Leptin gene (Lep) G-2548A polymorphism in patients with and without osteoporosis

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Abstract. Leptin plays several important roles in the body, such as body mass control and bone remodeling. Previous research suggests that LEP G-2548A gene polymorphism may be associated with increased risk of osteoporosis. This study aimed at analyzing LEP G-2548A polymorphism in Indonesian patients with and without osteoporosis. DNA samples were collected from 50 subjects with osteoporosis and 50 subjects without osteoporosis. The DNA fragments were amplified with LEP G-2548A primers using polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis using the HhaI restriction enzyme. The distribution of the genotypes and alleles in the test and control groups were determined and were compared. The frequency of the AA genotype of LEP G-2548A was higher in the osteoporosis group than in the control group. However, the differences in genotype and allele frequencies between the two groups were not statistically significant (p > 0.05). The genotypes and alleles of LEP G-2548A gene polymorphism did not differ significantly between Indonesian patients with and without osteoporosis.

1. Introduction

Leptin (LEP) is a 16 kDa protein that is synthesized and secreted by white adipose tissue when expressed by the corresponding LEP gene. Leptin plays several important roles in the body, such as body mass control, bone remodeling, and a role in hypothalamic regulation of food intake and body weight [1-3]. Previous studies have shown that LEP G-2548A single nucleotide polymorphism is significantly associated with obesity [4,5]. Other studies have suggested that LEP G-2548A is linked to an increased risk of osteoporosis [2].

Osteoporosis is an age-related disorder of bone mass loss, resulting from an imbalance in bone resorption and bone formation [6]. Typical signs of osteoporosis are decreased bone mineral density (BMD), destruction of bone microarchitecture, and increased bone fragility [7]. Locations typically
affected in osteoporosis include the distal radius, the proximal humerus, the pelvis, the vertebrae, and the jaw bone [8] According to PEROSI (Osteoporosis Society of Indonesia), in 2009, about 2.8% of men and 32.3% of women in Indonesia had osteoporosis [9] BMD is a useful indicator used to diagnose osteoporosis. Previous research has suggested that genotypes associated with osteoporosis can be used to predict osteoporosis risk.

Some researchers have noted previously that certain polymorphisms of genes, such as those in IL-6, IL-8, and IL-10 are found in postmenopausal women with an increased risk of osteoporosis [10]. One polymorphism of the LEP gene with possible association with osteoporosis is LEP G-2548A. The present study aimed at investigating the association between this polymorphism and BMD status in the subpopulation of Indonesian patients.

2. Materials and Methods

DNA samples were obtained from 50 patients with osteoporosis and 50 patients without osteoporosis at the Oral Biology Laboratory of the Faculty of Dentistry, University of Indonesia. The DNA samples were stored in a refrigerator at −20 °C until use.

LEP gene polymorphisms G-2548A was assessed by PCR-RFLP. DNA fragments were amplified with forward primer 5'-TAAGCCAAGCAGAAAATTGAG-3’ and reverse primer 5'-CTTCAAAAATTTATGTTCTCTGC-3’ using a PCR thermocycler. In 20 mL of reactants, PCR components included 10 mL of Kappa, 0.75 mL of forward primer, 0.75 mL of reverse primer, 7.5 mL of DDEH2O, and 1 mL of DNA templates mixed using a centrifuge. PCR included initial denaturation at 95 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, elongation (extension) at 72 °C for 1 min, and elongation and final extension at 72 °C for 10 min, and set of storage at 4 °C.

The PCR product (281 bp) was subjected to restriction enzyme HhaI cutting to examine the polymorphism by RFLP. The fragments were analyzed by electrophoresis in 2% agarose gel at 100 V for 35 min and, then, were visualized in GelDoc under UV light. The G allele was indicated by bands of 172 bp and 109 bp, while the A allele was indicated by a band of 281 bp.

SPSS 17.0 software was used to perform the statistical analysis. The chi-square test was used to assess the Hardy-Weinberg equilibrium (HWE). The Fisher exact test was used to evaluate the relationship between the polymorphic genotype/allele distributions and osteoporosis status.

3. Results

Figure 1 shows examples of the indicated PCR product of LEP G-2548A with a fragment length of 281 bp. Figure 2 shows examples of the HhaI restriction enzyme fragments as RFLP results.

![Figure 1](image-url)
Figure 2. Results of RFLP of LEP G-2548A using enzyme HhaI.

Lane 1 is a 50 bp marker ladder. Lanes 2 and 3 show bands with 281 bp fragments for the AA (homozygous) genotype. Lane 4 shows bands with 281 bp, 172 bp, and 109 bp fragments for the GA (heterozygous) genotype. Lane 5 denotes bands with 172 bp and 109 bp for the GG genotype (homozygous).

The control group (without osteoporosis) included 28 (56%) subjects with genotype AA, 20 (40%) subjects with genotype GA, and 2 (4%) subjects with genotype GG. The osteoporosis group included 35 (70%) subjects with genotype AA (70%), 15 (30%) subjects with genotype GA, and no cases of genotype GG (Figure 3). The genotype distributions of both the osteoporosis and control groups were formally consistent with HWE (Chi-square test, $p > 0.05$), although the low number of cases with genotype GG renders the HWE assessment inaccurate.

The allele frequency distribution showed that the control (normal) samples included 76 (76%) A alleles and 24 (24%) G alleles. The osteoporosis group included 85 (85%) A alleles and 15 (15%) G alleles (Figure 4).

The AA genotype and A allele occurred more frequently in the osteoporosis group than in the control group. However, there was no statistically significant difference between the two groups in the genotype or allele distributions (Fisher exact test, $p > 0.05$).

Figure 3. Genotype distributions in patients with and without osteoporosis
Figure 4. Allele frequencies in patients with and without osteoporosis.

4. Discussion
In all the samples, the intended polymorphic site was amplified successfully by PCR and was evaluated for the LEP G-2548A polymorphism using RFLP.

In both the osteoporosis and control groups, the AA genotype was the most frequent (Figure 3). This is not the case in all populations, when GA has been reported to be the most common genotype [2]. In the present study, the A allele dominated in both the osteoporosis and control groups (Figure 4).

Previous work has indicated a significantly elevated frequency of the A allele and reduced leptin expression in patients with osteoporosis [2]. Although there are differences between the results of previous studies and the present work, e.g., in the observed genotype frequencies, the dominance of the A allele is consistent with previous work.

5. Conclusion
The AA genotype and A allele of LEP G-2548A polymorphism occurred more frequently in the osteoporosis group than in the control group. However, there was no statistically significant difference between the two groups in the genotype or allele distributions.

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