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ORIGINAL ARTICLE

Analysis of the relationship between three coding polymorphisms in *LEPR* gene and obesity in northern Chinese☆

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Summary To determine the effect of variants in *LEPR* gene on obesity in northern Chinese, three coding polymorphisms Arg109Lys (A/G), Asn656Lys (C/G) and Pro1019Pro (C/T) were investigated for association with overweight and obesity. By a case control design, 248 overweight or obese subjects and 351 lean normal controls were recruited in Harbin region in north China. All three polymorphisms were genotyped by Sequenom single nucleotide polymorphism (SNP) detection system in both cases and controls. Genotypes for all three polymorphisms were in Hardy—Weinberg equilibrium in control subjects. Both groups had similar distribution of alleles and genotypes created by the three coding polymorphisms of *LEPR* gene. No differences in frequencies of genotypes or alleles between cases and controls for any polymorphism individually were found by χ^2 analysis ($p = 0.444$, $p = 0.507$ and $p = 0.662$, respectively). Further, when the haplotypes of three polymorphisms were assessed, no association for any haplotype of three polymorphisms was revealed.

Abbreviations: *LEPR*, leptin receptor gene; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; BMI, body mass index; STAT, signal transducer and activator of transcription; WHR, waist hip ratio.

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In the present study, the three coding polymorphisms in *LEPR* gene were firstly investigated in a population of northern Chinese. It was suggested that the three coding polymorphisms in *LEPR* gene were unlikely to have major effects on susceptibility to obesity in northern Chinese.

Introduction

Leptin receptor is a member of cytokine receptor family, which plays a critical role in leptin signal transduction and the regulation of body weight by activating signal transducer and activator of transcription (STAT) proteins STAT3, STAT5 and STAT6. Leptin receptor gene (*LEPR*) was considered as an important candidate of overweight and obesity in recent years. In animal models, *LEPR* gene mutation can lead to serious obesity and diabetes, and these complicated syndromes can be completely rescued by introduction of a neuron-specific *LEPR*-B trans-gene in *db*/*db* mice [1], which confirmed the critical role of *LEPR* gene in the etiology of obesity and diabetes. It was also suggested LEPR can help recombinant leptin across the human blood—brain barrier by binding and endocytosis [2]. Since Gotoda et al. found five common DNA sequence variants on entire coding sequence of the human leptin receptor cDNA from 22 morbidly obese patients [3], some association studies on common polymorphisms in *LEPR* gene were conducted in different populations in order to find the role of these variants in obesity or related metabolic phenotypes [4—7]. Part of them focused on Arg109Lys, Asn656Lys and Pro1019Pro polymorphism and suggested their association with metabolic phenotypes and obesity related disorder. For examples, Loos et al. found that Arg109Lys polymorphism was significantly associated with respiratory quotient while walking at 4.5 km/h, and Asn656Lys polymorphism was associated with resting metabolic rate in the Quebec Family Study [8]. In 630 Caucasian individuals, Zhang et al. found Pro1019Pro (T/C) polymorphism was associated with inflammatory traits including plasma fibrinogen and C reactive protein levels [9]. de Luis Roman et al. found that Asn656Lys variation was associated with decreased leptin response and weight loss secondary to life style modification in obese patients [10].

Overweight and obesity become a serious problem menacing health of more and more people. Though a few association studies were conducted on the association of *LEPR* variation with overweight and obesity in several populations including southern Chinese. The effects of coding polymorphisms Arg109Lys, Asn656Lys and Pro1019Pro have not been clearly defined in northern Chinese population with a high frequency of overweight and obesity. In this study, we attempted to test the association of the three coding polymorphisms in *LEPR* gene with overweight and obesity in northern Chinese.

Subjects and methods

Study subjects and clinical characteristics

Two hundred and forty-eight overweight or obese subjects (47.8 ± 10.5 years old) from Harbin region were recruited. Obesity was diagnosed based on the standard set up by the World Health Organization (WHO) with minor modification. Subjects were defined as overweight or obesity when their body mass indexes (BMI) were more than 25.0 kg/m^2. Three hundred and fifty-one normal lean subjects (46.7 ± 11.3 years old) were recruited through a routine physical examination. All subjects are from unrelated Han ethnic group. Height, weight and blood pressure were obtained in all subjects. BMI was calculated as weight (kg) divided by height squared (m^2). Informed written consent was obtained from all subjects before participation. The study was approved by the ethics committee of Beijing Hospital, Ministry of Health, PR China.

Genotyping of the three polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes by phenol—chloroform method. Sequenom SNP detection system was used for the genotyping of Arg109Lys, Asn656Lys and Pro1019Pro polymorphisms in *LEPR* gene. Sequenom SNP detection system is based on MALDI-TOF MS technique. The primer sequences for PCR and extension were as indicated in Table 1. The genotyping results were confirmed by direct sequencing of 20 randomly selected subjects.

Statistical analysis

All statistical analyses were conducted using SPSS 11.5 software package. Hardy—Weinberg equilibrium of the genotypes was assessed in control

Table 1 Primer sequences for Sequenom polymorphisms genotyping

Polymorphism	Primer sequence
Arg109Lys	Forward: ACGTTGGATGGCTCCTTATGTGCAGACAAC Reverse: ACGTTGGATGAGCTAATGCTTACCTATTTG Extension: TGCAGACAACATTGAAGGAA
Asn656Lys	Forward: ACGTTGGATGACCTTCCAAAGTAAAGTGAC Reverse: ACGTTGGATGAGTTCCTATGAGAGGACCTG Extension: AAAGTAAAGTGACATTTTTCTC
Pro1019Pro	Forward: ACGTTGGATGTCAGTCACCAAGTGCTTCTC Reverse: ACGTTGGATGAAAATGCCTGGGCCTCTATC Extension: TTCTCTAGCAAAAATTCTCC

Table 2 The clinical characteristics of overweight or obese patients and control subjects

Phenotypes	Controls	Cases
Age (years)	47.8 ± 10.5	46.7 ± 11.3
Sex (M/F)[a]	144/104	181/170
BMI (kg/m^2)	22.3 ± 1.9	28.1 ± 2.9**
WHR	0.8 ± 0.1	0.9 ± 0.1**
SBP (mmHg)	112.4 ± 19.5	122.1 ± 19.6**
DBP (mmHg)	81.5 ± 13.0	85.5 ± 13.1**

Note. Continuous variables are expressed as mean ± S.D. values; **, $p \leq 0.01$; BMI, body mass index; WHR, waist hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

population. Genotype and allele frequencies in different groups were compared through χ^2 analysis and quantitative phenotypes were analyzed with Student's *t*-test and expressed as mean ± S.D. $p \leq 0.05$ was taken as significant threshold. Haplotype of the three polymorphisms were analyzed by using HAP-haplotype resolution: version 3.0 [11].

Results

Clinical characteristics of obese and control subjects

The clinical characteristics of obese and control subjects are summarized in Table 2. In addition to significantly higher BMI, the obese subjects also had a higher waist hip ratio and blood pressure. No significant differences for the distribution of age and sex were found between case and control.

Distribution of LEPR genotypes and alleles in obese and control subjects

Overall, the alleles of three polymorphisms were determined in 599 individuals including 248 obese and 351 normal control subjects. Genotypes for all three polymorphisms were in Hardy—Weinberg equilibrium in control subjects ($p = 0.779$, 0.351 and 0.716, respectively). Both groups had similar distribution of alleles and genotypes created by the three coding polymorphisms of *LEPR* gene (Table 3).

Table 3 Genotype and allele frequencies of the three polymorphisms in *LEPR* gene in overweight or obese patients and control subjects

Variations	HWE (*p*)	AA	AB	BB	A/B
Arg109Lys (G/A)					
Cases	0.574	174 (70.2%)	66 (26.6%)	8 (3.2%)	0.83/0.17
Controls	0.779	242 (69.0%)	98 (27.9%)	11 (3.1%)	0.83/0.17
Gln656Lys (G/C)					
Cases	0.423	224 (90.3%)	24 (9.7%)	0 (0.0%)	0.95/0.05
Controls	0.351	314 (89.4%)	35 (10.0%)	2 (0.6%)	0.94/0.06
Pro1019Pro (T/C)					
Cases	0.623	198 (79.8%)	48 (19.4%)	2 (0.8%)	0.89/0.11
Controls	0.716	271 (77.2%)	74 (21.1%)	6 (1.7%)	0.88/0.12

For the Arg109Lys, Asn656Lys and Pro1019Pro polymorphisms in *LEPR* gene, major allele G, G, T, respectively, were designated as A; while minor allele designated as B; the genotype and allele frequencies between case and control were compared by Pearson χ^2 analysis ($p > 0.05$). Number and percentage were shown for genotypes while only frequency was shown for alleles. HWE, Hardy—Weinberg equilibrium.

Table 4 Haplotype frequencies of the three coding polymorphisms in *LEPR* gene in overweight or obese patients and control subjects

Haplotype	Controls ($n = 702$)	Cases ($n = 498$)	p, OR (CI)
GGT	543 (77.4%)	395 (79.3%)	0.63, 1.07 (0.81—1.43)
AGC	57 (8.1%)	39 (7.8%)	0.87, 0.97 (0.63—1.48)
AGT	37 (5.3%)	26 (5.2%)	0.98, 0.99 (0.59—1.66)
ACT	26 (3.7%)	17 (3.4%)	0.80, 0.92 (0.50—1.72)
GGC	26 (3.7%)	12 (2.4%)	0.21, 0.65 (0.32—1.29)
GCT	10 (1.4%)	6 (1.2%)	0.75, 0.85 (0.31—2.35)

The p value, OR and 95% CI of each haplotype relative to other haplotypes as a group are shown. The results for very rare haplotypes (no more than 1%) in both groups are not shown.

Distribution of haplotypes for three polymorphisms in LEPR gene in obese and control subjects

When haplotypes of the three polymorphisms were assessed, only six out of eight possible haplotypes occurred in a more than one percentage frequency in northern Chinese. No significant differences in distribution of haplotypes of three polymorphisms and haplotypes combination of them were found between cases and controls. It was suggested the haplotypes of three polymorphisms unlikely have a major effect on the susceptibility of obesity in northern Chinese. The results of haplotype analyses were shown in Tables 4 and 5.

Discussion

In the present study, three coding polymorphisms in *LEPR* gene were firstly investigated in a population of northern Chinese. We have not found association between obesity and polymorphisms in *LEPR* gene and failed to find powerful evidence supporting our hypothesis that these polymorphisms may have a major effect on obesity in northern Chinese.

According to our present knowledge, this is the first investigation of the three polymorphisms in northern Chinese population. Genotype distributions for all three polymorphisms were consistent with Hardy—Weinberg equilibrium in control subjects which eliminated the possibility of sampling divergence. All three polymorphisms are with a frequency of not less than 5% and can be used as reasonable susceptible markers for common disease, such as obesity and type 2 diabetes.

The coding polymorphisms in *LEPR* gene were investigated in many different ethics and populations for their roles in obesity. Yiannakouris et al. found association only for Gln223Arg polymorphism and body composition variables, while not for Arg109Lys and Asn656Lys polymorphisms [12]. The results were agreed with our ones. The study in Korean population suggested *LEPR* gene polymorphisms were not associated with type 2 diabetes, but Arg109Lys polymorphism was marginally associated with BMI of the subjects [13]. In 204 southern Chinese, Lu et al. found Pro1019Pro (T/C) polymorphism have an effect on lipid metabolism and body fat distribution of type 2 diabetes patients [14]. Both of which were divergent from our results in northern Chinese. The inconsistence of the association studies on these polymorphisms was due to multiple factors. Firstly, it was partly up to the ethic difference or genetic diversity of the research subjects, even genetic background of northern Chinese

Table 5 Haplotype combination frequencies of the three coding polymorphisms in *LEPR* gene in overweight or obese patients and control subjects

Haplotype combination	Controls ($n = 351$)	Cases ($n = 248$)	p, OR (CI)
GGT/GGT	209 (59.5%)	157 (63.3%)	0.35, 1.17 (0.84—1.64)
GGT/AGT	31 (8.8%)	21 (8.5%)	0.88, 0.96 (0.54—1.71)
GGT/AGC	45 (12.8%)	32 (12.9%)	0.98, 1.01 (0.62—1.64)
GGT/GGC	20 (5.7%)	10 (4.0%)	0.36, 0.70 (0.32—1.51)
GGT/ACT	17 (4.8%)	11 (4.4%)	0.82, 0.91 (0.42—1.98)
GGT/GCT	9 (2.6%)	6 (2.4%)	0.91, 0.94 (0.33—2.68)
AGT/ACT	2 (0.6)	3 (1.2%)	0.65, 2.14 (0.35—12.88)

The p value, OR and 95% CI of each haplotype combination relative to other haplotype combinations as a group are shown. The results for very rare haplotype combinations no more than 1% in both groups are not shown.

was different from that of southern Chinese. Secondly, phenotypic measurement of obesity in this study was not the same as the study in southern Chinese. We focused on body mass index as a whole while Lu et al. paid more attention to biochemical measurement and fat distribution in different part of body. Finally, the standard for selection of subjects was not in accord. The subjects from southern Chinese were complicated with type 2 diabetes, a syndrome demonstrated to be closely associated with obesity.

Hekerman et al. suggested that three intracellular tyrosines at position 985, 1077 and 1138 were responsible for the signal transduction from *LEPR* to STATs. Several other important amino acids, for example, Met1139 and Gln1141 are determinants defining the specificity toward the different STAT factors and can also affect leptin response and signal transduction [15]. The absence of association for three common cSNPs we test may due to these polymorphisms were not at functional active site of the protein and with little modification on the structure or configuration of the active site. Chung et al. suggested the Asn656Lys polymorphism can result in positive to neutral change of charge, which may lead to modification of the structure and function of *LEPR* protein. Besides another polymorphism, Gln223Arg, which was adjacent to *fa* mutation in animal model and strongly supported being associated with obesity and insulin response, also resulted in change of charge from neutral to positive and most likely have some functional consequences [16]. Studies also suggested Gln223Arg polymorphism in the leptin receptor is associated with familial combined hyperlipidemia [17]. These studies further suggested only those polymorphisms that modify the structure of functional active site can be possibly closely associated with complicated phenotypic measurement such as obesity.

We inferred that three polymorphisms we investigated can be regarded as balanced polymorphisms that escaped natural selection, which did not play a central role in body composition and the development of obesity, instead, they only slightly modified the progress of obesity and related disorder. Further work is necessary to discern the confound effect of genetic background of different population and find gene variations with critical role, as well as their molecular mechanism in overweight and obesity.

In summary, no association with obesity was revealed for both three coding polymorphisms individually and their haplotypes. It was unlikely these polymorphisms played an important role in etiology of obesity in northern Chinese.

Competing interests

None declared.

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