Leptin receptor gene Gln223Arg polymorphism is not associated with obesity and metabolic syndrome in Turkish children

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SUMMARY: Komşu-Örnek Z, Demirel F, Dursun A, Ermiş B, Pişkin İE, Bideci A. Leptin receptor gene Gln223Arg polymorphism is not associated with obesity and metabolic syndrome in Turkish children. Turk J Pediatr 2012; 54: 20-24.

The aim of the study was to investigate the relationship between leptin receptor gene (*LEPR*) Gln223Arg polymorphism and obesity in Turkish children. Ninety-two obese and 99 lean children (between 5-15 years) were included in the study. Twenty-three of the obese children were diagnosed with metabolic syndrome. Blood samples were collected for morning fasting blood glucose, insulin, leptin, and lipid level measurements. LEPR Gln223Arg polymorphism was analyzed by restriction fragment length polymorphism. Significant differences were observed in anthropometric measurements, fasting blood glucose, insulin, leptin, and lipid levels between obese and lean children. Serum leptin levels were markedly higher in obese children. No significant association was noted between Gln223Arg polymorphism and serum leptin, insulin and lipid levels. There were no differences in the genotype frequencies or allele distribution for Gln223Arg polymorphism among obese, obese with metabolic syndrome and lean children. Our findings suggest that there is no association between Gln223Arg polymorphism and obesity in Turkish children.

Key words: obesity, polymorphism, leptin receptor gene, Gln223Arg.

Obesity is becoming an increasingly important clinical and public health problem throughout the world. The disorder occurs as a result of complex interactions between genetic and environmental factors. Several genes are known to contribute to obesity. The leptin receptor gene (*LEPR*) is extensively studied among these genes[1-4]. Leptin is an adipocyte-derived hormone, which exerts its effect on food intake and energy expenditure by binding to specific receptors in the hypothalamus[4,5]. Leptin receptors are primarily expressed in the brain and hypothalamus, but they are also widely distributed in peripheral tissues including the adipose tissue, liver, kidneys, pancreas, and gonads. LEPR is located at chromosome 1p31 and recognized as a member of the cytokine family receptors[5]. Homozygote LEPR mutation is a very rare condition, and is not observed in many obese individuals[6]. However, there were many studies that indicated an association between some polymorphisms of LEPR and obesity and obesity-related disorders[1,2,5,7,8]. Gln223Arg polymorphism is, in particular, one of the most studied LEPR polymorphisms in obese individuals, with several reports on its relation to obesity[1,2,5,7-13]. In Turkey, there have been no studies investigating the relationship between LEPR polymorphism and obesity in Turkish children. Therefore, the aim of this study was to evaluate the relationships between LEPR Gln223Arg polymorphism and obesity in Turkish children.

Material and Methods

Subjects

This study included 92 obese children and adolescents (48 females, 44 males) with no

Table I. Anthropometric and Metabolic Data of the Obese and Control Groups

	Obese children (n=92)	Lean controls (n=99)	P value
Age (year)	11.3 ± 0.3	10.3± 0.3	NS
Males/Females (n)	44 / 48	57 / 42	NS
BMI (kg/m^2)	28.8 ± 0.5	16.9± 0.2	0.0001
Waist/hip ratio	0.9 ± 0.1	0.8 ± 0.1	0.0001
Fasting glucose (mg/dl)	88.0 ± 0.8	87.0± 0.8	0.036
Fasting insulin (IU/ml)	16.9 ± 1.1	4.9 ± 0.4	0.0001
HOMA-IR	3.6 ± 0.3	1.0 ± 0.1	0.0001
Triglycerides (mg/dl)	114.1± 5.0	75.0± 4.2	0.0001
Total cholesterol (mg/dl)	150.5± 3.1	146 ± 3.0	NS
LDL-C (mg/dl)	86.0 ± 2.4	75.0± 2.1	0.012
HDL-C (mg/dl)	45.1 ± 1.1	53.0± 1.1	0.0001
Leptin (ng/ml)	14.1 ± 0.8	1.7 ± 0.4	0.0001

Values are expressed as median ± SEM.
NS: Not significant. BMI: Body mass index. HOMA-IR: Homeostatic model assessment of insulin resistance. LDL-C: Low-density lipoprotein cholesterol. HDL-C: High-density lipoprotein cholesterol.

familial relationship. Ninety-nine healthy children (42 females, 57 males) with normal weight (body mass index [BMI] <85%) who were matched for age and sex were recruited to the control group. Their ages ranged from 5.0 to 15.0 years. Height of all the participants was measured using a wall-mounted stadiometer (Harpenden®), while weight was measured to the nearest 0.01 kg with a digital scale (Seca®). Obesity was defined as BMI [BMI = weight (kg)/height (m)^2] being over the 95th percentile. Obese children with genetic syndrome, hypothalamic tumor or central nervous system disorder were excluded from the study. The obese individuals were recruited from the pediatric endocrinology clinics, and subjects in the control group were selected from general pediatric outpatient clinics at Karaelmas University Faculty of Medicine.

Birth weights of subjects and parental obesity were recorded. Birth weights above the 90th percentile or 2 SD by gestational week were considered as large for gestational age (LGA), while those with birth weights below the 10th percentile were considered as small for gestational age (SGA)[14,15].

The incidence of metabolic syndrome in obese children was determined according to the 2007 International Diabetes Federation (IDF) criteria[19]. Written informed consent was provided by all parents, and the local ethics committee approved the study.

Laboratory Analyses

After a 10-hour overnight fasting, blood samples for glucose, insulin, lipids [triglycerides (Tg), cholesterol total (Tc), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C)], and leptin were obtained from all the children.

Fasting glucose concentration was determined by an enzymatic colorimetric method (Roche Diagnostics, Mannheim, Germany), and fasting insulin concentration was determined using a chemiluminescent assay (Roche Diagnostics, Mannheim, Germany).

Lipids were measured by an enzymatic colorimetric method with available kits (Roche Diagnostics, Mannheim, Germany).

Plasma leptin levels were evaluated using a commercially available radioimmunoassay (RIA) (Lince Research, Inc, St. Louis, MO) with a sensitivity of 0.5 ng/ml.

Insulin resistance was estimated using the homeostasis model for insulin resistance index (HOMA-IR), which is defined as fasting insulin (IU/ml) times fasting glucose (mmol/L) divided by 22.5[20].

DNA Study

Genomic DNA was obtained from peripheral blood leukocytes. The LEPR Gln223Arg polymorphism was analyzed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay, using primer pairs previously described[3,7].

Table II. Anthropometric and Metabolic Data of the Obese Subjects with or without Metabolic Syndrome

	With MS (n=23)	Without MS (n=69)	P
Age (year)	12.0 ± 0.5	11.1 ± 0.3	NS
Males/Females (n)	6 / 17	38 / 31	0.018
BMI (kg/m^2)	30.9 ± 1.1	28.0 ± 0.5	0.005
Waist (cm)	102.0 ± 3.4	89.0 ± 1.5	0.005
Systolic BP (mmHg)	130.0 ± 4.7	110.0 ± 1.3	0.0001
Diastolic BP (mmHg)	80.0 ± 1.0	77.0 ± 1.0	0.021
Triglycerides (mg/dl)	153.0 ± 12.3	106.0 ± 4.5	0.0001
Total cholesterol (mg/dl)	157.0 ± 6.7	148.0 ± 3.3	NS
HDL-C (mg/dl)	41.0 ± 1.9	47.0 ± 1.3	0.004
LDL-C (mg/dl)	94.0 ± 5.2	84.0 ± 2.6	NS
Fasting glucose (mg/dl)	89.0 ± 1.7	88.0 ± 0.8	NS
Fasting insulin (IU/ml)	22.9 ± 2.8	14.3 ± 1.1	0.0001
HOMA-IR	4.8 ± 0.8	2.9 ± 0.3	0.0001
Leptin (ng/ml)	17.4 ± 6.1	11.9 ± 1.0	0.026

Values are expressed as median ± SEM.

NS: Not significant. BMI: Body mass index. BP: Blood pressure. LDL-C: Low-density lipoprotein cholesterol. HDL-C: High-density lipoprotein cholesterol. HOMA-IR: Homeostatic model assessment of insulin resistance.

Statistical Analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 11.0 (SPSS Inc, Chicago, IL, USA). Values are expressed as percentages and median±SEM (standard error of mean) due to the high individual variability. P values of <0.05 were considered statistically significant. Mann-Whitney and chi-square tests were used where appropriate in order to compare independent groups. Pearson correlation coefficient was used in order to investigate the effects of independent variables on the dependent variable.

Results

Subject Characteristics

There was a significant difference between the groups in the anthropometric parameters, glucose, insulin, Tg, LDL-C, HDL-C, and leptin levels ($p<0.05$). Serum leptin levels were significantly higher in obese children than in the control group. Anthropometric and metabolic data of the obese and control groups are shown in Table I.

The rates of SGA infants did not differ significantly between the obese and control groups, while a significantly higher number of obese children were born LGA [21.7% (n: 20) and 9.1% (n: 9), respectively] ($p<0.05$).

Obesity in both parents was identified in 35 (38.0%) obese and in 23 (23.2%) control subjects, with a statistically significant difference between the two groups ($p<0.05$).

Incidence of metabolic syndrome was 25.0% (n=23) in obese children. Comparative anthropometric and laboratory data of obese children with and without metabolic syndrome are presented in Table II.

Genotype and Allele Frequency

Genotype and allele frequencies of LEPR Gln223Arg polymorphism in obese and control groups are presented in Tables III and IV. No significant differences were found between the obese and control groups in genotype or allele frequency. There were also no significant differences between children with metabolic syndrome and other obese children and the control group ($p>0.05$).

There were no significant differences in Gln223Arg polymorphism between LGA- and normal- or SGA-at-birth children.

Comparison of obese children of obese and non-obese parents we did not demonstrate a significant difference in LEPR Gln223Arg polymorphism or allele frequency ($p>0.05$).

Table III. LEPR Gene Gln223Arg Polymorphism in the Study Groups

	Obese children (n=92)	Lean controls (n=99)	P value
AA	25 (27.2%)	24 (24.2%)	NS
CC	37 (40.2%)	44 (44.5%)	NS
AC	5 (5.4%)	6 (6.1%)	NS
CA	25 (27.2%)	25 (25.2%)	NS

No relationship was observed between LEPR Gln223Arg polymorphism and serum leptin, insulin, lipid levels, or HOMA-IR.

Discussion

In this study, we investigated the relationship between LEPR Gln223Arg polymorphism and obesity in Turkish children. No relationship was identified between Gln223Arg polymorphism and obesity and obesity-related metabolic disorders, including IR, hyperlipidemia and hyperleptinemia. Obese children with or without metabolic syndrome did not differ significantly in terms of the polymorphism. There was a significant difference between obese children and the control group in parental obesity and LGA at birth. Obese subjects who were LGA at birth and whose parents were both obese did not differ significantly from other obese children or the control group in LEPR Gln223Arg genotype or allele distribution.

Some earlier studies have reported varying results on relationships between LEPR polymorphism and obesity and related disorders[2,5,7,9,13]. Some of these studies indicated a significant relationship. The Quebec Family Study reported a relationship between Gln223Arg and BMI, skin thickness, fat mass, and lean muscle mass[2]. The Heritage Family Study noted a significant relationship between Gln223Arg polymorphism and BMI in Caucasian men[3]. A relationship was defined between Gln223Arg polymorphism and abdominal fat mass in postmenopausal Dutch women[18]. A study from Brazil with a multi-ethnic adult group indicated a strong correlation between obesity and Gln223Arg polymorphism[8]. Another study with obese Mexican adolescents determined relationships between Gln223Arg and hemodynamic and metabolic changes associated with obesity[7]. On the other hand, according to the results of a meta-analysis, there were no significant associations between LEPR genotypes and BMI or waist circumference, after adjustments for age and sex[19].

Some recent reports emphasized a weak or no relationship between Gln223Arg polymorphism and associated comorbidities. In a unique study of a Turkish adult population, Mergen et al.[13] did not identify a relationship between Gln223Arg polymorphism and BMI. A study in Japanese children and adolescents could find no relationship between Gln223Arg and obesity, IR and hyperlipidemia, but an association of serum lipids with LEPR Lys656Asn, Lys109Arg and Ser343Ser polymorphisms was reported[9]. A LEPR polymorphism study in Thai obese children obtained results similar to our study[11].

Available reports suggest the lack of a strong causal relationship between LEPR Gln223Arg polymorphism and predisposition to obesity, but indicate a modest relationship with comorbidities including changes in regional fat distribution, hypertension and dyslipidemia. It should be noted that different ethnicities may be responsible for the inconsistent results reported in these studies.

In conclusion, this is the first report to investigate the relationship between LEPR Gln223Arg polymorphism and obesity in Turkish children. We believe future studies on obesity genetics will help to develop preventive strategies.

Table IV. LEPR Gene Gln223Arg Allelic Frequencies in the Study Groups

	Obese children (n=92)	Lean controls (n=99)	P value
A	80 (43.4%)	79 (39.8%)	NS
C	104 (56.6%)	119 (60.2%)	NS

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