Vol.5, No.2, 285-291 (2013)
http://dx.doi.org/10.4236/health.2013.52038

The common pentanucleotide polymorphism of the 3'-untranslated region of the leptin receptor gene is associated with obesity in Saudi females*

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Received 5 November 2012; revised 4 December 2012; accepted 12 December 2012

ABSTRACT

Obesity is due to the combined effects of genes, environment, lifestyle, and the interactions of these factors. Leptin receptor (LEPR) gene has been intensively evaluated in the search of variants that could be related to obesity. The results of most of these studies have been controversial. We investigated the effects of leptin receptor gene 3'-untranslated region (3'-UTR) polymorphism on phenotype, metabolic parameters and anthropometric measurements of obese Saudi females. 122 healthy women aged 19 to 36 years. The subjects were divided into 3 groups according to their body mass index BMI; lean (BMI 18 - 24)' overweight (BMI 25 - 29) and obese (BMI ≥ 30). There were 13 homozygotes and 34 heterozygotes for the 3'-UTR insertion allele amongst all 122 women. The results of this study show that the allele frequency of the insertion allele (I) of 3'UTR was significantly higher in overweight (35.3) and obese females (32.2) compared to the frequency in lean females (15.6). The insertion allele was associated with increased BMI in obese groups. The results obtained from this study indicated that in the obese subjects most variable values increased in I/I homozygote but the significant high value recorded among BMI (40.9 ± 7.11 kg/m^2, P = 0.042). Our findings indicated that, the obesity in Saudi females is influenced by alteration in the leptin receptor gene 3'-UTR polymorphism.

Keywords: Pentanucleotide; Polymorphism; Leptin; Obesity; Saudi Females

1. INTRODUCTION

Leptin, a peptide hormone encoded by the obesity gene [1], is produced by adipocytes to regulate body weight and energy balance [2,3]. In the fed state, circulating leptin concentrations reflect the magnitude of fat stores [4,5], and leptin levels are elevated in many models of animal obesity and in obese humans, correlating strongly with the degree of obesity [4-6]. Leptin acts through the leptin receptor (OB-R) [7] that is expressed in the nervous system and peripheral tissues such as adipose tissue, skeletal muscles, pancreatic β-cells, and liver. A single transcription unit of leptin receptor may serve to generate more than one protein. For instance, several isoforms can be derived from a single gene locus by alternative hnRNA splicing, including a long isoform expressed primarily in the hypothalamus and several short-isoforms with a much wider tissue distribution [7]. Although the frequency of such mutations is very low, common polymorphisms of the leptin and OB-R may well contribute to a common form of obesity and, as a consequence, obesity-related diseases [8,9].

Mutations in the genes encoding leptin (ob) and its receptor (db) have been found to cause early onset morbid obesity, with related phenotypes such as type 2 diabetes

*The authors declare no conflict of interest.

in animals [10] and humans [11,12]. The *OB-R* gene is a polymorphic gene. Q223R (rs59347832), K109R (rs59932898), and K656N (rs8179183) are among the principal polymorphisms of this gene and are localized in the extracellular region of the receptor in exons 6, 4, and 14, respectively. The trans-membrane region of the receptor is encoded by exon 18. A large number of studies have been able to associate some of these polymerphisms with body weight gain [13], metabolic carbohydrate reduction [14], lower body weight, body mass index (BMI), body fat, and smaller waist circumference [15]. Low body fat levels and elevated high-density cholesterol [16]. The Q223R polymorphism appears to contribute to a common form of obesity. In contrast, the K109R and K656N polymorphisms have been observed to be involved in the response to glucose and insulin [17].

Studies in obese subjects have reported that heterozygous carriers of a pentanucleotide insertion in the 3'-UTR of the leptin receptor gene had lower serum insulin concentrations than the homozygous carriers of the more common deletion allele [18,19].

Obese humans are characterized by increased circulating leptin levels [5,6], suggesting that human obesity is associated with an insensitivity to endogenous leptin. Since a defect in the leptin-mediated signaling pathway, including the leptin receptor, could be the cause of leptin resistance, the leptin receptor gene has been a plausible candidate gene for obesity [6-20]. Therefore, we investigated the effects of leptin receptor gene 3'-UTR polymorphism on phenotype, metabolic parameters and anthropometric measurements of obese Saudi females.

2. METHODS

2.1. Subjects

Inclusion Criteria: Saudis, age above 18 years, gave consent to participate in the study, with no known illness. Exclusion criteria: non-Saudis, children, suffering from endocrine or genetic causes of obesity. One hundred and twenty two Saudi females' volunteers were participated in this study. After being informed of the purpose and procedures of the study, all subjects signed a consent form. The study protocol was approved by the KFSH & RC Research Ethical committees. The subjects were divided into 3 groups according to their body mass index BMI; lean (n = 60, BMI 18.5 - 24 kg/m^2), overweight (n = 17, BMI 25 - 29 kg/m^2) and obese (n = 45, BMI ≥ 30 kg/m^2). The general characteristics of the subjects are summarized in **Table 1**. All subjects were healthy, free of any medication with regular menstrual cycle, and no history of gastrointestinal or endocrine disorders. After an overnight fast (12 hrs) a venous blood sample was obtained from all subjects in the morning between 08.00 h and 09.00 h for the determination of fasting serum leptin, insulin and glucose. Serum was aliquoted, and stored at −80˚C until required for assay.

2.2. Anthropometry and Body Composition Measurement

For all subjects, weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively. Body mass index (BMI) was calculated as weight (kilograms)/height (meters)^2. Waist and hip circumferences were measured to a precision of 0.1 cm, and the waist to hip ratio was calculated [20].

2.3. Analytical Method

Plasma glucose was measured by a glucose oxidase method using the Roche Glucose HK liquid assay on Roche/Hitachi 917 automatic analyzer. Serum insulin was measured with electrochemiluminescence immunoassay "ECLIA" technique, using Elecsys insulin kit on E 170 immunoassay analyzer (Roche). Serum leptin was measured in duplicate using human leptin ELISA kit from Linco Research, Inc. (St. Charles, MO), with a lower limit of detection of 0.5 ng/mL.

Table 1. Mean comparisons of clinical and endocrine-metabolic characteristics between lean, overweight and obese groups.

Variable	Control lean (n = 60) mean ± SE	Overweight (n = 17) mean ± SE	p value	Obese (n = 45) mean ± SE	p value
Age (yr.)	23.95 ± 0.60	21.59 ± 0.94	0.06	26.49 ± 0.96	0.028
BMI (kg/m^2)	20.85 ± 0.25	27.38 ± 0.37	<0.0001	35.90 ± 0.92	<0.0001
Waist (cm)	66.85 ± 0.70	81.59 ± 1.82	<0.0001	100.29 ± 2.14	<0.0001
Hip (cm)	94.59 ± 0.91	105.29 ± 1.78	<0.0001	121.96 ± 2.14	<0.0001
WH ratio	0.71 ± 0.01	0.78 ± 0.01	<0.0001	0.82 ± 0.01	<0.0001
Leptin (ng/ml)	11.70 ± 0.46	23.79 ± 1.55	<0.0001	46.04 ± 3.07	<0.0001
Fasting insulin (pmol/L)	52.57 ± 2.29	63.93 ± 9.95	0.09	104.69 ± 5.58	<0.0001
Fasting glucose (mmol/L)	4.54 ± 0.05	4.66 ± 0.15	0.32	4.99 ± 0.07	<0.0001

2.4. DNA Analysis

The 3'-UTR pentanucleotide insertion/deletion polymorphism of the leptin receptor gene was analysed from DNA extracted from EDTA-anticoagulated venous blood samples using the QIAamp DNA Blood Mini Kit (QIAgen Inc., Santa Clarita, CA). A DNA fragment of 114 or 119 bp in size (depending on the absence or presence of the insertion) was generated by polymerase chain reaction using 5' mismatch primer ATAATGGGTAATATAAAGTGTAATAGAGTA and 3' primer AGAGAACAAACAGACAACATT, and analysed as described in detail previously [19]. To confirm the results, all heterozygous, homozygous and 10 samples from each group of common deletion allele were sequenced. Oligonucleotides primers were designed using web primer DNA and purpose entry (http://seq.yeastgenome.org/cgi-bin/SGD/web-primer) Forward primer II. 5'-GACTTTTGCATCTTACATGCC-3'Reverse primer II. 5'-ATTGGTAGGCTTATGAA-3'. The PCR conditions consisted of an initial denaturation step at 95˚C for 15 minutes, followed by 34 cycles of denaturation at 95˚C for 1 minute, annealing at 60˚C for 1 minute, and extension at 72˚C for 1 minute, with a final extension of 10 minutes at 72˚C, sequencing was performed with the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) on the ABI 3100 Genetic Analyzer (Applied Biosystems).

2.5. Statistical Analysis

Data are presented as mean ± SEM. The comparisons between obese subjects and matched control were done using the independent t-test and ANOVA test. The distributions of the age, BMI, anthropometric measurements, fasting serum insulin, leptin and glucose values of the study subjects according to their leptin receptor 3'-UTR genotype of each group were compared by the U Mann-Whitney test. Frequency distribution analysis was performed with chi square test. A probability value $p \leq 0.05$ was considered statistically significant. All analyses were run using the StatView program for Windows (version 8.0; SAS).

3. RESULTS

The mean age, BMI, anthropometric, mean leptin, insulin concentrations, and fasting glucose of the groups are shown in **Table 1**. As presented in **Table 1** student's t-test was applied and significant differences were found in the waist, hip and waist/hip ratio among over weight and obese subjects compared with lean control group. Serum concentrations of leptin were markedly and significantly higher in obese, overweight subjects compared with lean controls. Serum fasting glucose and insulin levels were significantly higher in obese group compared to controls, no significantly different between overweight and lean groups (**Table 1**).

The genotype distribution and allele frequencies for the insertion/delition 3'UTR polymorphisms of the LEPR gene are presented in **Table 2**. The genotype distribution was governed by Hardy-Weinberg equilibrium law in this study. As shown in table there were 13 homozygotes and 34 heterozygotes for the 3'-UTR insertion allele amongst all 122 women. The results of this study show that the allele frequency of the insertion allele (I) of 3'UTR was significantly higher in overweight (35.3) and obese females (32.2) compared to the frequency in lean females (15.6). The frequency of the (D) allele in the lean group was 84.2%, 64.7% in the overweight group and 67.8% in the obese group, whereas the frequency of the (I) allele in the different weight groups was 15.8% in the lean group, 35.3% in the overweight group and 32.2% in the obese group. When overweight and obese results were compared to lean, the x^2 was $p = 0.01$ and $p = 0.005$, respectively, and the difference was statistically significant (**Table 2**).

The distributions of the age, BMI, anthropometric measurements, fasting serum insulin, leptin and glucose values of the study subjects according to their leptin receptor 3'-UTR genotype of each group are presented in **Tables 3-5** in the obese subjects most variable values

Table 2. 3'UTR pentanucleotide insertion/deletion polymorphism of the human leptin receptor gene. Genotype and allele frequencies in lean, over weight and obese subjects.

3'UTR	Control lean	Overweight		Obese	
	Frequency (%)	Frequency (%)	p value	Frequency (%)	p value
Genotype D/D	43 (71.67%)	9 (52.94%)		23 (51.11%)	
Genotype D/I	15 (25%)	4 (23.53%)	0.02	15 (33.33%)	0.03
Genotype I/I	2 (3.33%)	4 (23.53%)		7 (15.56%)	
Allele D	(84.2%)	(64.7%)	0.01	(67.8%)	0.005
Allele I	(15.8%)	(35.3%)		(32.2%)	

Table 3. Leptin receptor gene 3'UTR deletion/insertion polymorphism in relation to anthropometry, hormone and metabolic variables in control lean group.

Variables	D/D (n = 43)	D/I (n = 15)	I/I (n = 2)	p-value
Age (years)	23.0 ± 4.35	26.0 ± 4.50	27.0 ± 9.90	0.064
BMI (kg/m^2)	20.7 ± 1.95	21.1 ± 1.95	21.0 ± 1.41	0.768
Waist (cm)	67.2 ± 5.39	65.7 ± 5.65	66.0 ± 5.66	0.627
Hip (cm)	94.5 ± 7.12	95.1 ± 6.94	92.5 ± 10.6	0.877
W/H ratio	0.71 ± 0.042	0.69 ± 0.049	0.71 ± 0.014	0.302
Leptin (ng/ml)	11.7 ± 3.86	11.4 ± 3.13	11.7 ± 0.35	0.948
Fasting insulin (pmol/L)	53.1 ± 18.9	50.0 ± 15.0	59.2 ± 7.42	0.736
Fasting glucose (mmol/L)	4.56 ± 0.39	4.50 ± 0.40	4.35 ± 0.21	0.700

Table 4. Leptin receptor gene 3'UTR deletion/insertion polymorphism in relation to anthropometry, hormone and metabolic variables in overweight group.

Variables	D/D (n = 9)	D/I (n = 4)	I/I (n = 4)	p-value
Age (years)	22.4 ± 5.13	20.75 ± 1.26	20.50 ± 1.73	0.640
BMI (kg/m^2)	27.1 ± 1.63	27.90 ± 1.33	27.48 ± 1.60	0.471
Waist (cm)	80.8 ± 7.52	85.50 ± 7.14	79.25 ± 8.22	0.380
Hip (cm)	103.5 ± 8.68	107.75 ± 3.77	106.75 ± 7.32	0.576
W/H ratio	0.78 ± 0.047	0.80 ± 0.055	0.74 ± 0.075	0.341
Leptin (ng/ml)	22.7 ± 6.28	24.2 ± 7.09	26.3 ± 7.37	0.716
Fasting insulin (pmol/L)	66.7 ± 45.7	69.5 ± 45.7	46.0 ± 17.7	0.729
Fasting glucose (mmol/L)	4.55 ± 0.65	4.78 ± 0.50	4.80 ± 0.75	0.755

Table 5. Leptin receptor gene 3'UTR deletion/insertion polymorphism in relation to anthropometry, hormone and metabolic variables in obese group.

Variables	D/D (n = 23)	D/I (n = 15)	I/I (n = 7)	p-value
Age (years)	26.9 ± 6.77	25.6 ± 6.23	26.8 ± 6.67	0.815
BMI (kg/m^2)	35.6 ± 6.33	33.9 ± 4.17	40.9 ± 7.11	0.042
Waist (cm)	101.0 ± 16.0	94.7 ± 6.47	109.7 ± 16.7	0.066
Hip (cm)	121.1 ± 16.1	118.7 ± 10.5	131.5 ± 12.6	0.137
W/H ratio	0.83 ± 0.044	0.80 ± 0.045	0.83 ± 0.068	0.163
Leptin (ng/ml)	44.1 ± 19.7	40.5 ± 12.7	64.1 ± 28.7	0.31
Fasting insulin (pmol/L)	111.0 ± 34.1	90.8 ± 37.2	113.4 ± 45.1	0.214
Fasting glucose(mmol/L)	5.03 ± 0.45	4.96 ± 0.40	4.88 ± 0.54	0.723

increased in I/I homozygote but the significant high value recorded among BMI (40.9 ± 7.11 kg/m^2, p = 0.042).

4. DISCUSSION

Since 1997, several single nucleotide polymorphisms (SNPs) in coding region and a pentanucleotide (CTTTA) insertion/deletion polymorphism in the 3'-untranslated region of the human OB-R gene have been reported in different ethnic groups [21-25]. Two studies confirmed the existence of the CTTTA pentanucleotide I/D polymorphism at the 3'UTR of the OB-R gene [18,19]. Leptin gene and leptin receptorgene products, respectively, have defined a new biological pathway for the regulation of food intake and energy expenditure. Leptin acts at distinct sites and through different mechanisms within the central nervous system (CNS) to mediate energy homeostasis and feeding behavior [26,27]. It appears that leptin controls feeding not just by providing physiological satiety signal, but also by mediating "synaptic plasticity" as well as modulating the perception of reward associated with feeding [26-28].

This is the first study to suggest an association between a polymorphism of the leptin receptor gene and

obesity. The present study revealed significant differences in allele and genotype frequencies between the normal group on one hand and overweight and obese groups on the other hand ($p < 0.05$). The allele frequency of the insertion allele was higher in overweight (0.35) and obese females (32.2) when compared to the frequency in lean females (15.6). In contrast to our findings a previous study performed on Finnish individuals showed that there was no significant difference in the frequency of the insertion allele (I) between obese (0.124) and normal weight subjects (0.120) [29]. In the Finnish study, four obese, but none of the normal weight subjects were homozygous for the insertion allele [29]. In our study, 5 of the overweight and 9 obese individuals were homozygous for the insertion allele and 3 of the normal weight individuals were also homozygous. Our results show significant differences in the genotype and allele frequency of leptin receptor gene pentanucleotide polymorphism in Saudi females compared to the Finnish population. This pointed to genetic variations in the leptin receptor gene polymorphism between populations. The results of this study show that the insertion allele could be considered as a marker for obesity in Saudi females. Even though, the insertion allele also occurs at a fairly high frequency in non-obese Saudis, these results indicate that it can be used as a significant marker of obesity in Saudis, due to the fact that, it may be possible that these normal weight individuals have a higher sensitivity to develop obesity, but since they are avoiding the precipitating environmental factors, they are not obese. In addition, they may develop obesity more easily in older age if the predisposing factors are present. This confirmation requires a long-term investigation and follow-up studies on individuals with different alleles of the leptin receptor gene, which is a major problem with a study of genetic markers for non-communicable diseases. A person with a particular genotype may not have the disease at present, but may be susceptible to it or may develop it later.

The association of pentanucleotide insertion/deletion polymorphism with metabolic parameters and anthropometric measurements was earlier studied by many researchers, [29] reported better insulin sensitivity in obese subjects carrying the I allele because they had lower insulin and insulin-to-glucose ratio levels. [25] concluded that obese heterozygous women had lower insulin values at 30 minutes in the oral glucose tolerance test (OGTT). [29] found that healthy men carrying the I allele had a 79% reduced risk of type 2 diabetes. A Finnish study had shown that the carriers of the insertion allele had a 79% reduced risk of diabetes when compared to non-carriers; this is due to the lesser extent of leptin's action on the pancreatic *ß*-cells to inhibit insulin secretion. The study revealed that this 3'-UTR insertion was common in the healthy population since the carrier frequency was a high as 23.5% amongst the controls [29].

In contrast to previous reports [20] did not find a relationship between the I/D LEPR polymorphism and the con- version to type 2 diabetes. However, they found that the I allele was associated with greater reduction in weight, BMI, and waist circumference during the 3-year follow-up. Similarly, [31] reported that the association between I/D-LEPR and prevalent impaired glucose tolerance or type 2 diabetes did not reach statistical significance, and they did not find significant associations with other features of the metabolic syndrome.

Interestingly, the results of this study demonstrate that there was no association of serum leptin, glucose or insulin levels with the pentanucleotide genotype in the obese individuals. However, most variable values increased in I/I homozygote but the significant high value recorded among BMI (40.9 ± 7.11 kg/m^2, $P = 0.042$). It is unfortunate that there have been no functional studies on the effects of this 3'-UTR polymorphism. Although stem-loop sequences usually de-stabilize mRNA [30-32], it is unclear whether those in 3'-UTR of the leptin receptor gene, which would be formed by the pentanucleotide insertion allele, might stabilize or de-stabilize the mRNA. [19] hypothesized that this polymorphism might stabilize the mRNA level of the leptin receptor.

5. CONCLUSION

The results obtained from this study indicated that in the obese subjects most variable values increased in I/I homozygote but the significant high value recorded among BMI (40.9 ± 7.11 kg/m^2, $p = 0.042$). Our findings support the hypothesis that alterations in the leptin signaling system could contribute to the obesity in Saudi Females.

6. ACKNOWLEDGEMENTS

This research project was supported by a grant from the "Research Center of the Center for Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

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