

The LEPR Q223R Polymorphism as a Potential Bioindicator of Class II Malocclusion

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Abstract

Malocclusions are known to have multifactorial etiology that includes a strong hereditary linkage. One of the genes involved is the LEPR gene on chromosomal locus 1p31, suggested to affect the occurrence of retrognathic mandible.

The present work aimed to determine the LEPRQ223R (rs1137101) polymorphism status and its possible association with class II malocclusions in an Indonesian population.

For this purpose, LEPR Q223R polymorphism status was determined using the PCR-RFLP technique from peripheral blood of 110 consenting Indonesians, including 47 subjects with Class II malocclusion (MO group) and 63 subjects with Class I malocclusion (control).

The Genotyped AA of the LEPR Q223R polymorphism occurred at significantly ($p = 0.048$) and also dominant allele A of the LEPR Q223R polymorphism occurred at significantly ($p = 0.05$) higher frequency in the MO group than in the control group.

Therefore genotyped AA and allele A can be considered to carry a risk of Class II malocclusion. The difference is not large (p -value close to 0.05) and it is recommended to repeat the exercise with a substantially larger sample of subjects and in combination with other potentially relevant polymorphisms.

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Introduction

Angle (1890) defined occlusion as the relationship of the occlusal plane of the teeth at the time of closing the upper and lower jaw, and classified irregularities to the normal occlusion in the sagittal direction, or malocclusions into skeletal Classes I, II and III. The classification is based on the key principle of occlusion in the relationship of the first molars with the jaws also describing the skeletal pattern.^{1,2} For dental practice the skeletal growth can be modified by orthodontic treatment and suitable appliances to improve, control and modify growth and skeletal form.¹

Malocclusions are fairly common, with a Class II prevalence of about one third of Class I

malocclusion cases in various populations of the world. Studies in Europe, United States and Asia suggest a prevalence of skeletal Class II malocclusions ranging from 21 to 33%, although from Iran much larger prevalence of 70% has been reported.^{2,3,4}

Class II malocclusions often occur because of a combination of maxillary protrusion and mandibular retrusion, resulting in a very convex facial profile.^{5,6} By intra-oral appearance Class II malocclusions are divided into two major groups, distinguished by inclination of the upper anterior teeth, so that division 1 malocclusions show a large inclination angle of the upper anterior teeth (proclination) while division 2 is characterized by a small inclination angle of upper anterior teeth (retroclination) with a small bite distance.^{1,2,7,8}

In general, malocclusions have multifactorial etiology with both hereditary and environmental factors strongly affecting the outcome, although the environmental modifications are not considered to change the basic morphology.⁸⁻¹² Studies on dentocranial

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disorders manifested in skeletal class III malocclusions have indicated associated genes in the chromosomal loci of 1p22.1, 1p35, 1p35.2, 1p36, 2p13, 3q26.2, 6q25, 11q22, 12q13.13, 12q21, 12q23, 12q24.11, 14q24.3-31.2 and 19p13.2.¹³⁻¹⁹ A study on Class II malocclusions in southern Chinese population suggests that the A/G polymorphisms rs7418057 and rs10449758 of the *LEPR* (leptin receptor) gene at the chromosomal locus 1p31 will significantly affect the occurrence of retrognathic mandible.²⁰ The *LEPR* gene encodes the leptin receptor LEPR, which is a trans membrane receptor in the family of type I cytokine receptors. Leptin, a 16 kDa hormone encoded by the *leptin* gene, can bind to LEPR to stimulate a range of pathways. These pathways include suppression of bone modeling by leptin-activated hypothalamic signaling of the sympathetic nervous system that mediates the signaling through beta 2-adrenergic receptors to osteoblasts close to sympathetic nerve fibers. An opposite direct effect of leptin is to increase the rates of proliferation and differentiation of osteoblasts that trigger the growth of bone, and suppression of osteoclastogenesis. The net effect is thought to be balanced bone growth that can be affected by the genetic details like polymorphisms in the leptin-LEPR signaling pathway.²⁰ As it is essential also in the formation of mandibular bone, the leptin-LEPR activity is expected to be associated with skeletal Class II malocclusions on the genotype and allele levels.²⁰

The *LEPR* gene polymorphism of interest in this study is the rs1137101 SNP (Q223R) polymorphism of A/G type to identify the genotypes GG, GA and AA. Both GG and AA genotypes can occur at the highest frequency depending on the population.^{21,22}

Materials and methods

Subjects

The study was conducted at the Dental Hospital and Oral Biology Laboratory of the Faculty of Dentistry, University of Indonesia, Jakarta. Orthodontic Clinic patients with class I and II malocclusions were recruited after obtaining informed consent and after they completed a questionnaire on demographic information such as name, age, sex, ethnicity with pedigree of three generations, address, education and socioeconomic status. In total 110

consenting Indonesians were included in the study, with an age range of 20-45 years and mean age of 26 years. The subjects included 47 cases in the group of class II malocclusions (MO group) and 63 cases in the group of class I malocclusions (Control group). Ethical clearance was granted for the work by the Ethical Committee of the Faculty of Dentistry, University of Indonesia.

DNA Isolation, PCR Amplification and RFLP Genotyping

Genomic DNA was obtained and the *LEPR* gene Q223R polymorphism status determined from peripheral blood samples. Isolation of DNA was done by placing 3 ml of peripheral blood from each of 110 subjects in 15 mL tubes containing 9 mL of red blood cell lysis solution (1.45 M NH₄Cl, 5 mM anhydrous EDTA, and 0.1M KHCO₃), and incubating at room temperature for 10 min. The samples were centrifuged at 1500 rpm for 10 min at room temperature and the supernatant was discarded to remove part of the mononuclear leukocytes to obtain a supernatant layer which does not contain red blood cells. After that 2 mL of cell lysis solution was added to the pellet and incubated in a water bath at 37°C for 60 min. Then 1.3 mL protein precipitation solution was added into the tube that was vortexed and centrifuged at 4°C/3000 rpm for 5 min. The supernatant was poured into a new tube containing 2.3 mL of cold isopropanol, and the tube was inverted several times until DNA appeared white. After discarding supernatant, DNA was dried in open air by reversing the tube. Then DNA was rehydrated with 200-300 µL Tris-HCl EDTA and incubated in a water bath at 37°C for 2 hours. The solution was transferred into 5 mL sterile microcentrifuge tubes and stored at -20°C until further examination.²³⁻²⁵

Genotyping the *LEPR* gene for the Q223R (rs1137101) polymorphism was conducted by PCR-RFLP. For this purpose, PCR amplification was done with the following primers: forward 5'-AACTCAACGACACTCTCCTT-3' and reverse 5'-TGAAGTACATTAGAGGTGAC-3'. PCR amplification of DNA fragments was carried out by using Perkin Elmer gene Amp PCR system 9700 with PCR master mix. PCR mixture was made consisting of 10 µL of Kappa 2G, 1 µL of forward primer, 1 µL of reverse primer, 7.5 µL ddH₂O, and 0.5 µL of DNA. The initial

denaturation at 94°C for 5 min was followed by 35 cycles of denaturation phase at 94°C for 30 seconds, annealing at 58.5°C for 45 seconds, elongation at 72°C for 60 seconds, and a final elongation at 72°C for 5 minutes. The PCR results (80bp) were confirmed by 2% agarose gel electrophoresis at 80V/400mA for 35 min, visualized using gel doc. RFLP was conducted using the restriction enzyme MspI; with incubation at 37°C for 18 hours. The resulting DNA fragments were subjected to 2% agarose gel electrophoresis at 80V/400mA for 60 minutes, and visualized using gel doc; showed genotyped GG (80bp), GA (80, 59, 21 bp), and AA (59, 21 bp).^{26,27}

Statistical analysis

Statistical analysis of the observed genotypes and alleles with respect to the study grouping (MO and control) was conducted using Fisher exact test with SPSS 17.0 and also used logistic regression analysis to evaluated of malocclusion and genetic risk factors.

Results

The distribution of the 110 subjects for DNA sampling, including the malocclusion Class I or control group and malocclusion Class II or MO group, is shown in Table 1. An example of the visualized PCR results of the 80 bp LEPR gene product is shown in Figure 1.

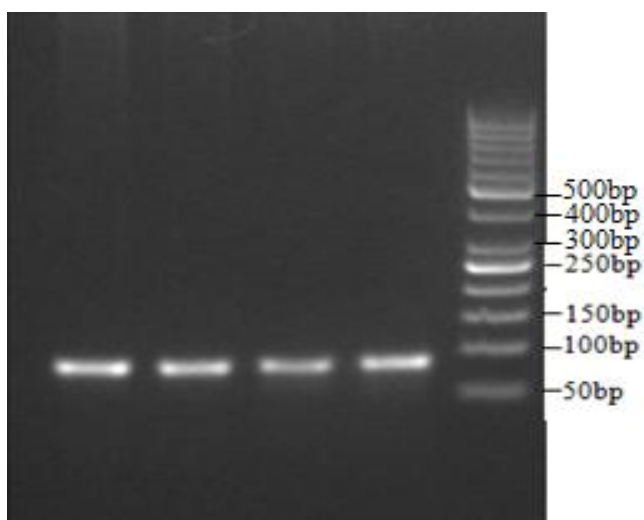


Figure 1. PCR results of the 80 bp LEPR gene product (four lanes) with 50bp ladder marker.

Furthermore, an example of the RFLP results using the restriction enzyme MspI is shown in Figure 2, with a 80bp fragment

representing the genotype GG (homozygous reference), two fragments of 59bp and 21bp in size the genotype AA (homozygous), three fragments of 80bp, 59bp and 21bp in size the genotype GA (heterozygous). The genotyped for LEPR Q223R among both Control group and MO group were distributed compliance and consistence with Hardy Weinberg equilibrium ($p = 0.06$; $p > 0.05$).

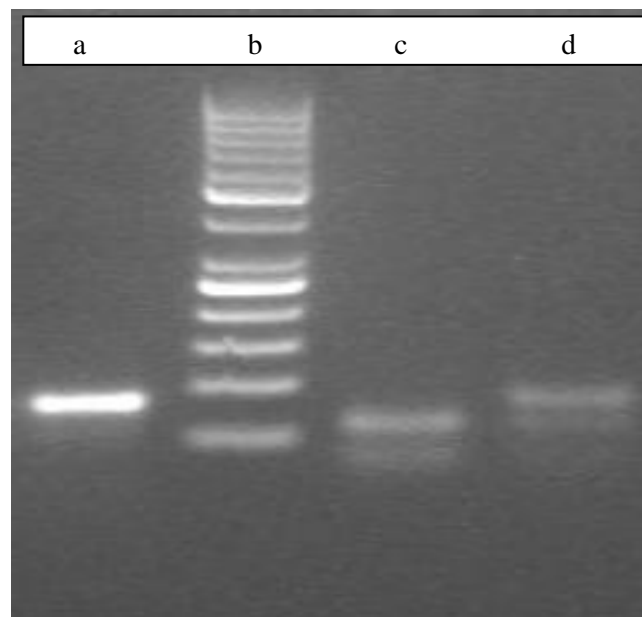


Figure 2. RFLP results with a 80bp fragment of the genotype GG (homozygous reference) on lane a, 50 bp ladder marker on lane b, 59bp and 21bp fragments of the genotype AA (homozygous) on lane c, and 80bp, 59bp and 21bp fragments of the genotype GA (heterozygous) on lane d.

Group	Total	%
MO Group	47	42.70%
Control Group	63	57.30%
Total	110	100%

Table 1. Subjects and DNA samples.

The genotype and allele distributions in the MO and control groups are presented in Table 2, the distributions of genotyped AA, GA, GG in MO group were 68.10%, 25.53%, and 6.37% if compared with Control group 44.44%, 39.68%, and 15.91% besides that distribution of Allele A more higher than allele G in both group. The results shown that for the tested subjects,

the genotype AA and allele A represented the majority of cases both in the MO and control group, and also shown significant difference between the MO group and the control group.

Genotype /Allele	MO group N=47	Control group N=63	p value
Genotype AA	32(68.10%)	28(44.44%)	0.048
Genotype GA	12(25.53%)	25(39.68%)	
Genotype GG	3 (6.37%)	10(15.91%)	
Allele A	76(80.85%)	81(64.28%)	0.05
Allele G	18(19.15%)	45(35.72%)	

Table 2. Distribution of genotypes and alleles of LEPR Q223R polymorphism.

Univariate logistic regression presented in Table 3 used to perceive association between genetic risk factor (allele A / Allele G) and class II malocclusion. In this test shown Odd Ratio 2.35.

Variables	p	Odd Ratio	95% CI
Allele A / Allele G	0.05	2.35	1.25 – 4.40

Table 3. Logistic regression analysis alleles of LEPR Q223R polymorphism with MO Group.

Discussion

The results for both MO and control groups are consistent with the Hardy-Weinberg equilibrium ($p > 0.05$), although for the control group the assessment is not accurate due to the small number of genotype GG cases, this means that genotyped and allele in a population will keep constant from generation to generation. For both groups the genotype AA (68.10% in MO group, 44.44% in control group) occurred at significantly ($p = 0.048$) and allele A (80.85% in MO group, 64.28% in control group) were dominating and occurred at significantly ($p = 0.05$) higher frequency in the Class II malocclusion (MO) group than in the Class I malocclusion (control) group. Univariate logistic regression used to evaluate the association between class II malocclusion and genetic risk factor (allele A / allele G), the result showed that allele A had higher risk of class II malocclusion ($p < 0.05$, OR = 2.35, 95% CI 1.25 – 4.40), so that genotyped AA and allele A of the LEPR Q223R (rs1137101) polymorphism could be considered at least to some extent risk of Class II malocclusion.

The difference is not large (p-value close

to 0.05) and hence it can be recommended to repeat the exercise with a substantially larger sample of subjects. The targeted polymorphism has been shown previously to be significantly associated with bone mineral density and bone growth but the relationship is complex due to many other associated genes and pathways.^{20,22}

In addition, the *LEPR* gene and the same polymorphism are involved in adipocyte signaling and possibly associated with other clinical manifestations such as the metabolic syndrome (obesity).^{20,28,29,30}

In general, orthodontic practice is not differentiated according to the details of etiology of malocclusion. This may well be appropriate for adults with fully developed skeletal structure, but for still developing stages the responses to treatment can depend on the details and extent of the involved hereditary drivers.²¹ Apart from general understanding of the hereditary component, this is taken to justify further effort to clarify the genetic (and epigenetic) mechanisms of malocclusions.

Conclusions

In the tested Indonesian population the genotype AA and allele A of the LEPR Q223R (rs1137101) polymorphism represent the majority of cases both in the Class II malocclusion (MO) and Class I malocclusion (control) groups, appear at higher frequency in the MO group than in the control group. The genotyped AA and dominant allele A of the polymorphism occurred at significantly higher frequency in the MO group than in the control group. Therefore this genotyped allele could be considered to carry a risk of Class II malocclusion. As the p-value is close to 0.05, it is recommended to extend the study to larger sample of comparable subjects and also in combination with other potentially relevant polymorphisms.

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Declaration of Interest

The authors report no conflict of interest.

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