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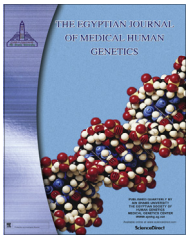

ORIGINAL ARTICLE

Polymorphism in leptin receptor gene was associated with obesity in Yogyakarta, Indonesia

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KEYWORDS

Leptin;
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Abstract *Background:* Leptin is a hormone that regulates homeostasis energy through the central–peripheral mechanism as well as regulates hunger and satiety. Leptin receptor is important in leptin signal transduction that is located mainly in the hypothalamus. The mutation in leptin receptor (LEPR) gene causes splicing abnormality that resulted in truncated receptor, aberrant signal transduction, leptin resistance, and obesity.

This study aims to determine the association of *LEPR* gene polymorphisms, rs1137100 and rs1137101, on phenotype and leptin level between obese and control groups in Yogyakarta population.

Methods: We examined two polymorphisms of *LEPR* gene, rs1137100 (K109R) and rs1137101 (Q223R) in 110 subjects consisting of 55 obese and 55 healthy adult subjects as controls from Yogyakarta. A correlation study was done between body mass index (BMI), waist and hip circumference, waist to hip ratio (WHR), and leptin level with their genotypes. Statistical analysis was performed using *t*-test to show the significant difference between the groups. SNPs of *LEPR* gene for obese and control groups were compared using chi-square analysis.

Results: Body mass index, waist circumference and leptin level in obese group were significantly higher than those in the control group. The frequency of R103R homozygote in obese group was higher than in the control group, whereas Q223Q homozygote in obese group was lower than in the control group. Polymorphism of rs1137100 (K109R) and rs1137101 (Q223R) were associated with obesity and leptin level.

Conclusion: In Yogyakarta population, polymorphisms of rs1137100 (K109R) and rs1137101 (Q223R) *LEPR* gene are associated with obesity. Thus further study on another Indonesian ethnic population is required to investigate the variation in *LEPR* gene.

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1. Introduction

Obesity is caused by an imbalance between food intake and energy expenditure. The etiology of obesity involves genetic, environmental, and lifestyle factors [1,2]. Obesity causes excessive accumulation of fat in adipose tissue, liver, muscle, pancreatic islets, and other organs, leading to an increased risk of metabolic diseases [3]. Approximately 118 candidate genes are associated with obesity [4]. Some of them are genes encoding leptin (*LEP*), leptin receptor (*LEPR*), melanocortin four receptor (*MC4R*), adiponectin (*ADIPOQ*), corticotrophin-releasing hormone1 (*CRHR1*), prohormone convertase1 (*PC1*), pro-opiomelanocortin (*POMC*) and resistin (*RETN*) [5].

Leptin regulates satiety, energy expenditure, and neuroendocrine function. Its level is highly dependent on the presence of fat in the cells [2]. The biological activities of leptin on target tissues are carried out through selective binding to a specific receptor, LEPR [6]. The leptin receptor is a single transmembrane protein belonging to a superfamily of cytokine receptors and has several alternative spliced isoforms. These include one long isoform predominantly located in the hypothalamus and several short isoforms that are distributed in many tissues [7]. Leptin receptors form homodimers, capable of activating Janus kinases to activate transcription [2].

Potential associations with obesity for several common polymorphisms and rare variants of the long isoform of the human *LEPR* gene have been reported and evaluated in different populations [8]. There are some conflicting results regarding the correlation of the *LEPR* gene with obesity. Some studies have found significant associations between obesity and polymorphisms of the *LEPR* gene [9–13], but some other studies failed to find significant correlations [14–21]. It is possible that these inconclusive results might be due to differences in the studied populations and relatively limited numbers of study subjects.

This study aims to determine the correlation of the *LEPR* gene polymorphisms rs1137100 (K109R) and rs1137101 (Q223R) with BMI, waist circumference, WHR and leptin level in the obese and control groups in the Yogyakarta population.

2. Subjects and methods

2.1. Subjects

The study was approved by the Ethical Committee Faculty of Medicine, Universitas Gadjah Mada. The work has been carried out under The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans. Informed consent was obtained from all participants for this study. All participants were of Yogyakarta descent. The subjects included 110 unrelated healthy volunteers: 55 obese (39 males and 16 females) and 55 control (41 males and 14 females)

2.2. Phenotypic and leptin level

Body weight (kg), height (m), BMI (weight in kg divided by height in m^2), waist (cm) and hip (cm) circumference were measured with a calibrated scale. Leptin level in plasma was determined using an ELISA kit (Applied DRG Instruments GmbH, Germany).

2.3. LEPR gene genotyping

Venous blood samples were drawn. The puffy coat layer was collected for DNA extraction. It contains leukocytes, therefore upon centrifugation, only RBCs will sediment in the pellet and not leukocytes. For genotype analysis, DNA was extracted from leukocytes with a Promega kit according to the manufacturer's protocol (Applied Promega, Madison, WI, U.S.A.). Genotyping was performed according to Gotoda et al., 1997 [22], using the following primer sequences (Applied IDT Singapore): rs1137100; forward, -5′TTT CCA CTG TTG CTT TCG GA3′-; reverse -5′AAA CTA AAG AAT TTA CTG TTG AAA CAA3′-; and for rs1137101: forward, -5′AAA CTC AAC GAC ACT CTC CTT3′-; and reverse, -5′TGA ACT GAC ATT AGA GGT GAC3′-. The conditions for PCR were 94 °C for 3 min, and then 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s for 40 cycles, and extra extension at 72 °C for 10 min. The PCR results were 101 bp for rs1137100 and 80 bp for rs1137101. Polymorphism was determined by the digestion of PCR product for 17 h using HaeIII and MspI enzymes (Biolabs-New England Inc.) for rs1137100 (K109R) and rs1137101 (Q223R), respectively. Gel electrophoresis with 3% agarose was performed to analyze the digestion. The digested product for rs1137100 was 101 bp for the K allele and 70 and 31 bp for the R allele. The digested product for rs1137101, the Q allele was 80 bp and the R allele was 58 and 22 bp.

2.4. Data analysis

Unpaired *t*-test was performed to match the age, body weight, BMI, waist circumference, WHR, and leptin level of each group. Genotype distributions were examined for Hardy–Weinberg equilibrium (HWE), and SNPs of rs1137100 (K109R) and rs1137101 (Q223R) *LEPR* gene for the obese and control groups were compared using chi-square analysis. Correlations between phenotypic variables, including BMI, waist circumference, WHR and leptin levels, and SNPs were determined using variance analysis. The KK genotype in rs1137100 and QQ genotype in rs137101 *LEPR* gene were not found in the control group, to correlate the SNPs with obesity, the KK + KR and RR genotype in rs137100 and QQ + QR and RR genotype in rs137101 were used. All of the tests were performed using SPSS version 17. Statistical significance was defined at $P < 0.05$.

3. Result

The phenotype and leptin level of all subjects are shown in Table 1.

The phenotypes of the obese group were significantly higher than in the control group in age, BMI and waist circumference ($P < 0.05$). The leptin level is increased and significantly different in the obese group compared with the control group ($P < 0.001$).

Genotype frequency of rs1137100 (K109R) and rs1137101 (Q223R) in the obese and control groups is shown in Fig. 1.

The higher frequency of RR genotype in the obese group compared with that in the control group was observed. The results were shown to be significantly different in rs1137100 (K109R) ($P = 0.009$). When compared using the Hardy–Weinberg Equation, the significant difference in the frequency of the rs1137100 (K109R) genotype was found. This may be due to a small sample number. The RR genotype frequency of rs1137101 in the obese group was lower than that in the control group but not significantly different ($P = 0.484$).

Polymorphisms of rs1137100 (K109R) and 1137101 (Q223R) were correlated with the phenotype of obesity and leptin level in the obese and control groups as shown in Tables 2 and 3.

The body weight, BMI, waist circumference, hip circumference and leptin level were higher and significantly different in all genotypes of obese group than control group in rs1137100. In rs1137101, this polymorphism was significantly higher in body weight, BMI, waist circumference and leptin level in QQ + QR genotype and significantly higher in body weight, BMI, waist circumference and leptin level in the RR genotype of obese group than control group.

Table 1 Phenotype characteristic and leptin level in the obese and control groups.

	Obesity	Control	$P^{\#}$
Phenotype	55	55	
Age (year)	20.7 ± 3.7	19.3 ± 1.8	**0.001***
Male	39	41	0.67
Female	16	14	
BMI	34.2 ± 3.6	22.2 ± 2.1	**0.001***
Waist circumference (cm)	104.3 ± 10.4	75.9 ± 6.7	**0.000***
Hip circumference (cm)	114.9 ± 8.4	90.3 ± 6.5	0.352
WHR	0.91 ± 0.07	0.84 ± 0.08	0.309
Leptin level (ng/mL)	27.09 ± 13.50	12.29 ± 5.25	**0.000***

BMI = body mass index
WHR = waist–hip ratio
# Analyzed with *t*-test.
* Statistically significant.

4. Discussion

In our study, we demonstrated a significant difference in the phenotype of obesity and an increase in leptin level between the obese and control groups. Leptin levels were higher in the obese group compared with the control group, which might be caused by LEPR resistance in the obese group, leading to cell deficiencies of leptin and abnormal satiety regulation. Moreover, waist circumference is higher and significantly different in the obese group compared with the control group. This result is consistent with studies showing that, in some populations, obesity was correlated with a higher leptin level [10,13,23] and that leptin levels were correlated with type 2 diabetes mellitus patients in Iran [24]. However, other studies found that obesity did not correlate with leptin levels [18,20].

Our study shows that the frequency of R109R homozygote rs1137100 (K109R) in the obese group was 0.145. This genotype frequency varies from 0.05 in a Malaysian population [14], 0.06 in Indian [2] and Korean populations [25], 0.648 in Korean populations [26] and 0.678 in Chinese Han populations [27]. To the best of our knowledge, we report for the first time that the polymorphism rs1137100 is correlated with obesity and high leptin level in a Yogyakarta population. The correlation between genotype and obesity and leptin level was also found in middle-aged Caucasian males [5], and this genotype was correlated with systolic blood pressure in a Chinese Han population [27]. Another study correlated rs1137100 polymorphism with centenarian age in Polish populations and showed this polymorphism to play a role in the regulation of longevity [28]. On the other hand, research by de Oliveira [13] found a homozygous R109R to be correlated with reduced risk of obesity, and higher total cholesterol and triglycerides than other genotypes. Another research by Lakka [1] found that in white populations, the 109R allele was more often associated with an increased glucose disappearance index and a decreased fasting blood glucose than the 109 K allele. On the other hand, a study by Marti [19] in a Spanish population found no association between *LEPR* or the *Brain-derived Neurotropic Factor (BDNF)* gene and obesity.

The frequency of R223R rs1137101 in the obese group of our study was 0.654. The frequency of R223R in other obese

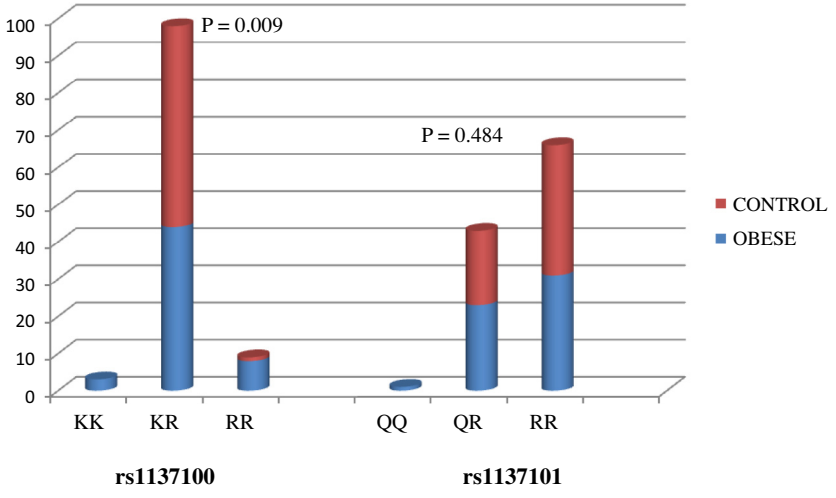

Figure 1 Genotype frequency of rs1137100 (K109R) and rs1137101 (Q223R) *LEPR* gene in obese and control group.

Table 2 Correlation between rs1137100 (K109R) *LEPR* genotype with phenotype and leptin level in obese and control groups.

Phenotype	KK + KR genotype		$P^{\#}$	RR genotype		$P^{\#}$
	Obese (n = 47)	Control (n = 54)		Obese (n = 8)	Control (n = 1)	
Body weight (kg)	96.38 ± 13.73	61.78 ± 7.00	**0.000***	100.25 ± 12.34	60	**0.018***
BMI	33.86 ± 3.54	22.17 ± 2.50	**0.001***	36.10 ± 3.93	22.04	**0.012***
Waist circumference (cm)	104.57 ± 10.95	75.91 ± 6.76	**0.000***	102.88 ± 6.31	73.50	**0.003***
Hip (cm)	114.23 ± 8.46	90.65 ± 6.93	0.559	119.81 ± 6.304	83.50	**0.001***
WHR	0.916 ± 0.069	0.840 ± 0.083	0.357	0.859 ± 0.044	0.880	0.669
Leptin (ng/mL)	26.00 ± 13.28	14.52 ± 8.21	**0.001***	33.49 ± 13.87	0.05	**0.000***

BMI = body metabolic index
WHR = waist–hip ratio
# Analyzed with the analysis of variance.
* Statistically significant.

Table 3 Correlation between rs1137101 (Q223R) *LEPR* genotype with phenotype and leptin level in obese and control groups.

Phenotype	QQ + QR genotype		$P^{\#}$	RR genotype		$P^{\#}$
	Obese (n = 24)	Control (n = 20)		Obese (n = 31)	Control (n = 35)	
Body weight (kg)	96.33 ± 11.97	61.55 ± 7.11	**0.036***	94.42 ± 14.75	61.86 ± 6.93	**0.000***
BMI	33.92 ± 3.16	21.99 ± 2.19	0.183	34.39 ± 4.02	22.27 ± 1.89	**0.001***
Waist circumference (cm)	104.77 ± 10.42	74.40 ± 5.31	**0.001***	103.98 ± 10.51	76.70 ± 7.32	**0.031***
Hip (cm)	112.90 ± 8.94	90.08 ± 6.69	0.317	116.71 ± 7.57	90.77 ± 7.16	0.979
WHR	0.929 ± 0.067	0.830 ± 0.078	0.502	0.891 ± 0.066	0.848 ± 0.085	0.273
Leptin (ng/mL)	26.88 ± 14.51	12.74 ± 5.65	**0.000***	27.26 ± 12.91	15.14 ± 9.59	**0.038***

BMI = body mass index
WHR = waist–hip ratio
# Analyzed with the analysis of variance.
* Statistically significant.

groups from other populations varies from 0.15 in Teheran [29], 0.156 in Spain [19], 0.169 in Brazilian [11], 0.18 in Kuala Lumpur [14], 0.227 in Jeddah city [12], 0.287, 0.261 and 0.412, respectively in Malay, Chinese and Indian ethnics of Malaysian populations [30], 0.35 in Coimbatore Indian [2], 0.755 in Korean population [26], and 0.776 and 0.75, respectively, in Japanese populations [31]. In this study, we found that there was a significant correlation between the polymorphism rs1137101 (Q223R) in the *LEPR* gene and obesity or leptin level. On the other hand, the R223R homozygote was correlated with body weight, BMI, waist circumference and leptin level. Our data in this research were corresponding with other researches regarding a correlation between the rs1137101 polymorphism in the *LEPR* gene and obesity [5,10,11,23,30,34]. Research by Enns [34] in a Canadian population found that the 223Q allele frequency was higher than the 223R allele frequency. This research found that the R allele of rs1137101 was correlated with an increase in obesity, BMI, fat mass, insulin and leptin level. Guizar-Mendoza [10] found that the polymorphism of rs1137101 correlated with hemodynamic and metabolic disturbances related to obesity. Further investigations conducted by Etemad [30] in three ethnic groups of Malaysian and by Al-Harithy [35] in Saudi women found that Q223R was a risk factor for type 2 DM. Research in children [18,20], conducted in Asian [12,14,16,31,32] and Caucasian [17,33] populations, showed that R223R in rs1137101 genotype was not correlated with obesity. These results were contrary to our data. Research using statistical meta-analysis [36,37] found that polymorphism of *LEPR* was not related to obesity because of the complexity involved in the pathogenesis of obesity, including multiple genes and environmental factors. In contrast, gender differences influenced leptin levels and fat distribution [38].

Our study has limitations due to the small sample number and lack of gender grouping. Therefore, future studies need to include gender as a grouping criterion. Importantly, research into the genes related to obesity in various ethnic populations of Indonesia, especially in eastern Indonesia, such as the Melanesian ethnic group, is needed.

5. Conclusion

Polymorphisms rs1137100 (K109R) and rs1137101 (Q223R) in *LEPR* gene correlate with the phenotype of obesity and leptin level. Therefore, it can be concluded from this research that both polymorphisms are obesity risk factors in the Yogyakarta population.

Conflict of interest

The authors declare no conflict of interest.

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