconds, and 72°C for 30 seconds, ending at 72°C for 10 minutes. After gel electrophoresis the end products of the reactions were 141bp for NA1 and 169bp for NA2, respectively.

III. RESULTS

All periodontally healthy subject's clinical evaluation data was shown in Table 1. Mean probing depth was 2.04mm (SD: 0.21) and mean clinical attachment loss was 2.08mm (SD: 0.23). The percentage sites of bleeding on probing was 7.53±5.49 and gingival index was 0.16±0.11.

<table>
<thead>
<tr>
<th>Table 1, Clinical characteristic of subjects (mean±SD)</th>
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<tbody>
<tr>
<td>Clinical Parameters</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Male/Female</td>
</tr>
<tr>
<td>Probing depth mm (mean±SD)</td>
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<tr>
<td>Clinical attachment loss mm (mean±SD)</td>
</tr>
<tr>
<td>% sites with BOP</td>
</tr>
<tr>
<td>Gingival index</td>
</tr>
</tbody>
</table>

The genotype frequencies of Fc γRIIa, Fc γRIIIa, Fc γRIIb were shown in Table 2.

The genotype frequency of Fc γRIIa, and Fc γRIIIa showed similar distribution. More than 50% of the subjects were Fc γRIIa-H131 homozygous and Fc γRIIIa-158F homozygous, And only 5 (7.7%) of 65 subjects were Fc γRIIa-R131 homozygous and Fc γRIIIa-158V homozygous, In genotype frequency of Fc γRIIb, 35 subjects (53.8%) were Fc γRIIb NA1/NA2 heterozygous, 22 subjects (33.9%) were
Table 2. Distribution of genotype frequency of Fc γRIIa, Fc γRIIIa, Fc γRIIIb

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n = 65</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc γRIIa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/R131</td>
<td>5</td>
<td>7.7</td>
</tr>
<tr>
<td>R/H131</td>
<td>25</td>
<td>38.5</td>
</tr>
<tr>
<td>H/H131</td>
<td>35</td>
<td>53.8</td>
</tr>
<tr>
<td>Fc γRIIIa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>158V/V</td>
<td>5</td>
<td>7.7</td>
</tr>
<tr>
<td>158V/F</td>
<td>23</td>
<td>35.4</td>
</tr>
<tr>
<td>158F/F</td>
<td>37</td>
<td>56.9</td>
</tr>
<tr>
<td>Fc γRIIIb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA1/NA1</td>
<td>22</td>
<td>33.9</td>
</tr>
<tr>
<td>NA1/NA2</td>
<td>35</td>
<td>53.8</td>
</tr>
<tr>
<td>NA2/NA2</td>
<td>8</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Table 3. Distribution of allelic frequency of Fc γRIIa, Fc γRIIIa, Fc γRIIIb

<table>
<thead>
<tr>
<th>Genes</th>
<th>Allele</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc γRIIa</td>
<td>R131</td>
<td>26.9</td>
</tr>
<tr>
<td></td>
<td>H131</td>
<td>73.1</td>
</tr>
<tr>
<td>Fc γRIIIa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>158V</td>
<td>25.4</td>
<td></td>
</tr>
<tr>
<td>158F</td>
<td>74.6</td>
<td></td>
</tr>
<tr>
<td>Fc γRIIIb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA1</td>
<td>60.8</td>
<td></td>
</tr>
<tr>
<td>NA2</td>
<td>39.2</td>
<td></td>
</tr>
</tbody>
</table>

NA1/NA1 homozygous, and only 8 subjects(12.3%) were NA2/NA2 homozygous.

The allelic frequencies of Fc γRIIa, Fc γRIIIa, Fc γRIIIb were shown in Table 3.

The allelic frequencies of Fc γRIIa, Fc γRIIIa showed also similar distribution. Three quarters of the gene were Fc γRIIa-H131, Fc γRIIIa-158F. In Fc γRIIIb, Fc γRIIIb-NA1 showed more frequently than Fc γRIIIb-NA2 allele(60.8 vs 39.2).

IV. DISCUSSION

Fc γ receptors(Fc γRs) have been the subject of numerous investigations in periodontal research because of its ability of binding the Fc portion of IgG antibody. The binding the Fc portion of IgG antibody are important in the phagocytosis, cytotoxicity, enhancement of antigen presentation, and the release of inflammatory mediators.15

Polymorphism in the genes encoding the low affinity receptors Fc γRIIa, Fc γRIIIa, and Fc γRIIIb may effect on antibody binding, phagocytosis and hence susceptibility to periodontitis. The Fc γRIIa H-R131 polymorphism changes receptor affinity of IgG2 and IgG3 which can change the efficiency of phagocytosis by PMN. PMNs from Fc γRIIIb-NA2 individuals bind IgG1 or IgG3 less efficiently than those from Fc γRIIIb-NA1 individuals. The Fc γRIIIa-158V allotype exhibits higher affinity for both monomeric and immune complexed IgG1 and IgG3 than Fc γRIIIa-158F does, and is capable of binding IgG4.67,160

Frequencies of Fc γR genotypes have been shown to be influenced by race and ethnicity like IL-1, Fc γ RIIa and Fc γRIIIb genotype distributions were different significantly between Caucasians and Japanese. And the Japanese and Chinese have an increased frequency of the Fc γRIIa-HH131 allotype (61 and 50% respectively) as compared to the Caucasian group(23%) and the Asian Indian group. In our study, Korean have also increased frequency of the Fc γRIIa-HH131 allotype(53.8%). However in other study in Korean population frequency of the Fc γRIIa-HH131 allotype was only 41% and the frequency of Fc γRIIa-RH131 heterogenous type was greatest(49%).18

Fc γRIIa polymorphisms was not shown ethnic difference in several reports. Fc γRIIa genotype frequencies were not significantly different between Caucasian dutch and Japanese.19

Although the Fc γRIIIa genotype frequency of Korean people in our study was similar with Japanese, further research should have to be done. Fc γRIIIb genotype distribution in our study was

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somewhat different from that of the Japanese. The frequency of FcγRIIb-NA1/NA2 heterozygous was higher in Korean (53.8%) than in Japanese (45.3%). In Japanese population the frequencies of FcγRIIb-NA1/NA1 homozygous and FcγRIIb-NA1/NA2 heterozygous was similar.

V. CONCLUSION

In this study the distribution of FcγR polymorphisms was studied in periodontally healthy Korean population with PCR methods. The frequencies of FcγRIIa genotype-RR131, RH131 and HH131 were 7.7%, 38.5% and 53.8%. The frequencies of Fcγ RIllla genotype 158V/V, 158V/F and 158F/F were 7.7%, 35.4% and 56.9%. And the frequencies of Fcγ RIlllb genotype NA1/NA1, NA1/NA2 and NA2/NA2 were 33.9%, 53.8 and 12.3%.

The FcγR polymorphisms have been known to have relationship with periodontitis. So research on FcγR polymorphism related with periodontitis is needed in Korean population.

VI. REFERENCES

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치주적으로 건강한 한국인에서 FcγR 유전자
유전자 다형성 발생빈도에 관한 연구

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면역글로불린의 Fc 부분에 대한 수용기인 FcγR는 세균에 대한 인식, 결합과 포식작용과정에서 중요한 역할을 한다. 이 FcγR에서 FcγRIIa, FcγRIIb, FcγRIIIb의 유전자 다형성이 치의학 분야에서 연구되고 있다. FcγRIIa에서는 두번째 세포의 면역글로불린 ys가 양성의 131번째 아미노산에서 아르기닌(FcγRIIa-R131) 혹은 헤스테던(FcγRIIa-H131)을 갖고 있으며, FcγRIIIa에서는 두번째 세포의 양성의 158번째 아미노산이 발리(FcγRIIIa-158R) 혹은 페닐알라닌(FcγRIIIa-158F)을 갖고 있다. FcγRIIIb에서는 첫번째 세포의 면역글로불린 ys가 양성의 4개의 아미노산의 유전자 다형성으로 인해 FcγRIIIb-NA1과 FcγRIIIb-NA2의 두 가지 유전자 다형성을 보이고 있다.

이번 연구는 치주적으로 건강한 한국인에서 FcγRIIa, FcγRIIb, FcγRIIIb에 대한 유전자형의 분포를 조사하고자 한 것으로 서울대학교 치과병원에 근무하는 치과의사, 치과위생사, 간호조무사 및 서울대학교 치과대 4학년 학생 중 치주질환과 부작용이 4mm 이하인 치주적으로 건강한 한국인 65명을 대상으로 하였다.

FcγRIIa, FcγRIIb, FcγRIIIb의 유전자 다형성을 분리한 DNA에 각 대립유전자에 특이성을 가진 primer를 넣고 PCR(Polymerase Chain Reaction)법을 이용하여 중폭시키기 전기영동법을 이용하여 각 대립유전자와의 존재를 확인함으로써 결정하였다.

FcγRIIa의 유전자 다형성은 R/R131, R/H131, H/H131의 유전자형에 대하여 각각 7.7%, 38.5%, 53.8%의 분포를 보였으며, FcγRIIIa의 158V/V, 158V/F, 158F/F 유전자형에 대하여 각각 7.7%, 35.4%, 56.9%의 분포를 보였다. 또한 FcγRIIIb의 NA1/NA1, NA1/NA2, NA2/NA2 유전자형은 각각 33.9%, 53.8%, 12.3%의 분포를 보였다.

 이를 바탕으로 각 대립유전자의 발생빈도는 다음과 같이 나타났으며, FcγRIIa의 158V, 158F 유전자형이 각각 25.4%, 74.6%로 나타났다.

FcγRIIIb의 NA1, NA2 유전자형의 발생빈도는 60.8%, 29.2%로 나타났다.

이번 연구는 치주적으로 건강한 한국인에서의 FcγRIIa, FcγRIIb, FcγRIIIb에 대한 유전자형의 분포를 조사한 것으로, 이후 치주질환자의 유전자형 분포와의 비교로 치주질환과 FcγRIIa, FcγRIIIa, FcγRIIIb의 유전자형과의 관련성에 관한 추가적인 연구가 필요할 것으로 여겨진다.

주요어 : FcγR 유전자, 유전자 다형성, 대립유전자

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