

IL-10 C627A Polymorphism and BMD in Postmenopausal Indonesian Women

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Abstract

Osteoporosis is a disease of bone metabolism marked by severe loss of bone mass, degradation of bone microarchitecture, and increased risk of fracture. The disease is typically accompanied by a 50–85% reduction in bone mineral density (BMD), which is a good predictor of the osteoporotic condition and the likelihood of fracture. The multifactorial etiology of osteoporosis is known to include genetic factors, and one of the suspected genes encodes interleukin-10 (IL-10), a cytokine that is involved in the control of both cellular and hormonal immune responses. The aim of this study was to assess the potential association of the C627A polymorphism of the IL-10 promoter with osteoporosis. For genotyping, blood serum samples were obtained from 100 consenting postmenopausal Indonesian women. The subjects included 29 control cases with a normal range of BMD, 21 cases of osteopenia, and 50 cases of osteoporosis, as classified by T-scores based on ultrasound measurement of the calcaneus bone. The genotype status of IL-10 C627A polymorphism was examined by the PCR-RFLP technique. The observed genotype fractions (CC/CA/AA, in %) were 10.3/34.5/55.2 for normal control cases, 14.3/42.9/42.9 for osteopenia cases, and 16.0/48.0/36.0 for osteoporosis cases. Generally, the genotype distributions were consistent with the Hardy-Weinberg equilibrium. The results suggest that the genotypes or alleles of the IL-10 C627A polymorphism are not significantly associated with BMD or osteoporosis risk in postmenopausal Indonesian women.

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Introduction

Osteoporosis is a disease of bone metabolism marked by decreasing bone mass or bone mineral density (BMD), deteriorating bone micro architecture, and increasing bone fragility. The incidence and cost of osteoporosis increase with the rising proportion of elderly people in the world population, and most victims of the disease are postmenopausal women.¹⁻³ Normal bone remodeling is a totally renewal of the human skeleton approximately every 10 years by a tightly controlled process of new bone formation by osteoblasts and bone resorption by

osteoclasts. Both environmental and genetic factors are known to affect the etiology of osteoporosis that results from an imbalance in this remodeling, with an increased rate of resorption that is no longer compensated by new bone formation, usually after 40–50 years of age.^{4,5} One of the suggested susceptibility genes is the one that encodes interleukin-10 (IL-10), a cytokine that is involved in the control of inflammatory and immune responses, and in the synthesis of other cytokines and chemokines, including IL-1, TNF- α , IL-6, IL-8, and IL-12.⁶⁻⁹ IL-10 is produced by a variety of cells, such as T_H2 cells, eosinophils, and lymphoid cells, and has a role in obstructing replication of macrophage cells, such as osteoclasts. IL-10 can promote osteoblast differentiation in bone marrow, and in combination with inhibition of osteoclast formation, IL-10 activity is thought to be important in bone loss in inflammatory disorders.¹⁰⁻¹⁵

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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 increase the subsequent risk of osteoporosis. Although not without contradictions, an association of IL-10 gene polymorphisms with bone mineral density (BMD) has indeed been reported.^{16,17} The aim of the present study was to assess the potential association of the C627A polymorphism of the IL-10 promoter with BMD, and by implication, with osteoporosis.

Materials and methods

DNA extraction and genotyping for IL-10 C627A polymorphism was performed on whole blood samples from 100 consenting Indonesian women.¹⁸ The DNA samples were stored at -20°C in the Oral Biology Laboratory, Faculty of Dentistry, University of Indonesia. The sample population included 29 normal healthy subjects, 21 with osteopenia, and 50 with osteoporosis, as classified by T-scoring based on ultrasound measurements of calcaneus bone. The study was approved by the Research Ethics Committee of the Faculty of Dentistry, University of Indonesia.

IL-10 C627A genotypes were determined by the polymerase chain reaction (PCR) using primers 5'- CCT AGG TCA CAG TGA CGT GG - 3' and 5'- GGTGAGCACTACCTGACTAGC - 3'. The PCR mix, with 16 µL of reactants, consisted of 10 µL polymerase Master Mix, 0.5 µL forward primer, 0.5 µL reverse primer, and 6 µL ddH₂O. The PCR amplification consisted of initial denaturation at 95°C for 5 min and 35 cycles of denaturing at 94°C for 30 s, annealing at 54.9°C for 30 s, and extension at 70°C for 30 s. Final extension was at 72°C for 7 min, followed by cooling and holding at 4°C. The PCR products were checked by electrophoresis on 1.5% agarose gel at 60 V for 4 min, and visualized with GelDoc (Biorad).

The C627A polymorphism genotyping was performed by RFLP using the RsaI restriction cutting enzyme (New England Biolabs). The digested fragments were subjected to electrophoresis on 3% agarose gel at 60 V for 85

min, and visualized with GelDoc. The resulting products indicated a single 412 bp fragment for the CC genotype, three fragments (412, 236, and 176 bp) for the CA genotype, and two fragments (236 and 176 bp) for the AA genotype.

Chi-square testing, using SPSS 10.5 software, was applied for statistical analysis of the results, assuming significance for p-values less than 0.05.

Results

An example of the indicated genotypes of IL-10 C627A polymorphism after RFLP electrophoresis is shown in Figure 1. Table 1 shows the frequency and percentage of the genotypes in each BMD (T-scoring) group. In general, the genotype distribution is consistent with the Hardy-Weinberg equilibrium. In all BMD groups, the CC genotype (and C allele) represented a minority of the observed cases, but showed an increasing frequency from normal to the osteoporotic condition (Table 1). However, no significant association was found between the genotypes of IL-10 C627A polymorphism and BMD (p=0.133).

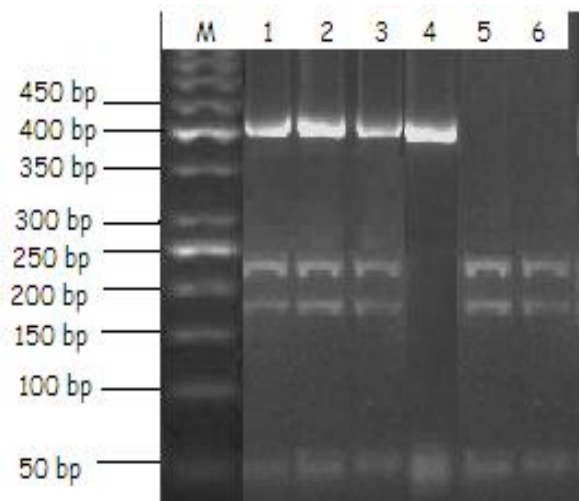


Figure 1. Examples of genotyping results: M = 50 bp marker ladder; lanes 1,2 and 3 = genotype CA; lane 4 = genotype CC; lanes 5 and 6 = genotype AA

Table 1. Genotype and BMD distributions; osteopn = osteopenia, osteoprs = osteoporosis

Type		BMD / T-score group			Total	p
		Normal	Osteopn	Osteoprs		
CC	Count	3	3	8	14	0.133*
	% within group	10.3	14.1	16.0	14.0	
CA	Count	10	9	24	43	
	% within group	34.5	42.9	48.0	43.0	
AA	Count	16	9	18	43	
	% within group	55.2	42.9	36.0	43.0	
Allele C	Count	16	15	40	71	
	% within group	27.6	35.7	40.0	35.5	
Allele A	Count	42	27	60	129	
	% within group	72.4	64.3	60.0	64.5	

*Fisher test

Discussion

The observed genotype distribution of the C627A polymorphism of IL-10 promoter (Table 1) showed no significant association between the genotypes and BMD, although a systematic trend was evident for an increasing frequency of the CC genotype and C allele from the normal to the osteoporotic condition. The CC genotype and C allele represented a minority of the observed cases in all BMD groups (normal, osteopenia, and osteoporosis). Currently, only a very few

reports appear to exist on the corresponding genotype (or allele) distributions of the same IL-10 polymorphism from elsewhere. However, the genotype fractions of the present work were not much unlike those reported from Taiwan by a study (Table 2) that suggested a significantly elevated risk of osteoporosis associated with the CC genotype.¹⁶ Judging from the trends of the present work, a similar conclusion could be possible for a larger sample of the Indonesian population.

Table 2. IL-10 C627A: genotype distributions and BMD

Region	Genotype fractions (%)						Reference	Notes ¹⁾ # ²⁾
	Cases			Control				
	CC	CA	AA	CC	CA	AA		
Taiwan	8.8	45.0	46.2	-	-	-	Chen et al.2005 ¹⁶	
Indonesia	16.0	48.0	36.0	10.3	34.5	55.2	Present study	*

1) significant association of genotypes and BMD: *no / # yes 2) CC: OR = 8.1 (95% CI: 1.5-42.8)

Conversely, the tested polymorphism (C627A) of IL-10 is not the only one of interest. For example, the A592C polymorphism of IL-10 has been reported to show an association with decreased BMD in Korean postmenopausal women.¹⁷

The overall impact of the IL-10 polymorphisms on the risk of osteoporosis clearly remains to be elucidated in further detail. The

total genetic component for the osteoporosis risk also includes many more genes, so the complete regulatory network involved is very complex, and is further complicated by the environmental contributing factors that partly interact with the genetic component.¹⁹⁻²¹

Conclusion

The results of the present work showed no significant association of the genotypes of the IL-10 C627A polymorphism with BMD (osteoporosis) in postmenopausal Indonesian women. Considering the relatively small sample size and the reported significant corresponding association from elsewhere in eastern Asia, our recommendation is that the study be extended to include a larger sample population and other polymorphisms of the IL-10 promoter gene.

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Declaration of Interest

The authors report no conflict of interest.

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