

## Interleukin-6-174 G/C Genetic Polymorphism in Indonesian Postmenopausal Women

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### Abstract

Osteoporosis is a multifactorial disease with a recognized, but not well defined, genetic component. Many contributing candidate genes have a role in bone metabolism, such as the gene encoding for interleukin-6 (IL-6). Previous work on the relationship between IL-6 with osteoporosis suggested that IL-6 can increase the number of osteoclasts and lead to lower bone mass density (BMD). However, the influence of IL-6 -G174C polymorphism on postmenopausal women with osteoporotic risk still needs further research. The aim of this study was to examine the relationship between the Interleukin-6 -G174C gene polymorphism and osteoporosis risk in postmenopausal women. We collected 100 blood samples from postmenopausal Indonesian women (23 with normal BMD and 77 with osteoporosis) for analysis using PCR-RFLP with the NlaIII enzyme. The results showed that 96 (96%) of the subjects in the study population carried the GG genotype, 4 (4%) had the GC genotype, and none had the CC genotype. No significant association was determined between osteoporosis risk in postmenopausal women and IL-6 -G174C polymorphism (Fisher test,  $p=0.571$ ).

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### Introduction

With age, the level of bone mineral density (BMD) is reduced. BMD reaches its peak at the age of 30–35 years and the decline begins at age 40–50 years, with the decline ranging from 0.3 to 0.5% per year for men and women. Postmenopausal women bone density will undergo a 10-fold decrease due to the reduced estrogen level as a result of the cessation of hormone production in the ovaries. Thus, postmenopausal women have a high risk of osteoporosis.<sup>1</sup>

Osteoporosis occurs when bone resorption is greater than bone formation. The reason could be multi factorial, such as hormonal factors, environment, nutrition, lifestyle habits, genetics, and epigenetic factors.<sup>2</sup>

One of the genetic factors that plays a role in osteoporosis is the interleukin-6 (IL-6) gene. IL-6 is an inflammatory cytokine whose production is increased if there is a shortage in estrogen hormone. This has been demonstrated by Manolagas in a study on cultured bone marrow cells treated with 1,25-dihydroxy vitamin D3 (1995). Similarly, when estrogen production is no longer sufficient, IL-6 increases in the bone marrow.<sup>1</sup>

Other studies on the relationship between IL-6 with osteoporosis have reported that IL-6 genetic polymorphisms triggered an increase in osteoclast differentiation and function that would result in lower bone mass density<sup>2</sup>. Apart from the IL-6 gene, polymorphisms in the vitamin D receptor, calcitonin (CT), estrogen receptor alpha (ERa), estrogen receptor beta (ERb), collagen type I alpha, apolipoprotein E, transforming growth factor-b1, parathyroid hormone, and other genes have been studied in relation with osteoporosis.<sup>3</sup>

In this study, we analyzed the association of the IL-6 -174 G/C gene polymorphism and

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bone mineral density in postmenopausal women in Indonesia.

### Materials and methods

#### Subjects

In this study, we used 100 DNA samples extracted from the serum of post-menopausal women with a recorded T-score using the techniques used by Auerkari *et al.*<sup>4-7</sup> and stored in a -20°C freezer at our university laboratory. The study was approved by the Ethics Committee of the Faculty of Dentistry, University of Indonesia.

#### IL6 genotyping

The IL-6 -174 G/C (rs1800795) genotype was determined by the polymerase chain reaction (PCR)/Restriction Fragment length

polymorphisms (RFLP) using the primers 5'-TTG TCA AGA CAT GCC AAA GTG-3' (forward) and 5'-TCA GAC ATC TCC AGT CCT ATA-3' (reverse).<sup>4-7</sup> The PCR reaction mixture contained 0.5µL of each primer, and 10µL of Mastermix (BioLine) in a reaction volume of 17µL. The thermal cycle used the following reaction conditions: initial step 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 58 °C for 30s, extension at 72°C for 30s, and final extension at 72°C for 5 min. The 300bp product was digested with NlaIII (New England Biolabs, Genetika Science Indonesia PT, Jakarta, Indonesia) for 16 hours at 37°C. The digested PCR products were three fragments (13, 54, and 233bp) for the G allele and four fragments (13, 54, 111, and 122bp) for the C allele. These fragments were visualized in a 3% agarose gel stained with GelRed.

### Results

This cross-sectional study of 100 DNA samples from postmenopausal women with T-scores, examined by genotyping for IL-6 -174 G/C, revealed 23 normal samples and 77

samples that had an osteoporosis risk. All samples were successfully analyzed for the SNP of IL-6 -174 G/C (Table 1).

**Table 1.** IL-6 -174 G/C genotypes and alleles in osteoporotic and normal BMD

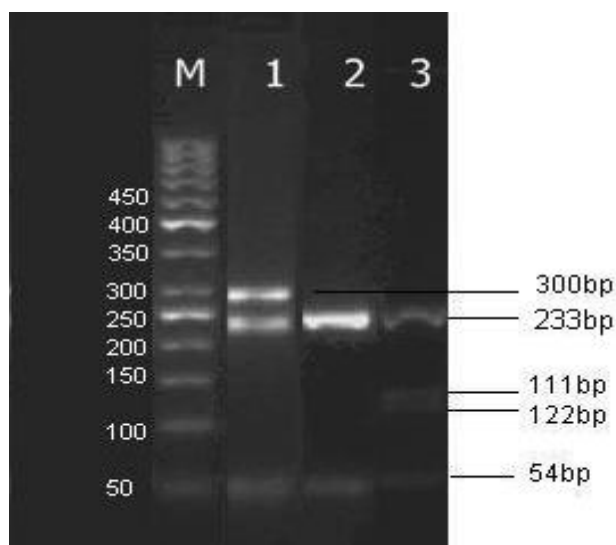
		Normal	Osteoporosis	P value
Genotype	GG	23 (23%)	73 (73%)	0.571*
	GC	0 (100%)	4 (4%)	
Allele	G	46 (23%)	150 (75%)	
	C	0 (0%)	4 (4%)	

\*Fisher test

The IL-6 polymorphism was analyzed using standard PCR-RFLP, digested using the NlaIII enzyme. The G/G homozygote was cut at 13, 54, and 233 bp, the G/C heterozygote was cut at 13, 54, 111, 122, and 233 bp, and the C/C homozygote was cut at 13, 54, 111, and 122 bp (Figure 1). The 13 bp cut is not shown in the electrophoresis image, because it is located within the sense primer location.<sup>8</sup>

The results of -174 G/C SNP showed that the allele G and GG genotype dominated in the

population studied. Of the 100 samples, 96 carried the GG genotype, 4 carried the GC genotype, and none carried the CC genotype in this study population. Calculation by the Hardy Weinberg Equation indicated that our population followed Hardy Weinberg Equilibrium ( $\chi^2 = 2.04$ ,  $p > 0.05$ ). However, no significant association was noted between BMD and the SNP of IL-6 -174 G/C according to the statistical analysis using the Fisher test ( $p = 0.571$ ).



**Figure 1.** Genotyping for the IL-6-174 G/C polymorphism, cut by the NlaIII enzyme and analyzed by agarose gel electrophoresis. M represents 50bp DNA ladder, lane 1 undigested PCR product at 300bp and G/G homozygote at 233 and 54bp. Lane 2 represents G/G homozygote at 233 and 54bp, and lane 3 G/C heterozygote at 233, 111, 122, and 54bp.

## Discussion

In our study, we examined the relationship between the IL-6 -174 G/C gene polymorphism and bone mineral density in postmenopausal women in Indonesia, and we found that no significant relationship. Our study showed very little variation, as only 4 G/C heterozygotes and no C/C homozygotes were detected in the 100 samples. However, the role of IL-6 as a stimulating factor for osteoclast has been proven to reduce bone mineralization.<sup>9</sup>

Several studies on IL-6 -174 G/C gene polymorphism also have been reported from other countries, and showed varied results (Table 2).

The frequency of the C allele is high in the Caucasian race,<sup>10</sup> with a frequency of about 0.4, and low in the negroid race and in Asia. In the negroid race, the frequency of the C allele is 0.059. In Asian countries, such as Korea<sup>11</sup>, Japan<sup>12</sup>, Taiwan<sup>13</sup>, and China,<sup>14,15</sup> the frequency for the C allele is below 0.01, and this fact can be interpreted as indicating no polymorphism. In our study (Indonesia) and a study in Thailand,<sup>16</sup> the frequency of the C allele is also low, but higher than 0.01. However, in India<sup>17</sup> the frequency of the C allele is more than 0.1, with a significant result.

**Table 2.** Genotype and C allele frequencies of IL-6 -174 G/C in various countries

Country/ ethnic	GG (%)	GC (%)	CC (%)	C allele frequency
Asia				
Indonesian (our study)	96 (95%)	5 (5%)	0 (0%)	0.025
Korean <sup>10</sup>	155 (98.7%)	2 (1.3%)	0 (0%)	0.006
Japanese <sup>11</sup>	362 (100%)	0 (0%)	0 (0%)	0
Taiwan <sup>12</sup>	207 (99%)	2 (0.97%)	0 (0%)	0.005
Chinese <sup>13</sup>	297 (97%)	3 (3%)	0 (0%)	0.005
Thai <sup>15</sup>	247 (97%)	8 (3%)	0 (0%)	0.016
Indian <sup>16</sup>	555 (75.8%)	138 (18.9%)	39 (5.3%)	0.148
Caucasian <sup>9</sup>	166 (36%)	216 (46.7%)	80 (17.3%)	0.407
Caucasian German <sup>8</sup>	123 (33.3%)	175 (47.4%)	71 (19.2%)	0.429
UK Caucasian <sup>8</sup>	144 (38%)	169 (44%)	70 (18%)	0.403
Negroid <sup>9</sup>	119 (88.2%)	16 (11.8%)	0 (0%)	0.059

We suppose that frequency of the C allele in India is high due to the presence of Caucasian ethnicity that are known as the Aryan people,<sup>18</sup> although Dravidian people are also present who are not Caucasian. In Korea, Japan, Taiwan, and China, the populations there are almost homogeneous and consist mainly of the mongoloid race,<sup>19</sup> compared to Indonesia and Thailand, which have a more varied population, especially in Indonesia.

The reason for the higher polymorphism frequency in Indonesia might be due to the presence of remnants of colonial Caucasian descendants in Indonesia, which are prone to the polymorphism. It also can be due to different environments, as polymorphism is evident in Indonesia, but not in China, Japan, Korea, and Taiwan. However, we did not examine the race and environment from our samples, which was a limitation of our study.

### Conclusion

No significant association was found between the IL-6-174 G/C gene polymorphism and BMD in postmenopausal Indonesian women ( $p=0.571$ ). The observed frequency of IL-6-174 G/C gene polymorphism (C allele) was about 2.5%, which is much lower than in Caucasian people but higher than in populations in eastern Asia.

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