Association of body mass index and Trp64Arg polymorphism of the β_3-adrenoreceptor gene and leptin level in Tehran Lipid and Glucose Study

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Accepted: 28 July 2007

ABSTRACT

In this study the association between β_3-adrenoceptor gene polymorphism and serum concentration of leptin with body mass index (BMI) is investigated. Using subjects in the Tehran Lipid and Glucose Study, genotyping of the Trp64Arg polymorphism of the β_3-adrenoreceptor gene was performed using a restriction fragment length polymorphism–polymerase chain reaction (RFLP-PCR) technique was used and the association with obesity was investigated. At total of 197 men and 204 women were divided into four groups (BMI<20, 20≤BMI<25, 25≤BMI<30, BMI≤30) and 97, 98, 104 and 102 subjects, respectively, were placed randomly in the four groups. Leptin level was determined by an enzyme immunoassay (EIA) method and FBS, HDL-C, triglyceride and total cholesterol levels were determined by an enzyme colorimetric method. Body mass index (BMI) was also measured. The A (Arg) allele frequency was 0.08 among the population and its presence was significantly associated with increase of leptin level (AA/TA, 30.5±24.8 ng/mL; TT, 22.6±20.9 ng/mL; P=0.014) but there was no significant association with increased BMI (AA/TA, 27±5.6 kg/m^2; TT, 25.4±5.5 kg/m^2; P=0.072). These data show that the presence of the Arg64 allele at the β_3-adenoceptor gene locus is related to increase in leptin level in this population, but is not related to body mass index.

KEY WORDS: Body mass index. Leptin. Polymorphisms, genetic. Receptors, adrenergic, beta-3.

Introduction

In modern societies, obesity has become an important health problem,[1] being a major prevalent problem in most industrialised countries, especially in regions with a recent change in lifestyle.[2] Human obesity is considered to be a large heritable complex of different factors.[3] Genetic and environmental factors affect human health.[4,5] The definition of obesity is an accumulation of adipose tissue, resulting from an imbalance of energy intake and expenditure.[6]

Many genetic polymorphisms are associated with the obesity phenotypes,[7] but little is known about the key roles of genes and interactions among polymorphisms in the progress of human obesity.[8] Adrenergic receptors are cell-surface proteins that bind adrenaline and/or noradrenaline with high affinity and trigger intracellular changes. The two major classes of adrenergic receptors, alpha and beta, were originally discriminated based on their cellular actions.[9] There is some evidence that β_3-adrenergic receptor activates lipolysis and energy expenditure,[10] and recently the β_3-adrenoreceptor gene (*ADRB3*) has become the centre of attention.

Expression of *ADRB3* in humans is predominantly in the adipose tissue.[11] It contains seven transmembrane domains and is coupled with G proteins.[12] β-adrenergic agonists stimulate lipolysis and thermogenesis through the activation of the enzyme adenylate cyclase, resulting in an increase in intracellular cyclic AMP. Therefore, *ADRB3* plays a role in the regulation of lipid metabolism, thermogenesis, resting metabolic rate and energy balance in human adipose tissue and thus may influence variation in body weight among individuals.[4,6,12]

The *ADRB3* gene is located on human chromosome 8p12-p11.2.[13] The Trp64Arg polymorphism is due to the replacement of thymidine (T) by cytosine (C) at nucleotide position 190,[4] resulting in replacement of tryptophan by arginine at codon 64 (*W64R*) in *ADRB3*.[13,14] Different reports have shown an association between Trp64Arg polymorphism and the increased capacity to gain weight.[13,14]

Leptin is an endocrine hormone that is highly conserved among different species, including man, and plays a role in different metabolic pathways.[15] It is primarily released from the adipocytes at levels approximately proportional to the body fat content and it signals to the brain in proportion to its plasma concentration. Thus, it is one of the obesity-related phenotypes that is thought to relate to body fat content.[15,16] A meta-analysis of the association between *ADRB3* polymorphism and body mass index (BMI) in 31 subjects demonstrated that subjects with the polymorphism had a BMI higher than those without the polymorphism.[17] In addition, Clement *et al*. showed that the Trp64Arg variant increases the capacity to gain weight.[14]

In the present study, the relationship between the *ADRB3* gene variant, BMI and leptin level is investigated in the Tehran Lipid and Glucose Study population.

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Materials and methods

Study population and phenotypic data collection

The Tehran Lipid and Glucose Study (TLGS) is designed to determine the risk factors for major non-communicable disorders including atherosclerosis among Tehran's urban population, in order to develop population-based measures to change the lifestyle of the population and prevent the rising trend of diabetes mellitus and dyslipidaemia. Details of the study are presented elsewhere.[18–20] Ethical clearance was obtained for the study from the Research Institute for Endocrine Science.

Subjects were screened using the following criteria: men and women over 18 years old, TG <400 mg/dL, not taking any medication, no cardiovascular disease or hypertension. Subjects satisfying these criteria were selected from among the 15,000 participants in TLGS. Based on their BMI (defined as weight [kg]/height^2 [m]), the subjects were selected randomly and divided into four groups by SPSS 11.5: BMI<20 (n=97); 20≤BMI<25 (n=98); 25≤BMI<30 (n=104); and BMI≥30 (n=102). In addition, they were matched and there was no significant difference between age in the four groups. Height, weight, waist and hip circumference, and blood pressure of each subject were measured. Their BMI and waist to hip ratio was calculated.

After a 12 h fast, blood samples were collected in plain and EDTA tubes. Following centrifugation for 10 minutes at 3000 rpm, serum was separated and stored at –70°C in 1.5 mL aliquots. Glucose and lipids were measured immediately from fresh samples. Serum glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), leptin and triglyceride levels were measured in fasting samples, as previously described.[19]

Levels of HDL-C were measured after precipitation of apolipoprotein B (apo-B)-containing lipoproteins with dextran-magnesium sulphate.[21] Low-density lipoprotein cholesterol (LDL-C) concentrations in samples with serum triglyceride <4.52 mmol/L were calculated using the Friedewald equation.[22]

Intra-assay and inter-assay coefficients of variation (CV) of total cholesterol, HDL-C and triglyceride measurements were all less than 5%. Blood samples collected in EDTA tubes were used for DNA extraction. Briefly, samples were washed in lysis buffer (10 mmol/L Tris-HCl, 5 mmo/L $MgCl_2$, 1% Triton X [pH 7.6]) and then with phosphate-buffered saline (PBS). Then DNA was extracted by the salting out/proteinase K method[23] and stored at –20°C. Serum concentration of leptin was measured by an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochemical, Canada).

Genotyping

The *ADRB3* Trp64Arg polymorphism was genotyped by amplifying genomic DNA and then digesting by restriction endonuclease. The PCR primers were 5′- CGCCCAATACCGCCAACAC -3′ and 5′- CCACCAGGAGTCCCATCACC -3′.[10] A polymerase chain reaction (PCR) was carried out in a volume of 25 µL containing 50 ng genomic DNA, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L deoxynucleotide triphosphate, 300 nmol/L each primer, one unit of *Thermus aquaticus* (*Taq*) DNA polymerase (Cinnagen, Iran) and a 10x PCR reaction buffer.

Cycling began with an initial denaturation at 95°C for 5 min, followed by 30 amplification cycles (94°C for 45 sec, 64°C for 45 sec and 72°C for 1 min) and an additional 5 min at 72°C. The PCR amplicon was digested with two units of BST N1 (Roche, Germany) at 37°C for 3 h.

The digested products were subjected to electrophoresis through a 2% agarose gel (Cambrex, Denmark). The gel was stained with ethidium bromide and DNA was visualised under ultraviolet (UV) transillumination. The expected sizes were 99, 62, 30, 12 and 7 bp for Trp64 homozygotes; 161, 30, 12 and 7 bp for Arg64 homozygotes; and 161, 99, 62, 30, 12 and 7 bp for heterozygotes.

Statistical analysis

Initially, a descriptive analysis of the clinical and sociodemographic characteristics of the study population was performed, including calculations of the means and SD of the phenotypes of interest by specific genotypes. Then, association analysis between the *ADRB3* W64R genotype and obesity-related phenotypes was carried out. Clinical, anthropometry and biochemical data were analysed by *t*-test. A one-way ANOVA test was performed, followed by post hoc multiple comparison (Tukey) between the four BMI groups and also between Arg carriers and Arg non-carriers. All data were analysed using SPSS 11.5. Statistical deviations of the W64R genotype distribution from Hardy-Weinberg equilibrium were tested using χ^2 analysis.

Table 1. Clinical, biochemical and anthropometry characteristics in males and females.

	Men (n=197)	Women (n=204)	P value
Age (years)	46±14	42±13	0.003
Waist (cm)	88±12	86±14	NS
Hip (cm)	95±8	102±11	<0.001
Waist/hip	0.9±0.1	0.8±0.1	<0.001
BMI (kg/m2)	24±5	27±6	<0.001
Systolic blood pressure (mmHg)	115±13	110±13	<0.001
Diastolic blood pressure (mmHg)	74±8	72±8	0.007
Fasting blood sugar (mmol/L)	5.2±1.1	5±1.4	NS
Triglyceride (mmol/L)	1.7±0.8	1.5±0.8	0.011
Total cholesterol (mmol/L)	4.7±1	5±1.1	0.008
HDL-C (mmol/L)	0.9±0.2	1.1±0.3	<0.001
Leptin (ng/mL)	10±11	37±21	<0.001

Results

Full characteristics of the study groups are summarised in Table 1. Women had significantly higher total cholesterol, HDL-C and leptin levels than men; however, men had significantly higher waist to hip ratio and systolic and diastolic blood pressure than women.

Table 2 shows clinical and biochemical variables of the study subjects according to the four BMI groups There were 340 subjects with TT genotype and 61 subjects with TA/AA genotype and the number of Arg

Table 2. Clinical, biochemical variables and β_3-adrenoceptor gene polymorphism frequency based on four groups of BMI.

	BMI<20 (*n*=97)	20≤BMI<25 (*n*=98)	25≤BMI<30 (*n*=104)	30≤BMI (*n*=102)
Genotype TA/AA (%)*	8(13.1)	17(27.9)	15(24.6)	21(34.4)
Genotype TT (%)*	89(26.2)	81(23.8)	89(26.2)	81(23.8)
Age (year)	42±18	44±13	44±12	44±12
Systolic blood pressure (mmHg)	107±15†	109±12§	116±12	117±13
Diastolic blood pressure (mmHg)	69±8†	71±9§	75±7	76±7
Fasting blood sugar (mmol/L)	4.8±0.7†	4.9±0.9	5.3±1.4	5.3±1.5
Triglyceride (mmol/L)	1.1±0.6‡	1.4±0.7§	1.8±0.8	1.9±0.8
Total cholesterol (mmol/L)	4.3±1‡	4.7±0.9§	5.3±1	5.1±1
HDL-C (mmol/L)	1.1±0.2¶	1±0.2	1±0.3	1±0.3
Leptin (ng/mL)	9±10‡	18±20§	26±19	41±20

*Figures in and out of parentheses indicate percentage and individuals, respectively.

†($P<0.05$) in comparison with 25≤BMI<30, 30≤BMI.

‡($P<0.05$) in comparison with 20≤BMI<25, 25≤BMI<30, 30≤BMI.

§($P<0.05$) in comparison with 25≤BMI<30, 30≤BMI.

¶($P<0.05$) in comparison with 20≤BMI<25, 30≤BMI.

carriers was higher in groups with higher BMI. Frequencies of R65 (mutant allele) and W64 (wild-type allele) in the overall study group were 0.08 and 0.92, respectively, and the genotype frequency distributions were in Hardy-Weinberg equilibrium.

Biochemical, clinical and anthropometry variables were investigated in two groups, Arg carriers and Arg non-carriers (Table 3). With the exception of diastolic blood pressure, all variables were higher in the Arg carriers.

Discussion

The β-adrenergic receptors regulate thermogenesis, resting metabolic rate and energy balance in human adipose tissue and thus may influence body weight variation among individuals.[24,25] In the β_3 receptor, a Trp→Arg substitution at position 64 seems to reduce lipid mobilisation and energy metabolism in visceral adipose tissue, thus increasing the risk of obesity in carriers of the Arg64 allele.[26] The present study investigated the association between *ADRB3* gene polymorphism (Trp64Arg), BMI and leptin level.

The polymorphism has been detected at an allelic frequency of about 0.08 in TLGS, which is lower than in Pima Indians (0.31), Mexican-Americans (0.13) and black populations (0.12). The frequency of this polymorphism in this Iranian cohort (0.08) is similar to that found among Caucasians in the USA.[13,24] The genotypes of the variants were found to be in Hardy-Weinberg equilibrium.

The primary finding in this study was that Arg carriers had significantly higher leptin levels than did Arg non-carriers, but BMI between these two groups was not significant. Also, serum leptin concentration, triglyceride and total cholesterol showed significant increases in the four groups according to their BMI. Leptin level, triglyceride, total cholesterol and fasting blood sugar showed significant differences between the Arg carriers and the Arg non-carriers, with higher rates in the Arg carrier group.

Yoshida *et al*. found that obese Arg allele carriers showed less weight reduction after a hypocaloric diet with exercise than did Trp carriers.[27] Techernof *et al*. showed that obese women with the Trp64Arg variant had an impaired capacity to lose visceral adipose tissue on a 13-month weight reduction programme, although 11 carriers and 13 non-carriers of the Trp64Arg allele showed a similar 17% reductions of initial body weight.[28]

Ramis *et al*. showed that the β_3-adrenoreceptor Trp64Arg polymorphism might have an impact on the mechanisms

Table 3. Clinical, anthropometry and biochemical variables in Arg carrier and Arg non-carrier groups (*P* values adjusted for gender).

	Arg carriers (*n*=61)	Arg non-carriers (*n*=340)	*P* value
Age (years)	45±12	43±14	0.241
Waist (cm)	91±13	87±13	0.021
Hip (cm)	100±11	99±10	0.387
Waist/hip	0.9±0.1	0.8±0.1	0.002
BMI (kg/m^2)	27±6	25±5	0.072
Systolic blood pressure (mmHg)	114±13	112±14	0.232
Diastolic blood pressure (mmHg)	73±8	73±8	0.933
Fasting blood sugar (mmol/L)	5.5±2.2	5±1	0.003
Triglyceride (mmol/L)	1.8±0.9	1.5±0.8	0.014
Total cholesterol (mmol/L)	5.2±1.1	4.8±1	0.023
HDL-C (mmol/L)	1±0.3	1±0.2	0.972
Leptin (ng/mL)	30±25	23±21	0.014

involved in leptin release from adipose tissue;[29] however, there have been some negative reports. Kurokawa *et al*. showed that the Trp64Arg variant of the β_3-adrenoreceptor gene does not seem to have a strong effect on the accumulation of body fat in Japanese junior high school students.[30] Janssen *et al*. reported heterozygosity for the Trp64Arg polymorphism of the β_3-adrenoreceptor gene was not accompanied by obesity.[31] These discrepant results may have been due to random sampling variations, small subject numbers, age or the degree of obesity.[32]

In summary, the results of the present study show an association between the Trp64Arg polymorphism of the β_3-adrenergic receptor and leptin levels in a Tehran Lipid and Glucose Study population. Arg carriers show significantly higher leptin levels than do Arg non-carriers, but there is not a significant association between their BMI. Further studies are needed to investigate the interaction between the Trp64Arg polymorphism of *ADRB3* and other polymorphisms involved in the development of obesity. □

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