The 10th Anniversary
FUKUOKA ACTIVE AGING CONFERENCE
IN ASIA PACIFIC 2016

Constructing an Age-friendly Collaboration among Academic, Industrial, Governmental and Civic Circles

CONFERENCE BOOK
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53. IL-10 627C>A Polymorphism in Postmenopausal Indonesian Women

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Abstract
Background: Polymorphism of numerous genes may have an impact in their functional performance in the complex regulatory and other processes that can affect bone turnover and maintenance, particularly in postmenopausal women who appear to face an increasing risk of osteoporosis, also in Indonesia. The multifactorial etiology of osteoporosis includes a known genetic component, which however itself includes many known and candidate genes with variation in their activities and roles. One suggested influential factor in this interactive environment is the IL-10 gene that encodes a cytokine controlling cellular and hormonal immune responses. Objective: This study was conducted to analyze the possible relationship between the IL-10 -627C>A polymorphism and the risk of osteoporosis in postmenopausal Indonesian women. Materials and methods: The study was approved by the Ethics Committee of the Faculty of dentistry, University of Indonesia. In total, 100 stored DNA samples were enrolled for the study. The samples originate from blood serum of postmenopausal women with known Bone Mineral Density (BMD), including 29 subjects with normal BMD and 71 subjects with a risk of osteoporosis. The status of IL-10 -627C>A polymorphism was evaluated for the samples by using the PCR-RFLP technique with Rsal restriction enzyme. Results: The genotype distributions of both samples from subjects with normal and reduced BMD were consistent with the Hardy-Weinberg equilibrium (p > 0.05). The minor homozygous CC genotype appeared in 10.3% and 15.5% of the normal and reduced BMD groups, respectively, and the major homozygous AA genotype was involved in 55.2% and 38% of the cases with normal and reduced BMD cases, respectively. The sample was not large, and showed no significant association between the polymorphism status and the indicated risk of osteoporosis. Conclusion: The study showed the genotype distribution of IL-10 -627C>A polymorphism in Indonesian women. No significant correlation was found between the genetic polymorphism IL-10 -627C>A and the risk of osteoporosis.

Keywords: Genetic polymorphism, IL-10, postmenopausal women, Osteoporosis
Poster Session

Appraisers

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- Dr. Tri Budi W. Rahardjo
- Dr. Siti Setiati
- Dr. Sungkook Lee
- Dr. Yoshiko Someya
- Dr. Takeo Ogawa
Introduction

Osteoporosis is a disease of bone metabolism marked by decreasing bone mass or bone mineral density (BMD), deteriorating bone and increasing bone fragility. The multifactorial etiology of osteoporosis is known to include genetic factors, and one of the suspected genes is that encoding for interleukin-10 (IL-10). IL-10 can promote osteoblast differentiation in bone marrow, and in combination with inhibition of osteoclast formation. Modification of the IL-10 activity by single nucleotide polymorphisms (SNPs) of the gene encoding for IL-10 could therefore shift the balance of bone metabolism and turnover, and the resulting risk of osteoporosis.

Objectives

To assess the potential association of the C627A polymorphism of the IL-10 promoter with BMD, and by implication, with osteoporosis in postmenopausal women.

Materials and Methods

100 extracted DNA samples from the blood serum of postmenopausal women. The inclusion criteria are women without menstrual cycle for more than one year and had signed the informed-consent form.

Table 1: Characteristics of 100 samples (T-score)

<table>
<thead>
<tr>
<th>Variation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>29</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>50</td>
</tr>
</tbody>
</table>

This study was approved by the Research Ethics Committee of the Faculty of Dentistry, University of Indonesia.

IL-10 C627A genotypes were determined by polymerase chain reaction (PCR). The PCR products were checked by electrophoresis and visualized with GelDoc (Biorad).

For C627A polymorphism genotyping, RFLP was applied using BsrI restriction cutting enzyme (New England Biolabs). The digested fragments were subjected to electrophoresis on 3% agarose gel and visualized with GelDoc. The resulting products indicated a single 412 bp fragment for the CC genotype, three fragment (412, 236 and 176 bp) for the CA genotype, and two fragments (236 and 176 bp) genotype. Chi-square testing, using SPSS 16.0 software, was mainly applied for analysis of the results, assuming significance for p-values less than 0.05.

Results

Table 2: Genotype and BMD distributions; normal, osteopenia, osteoporosis

<table>
<thead>
<tr>
<th>Type</th>
<th>Normal (n=50)</th>
<th>Osteopenia (n=50)</th>
<th>Osteoporosis (n=50)</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2C2</td>
<td>15</td>
<td>13</td>
<td>12</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>C2A1</td>
<td>18</td>
<td>16</td>
<td>8</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>A2A2</td>
<td>17</td>
<td>12</td>
<td>12</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>41</td>
<td>32</td>
<td>121</td>
<td></td>
</tr>
</tbody>
</table>

An example of the indicated genotypes of IL-10 C627A polymorphism after RFLP electrophoresis is shown in Figure 1. Table 2 shows the frequency and percentage of the genotypes in each BMD (T-scores) group. CC genotype (and C allele) represented a minority of the observed cases, but increasing frequency from normal to osteoporotic conditions. However, no significant association of the genotypes of IL-10 C627A polymorphism and BMD was found (p = 0.132).

Discussion

Genotype distribution of the C627A polymorphism of IL-10 promoter (Table 2) showed no significant association of the genotypes and BMD, although there was a systematic trend of increasing frequency of the CC genotype and C allele from normal to osteoporotic condition. The CC genotype and C allele represented a minority of the observed cases in all BMD groups (normal, osteopenia and osteoporosis). There appears to exist currently only very few reports on the corresponding genotype (or alleles) distributions of the same IL-10 polymorphism from elsewhere.

Table 3: IL-10 C627A genotype distributions and BMD Genotype distribution of the C627A polymorphism

<table>
<thead>
<tr>
<th>Genotype (n=121)</th>
<th>CC (n=48)</th>
<th>CA (n=42)</th>
<th>AA (n=32)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>% total group</td>
<td>39.6</td>
<td>34.6</td>
<td>25.8</td>
<td>100</td>
</tr>
<tr>
<td>% within group</td>
<td>30.0</td>
<td>26.0</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.132</td>
<td>0.132</td>
<td>0.132</td>
<td>0.132</td>
</tr>
</tbody>
</table>

The genotype fractions of the present work were not much unlike those reported from Taiwan by a study (Table 3) that did suggest a significantly elevated risk of osteoporosis associated with the CC genotype.

Conclusion

No significant association of the genotypes of the IL-10 C627A polymorphism with BMD (osteoporosis) in postmenopausal Indonesian women.
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