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Original Research Article

Association study of leptin and leptin receptor gene polymorphisms with diabetes type 2 and obesity

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ABSTRACT

The adiposity hormone, leptin, plays an important role in the control of glucose metabolism by its action in the brain. The effects of leptin are reducing body adiposity, food intake and improving insulin sensitivity in peripheral tissue by indirect mechanism. This study was performed to investigate the prevalence and association A19G and K109R polymorphisms in leptin (LEP) and leptin receptor genes (LEPR) with diabetes and obesity in Yazd, Iran. In this case control study, the case groups were 100 obese people with type 2 diabetes mellitus and the control groups were 100 healthy people. Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The allele frequency for A and G alleles in LEP gene (A19G) were 0.275 and 0.725, respectively. Also K and R alleles in LEPR gene (K109R) were 0.36 and 0.64, respectively. The

genotype and allele frequencies were not significantly different for patient and control groups. HBA1C and leptin were high in patient group. The LEP and LEPR SNPs in this study may not be useful markers for obesity or diabetes in Iranian population but with attention to the past studies these SNPs may have synergistic effects on obesity and diabetes.

Keywords: Polymorphism, diabetes, obesity, leptin, leptin receptor

INTRODUCTION

Diabetes and obesity are two complicated disorder with genetic background that increase the mortality [1-3]. The prevalence of type 2 diabetes has increased considerably since 1960 aligned with obesity [4]. Nearly to 80 % of the people with type 2 diabetes mellitus are overweight [5]. Studies have shown people with obesity for 10 years are two times high risk than people with obesity for less than 5 years [6]. Genetic predisposition plays an important role in creating these conditions [7]. Leptin was discovered in 1994 with isolation of obesity gene. Leptin is a 16 kDa glycoprotein hormone secreted by white adipose tissue (WAT) [8] which is in proportion to body fat mass, enters the central nervous system in proportion to its plasma level and interacts with its receptor, expressed in brain areas that regulate energy consumption, autonomic function and food intake. While the effect of leptin can improve insulin sensitivity in peripheral tissues by indirect mechanisms, many observations recommend that leptin can directly affect glucose metabolism and energy balance [9]. The gene is placed on chromosome 7 (7q31) [8]. This hormone binds to its receptor (LEPR) and has a important role in regulating of metabolism [10]. Leptin receptor also is produced by a gene on human chromosome1 [11]. Leptin obtained from the adipose tissue affect the insulin sensitivity, and impress the pathogenesis of disorder related obesity by stimulating vascular inflammation that may cause pathogenesis of atherosclerosis and other cardiovascular problems of obesity. Leptin has also a link with nutritional status and energy balance.

Various polymorphisms are existence in leptin and its receptor gene including A19G and K109R. The A19G SNP (rs 2167270A>G) is located in 19t nucleotide in the untranslated region (UTR) of the LEP gene and K109R SNP (rs 1137100A>G) is located in exon 4, and changes 109 amino acid codon from AAG to AGG in LEPR gene. K109R SNP causes a conservative change in conversion lysine amino acid to arginine amino acid (Lys/K to Arg/R) [12, 13]. In this study, we selected people who have

type 2 diabetes mellitus with obesity in order to investigate the leptin and leptin receptor polymorphisms with PCR method.

MATERIALS AND METHODS

Study population

In this case and control study, samples were collected from Yazd public center in Iran. All the participants in the case group had diabetes and obesity for at least 2 years, and they were 100 person. The patients in the case group were diagnosed according to body mass index (BMI) for obesity and laboratory tests for diabetes. The questionnaires were filled based on the ages, weights (kg) and BMI (kg/m2). BMI indexes were about 30, and laboratory test results showed diabetes in the case group. Also biochemical parameters include leptin and HbA1c were measured with laboratory tests and methods. Obesity and diabetes diagnosis were proved by physicians in the hospital. This proposal was proved by ethical committee and all subjects in the case and control groups were volunteers and briefed about the use of the results and they were also asked to agree with a consent form. Table 1 is a summary of the case and control group characteristics, such as BMI, HBA1C, wight, age and leptin level.

Table 1. Demographic characteristics of case and control groups

	case group	Control group	p-value
age	52.3±6.9	49.8±10.2	0.067
weight	85.32±11.2	81.3±8.5	0.063
BMI(KG/M^2)	37.5±5	25.1±4.2	0.029
Leptin (ng/ml)	35.2±18.9	27.8±16.3	0.041
HbA1c (%)	9.2±2.7	4.5±.9	0.023

Sample collecting and genotyping

5 mL of blood sample was collected from all participants for biochemical and genetic investigations. Serum levels of HBA1c and leptin were measured using the Pishtazteb kit and the enzyme-linked immune sorbent assay kit, respectively. DNA extractions were done manually with salting out method using cell lysis buffer, nuclei lysis buffer, proteinase K, ethanol, and some salts like Sodium Dodecyl Sulfate (SDS) and NaCl. DNA concentration, quality, and purity were checked using spectrophotometric methods [14]. Afterwards, polymerase chain reaction (PCR) was done for amplification of leptin gene promoter (contains rs 2167270-A19G) and exon 4 of leptin receptor genes (contains rs 1137100-K109R). Restriction length polymorphism (RFLP) was done using

Nsp BII and HaeIII restriction enzymes (New England Biolabs) for A19G (rs 1137100) and K109R (rs 1137100) Polymorphisms, respectively. We controlled PCR and digestion product size with agarose gel electrophoresis. The primers, PCR product sizes, restriction enzymes recognition sites and digestion products for two polymorphisms are shown in Table 2.

Table 2. PCR primers and product sizes

polymorphism	Primer sequence	Tm	PCR Product size	Restriction enzyme	RFLP product size
A19G	Forward 5′-CCCGCGAGGTGCACACTG-3′ Reverse 5′-AGGAGGAAGGAGCGCGCC-3′	55 55.5	221 bp	***Nsp BII***	AA 221 AG 221 ,38,183 GG 38,183
K109R	Forward 5′-TTTCCACTGTTGCTTTCGGA -3′ Reverse 5′-AAACTGAATTTACTGTTGA-3′	55.5 56	100 bp	***HaeIII***	KK 100 KR 100,68,32 RR 68,32

Statistical analysis

Statistical Package for Social Science (version 16.0, SPSS, Chicago, Illinois, USA) and X^2 test were used for data analysis. Allelic frequencies for each SNP were estimated and the distribution of genotypes frequencies within Hardy-Weinberg equilibrium rules was determined. The normality of sample population for variables was checked with the Kolmogorov-Smirnov test and the variables that were not normally distributed excluded from the study and substituted with other variables.

RESULTS

Table 1 shows characteristics of the samples, like average age and BMI, weight, and leptin level measures in both groups. Distributions of the genotypes and frequencies of the alleles for LEP gene polymorphism, A19G and LEPR gene polymorphism, K109R in the case and control groups are represented in Table 3. For leptin gene polymorphism, A19G, the prevalence of allele A in the patient and control groups were 27.5 % and 28.5 %, respectively. The G allele frequencies were 72.5 % and 71.5 % for the patient and control groups, respectively. No significant association was found between the risk of obesity related diabetes and homozygous or

heterozygous genotypes of this polymorphism (Table 3).

Table 3. SNPs allele and genotype frequencies

SNP/Genotype/Allele	case group	Control group
LEP A19G		
AA	6	11
AG	43	35
GG	51	54
p-value=0.598		
A	55	57
G	145	143
P value=0.783		
LEPR K109R		
KK	21	18
KR	30	35
RR	49	47
P value=0.366		
K	72	71
R	128	129
P value=0.819		

The genotype distributions of LEPR gene polymorphism K109R in the case and control groups are presented in Table 3. For leptin receptor gene, the frequency of allele R in the case and control groups were 36 % and 35.5 %, respectively. K allele frequencies were 64 % and 64.5 % in the case and control group, respectively.. No significant association was found between homozygous and heterozygous genotypes of this polymorphism with risk of obesity and diabetes.

The relationship between different genotypes of LEP gene polymorphism, A19G and LEPR gene polymorphism, K109R with plasma leptin levels was investigated (Table 4). No significant combination was found between the levels of leptin hormone and the two polymorphisms. In addition, no significant combination was found between the two polymorphisms and pathologic parameters in the case and control groups (Table 4).

Table 4. Genotypes and leptin concentration Correlations

Genotypes	Number	Mean leptin concentration ± SD(ng/mL)

LEP(-2548)G/A			
AA	6	29.8±22.3	P=0.271
GA	43	31.4±20.4	
GG	51	30.2±23.2	
LEPRQ223R			
KK	21	28.5±21.9	P=0.321
KR	30	33.0±22.7	
RR	49	29.7±20.5	

DISCUSSION

Diabetes and obesity are two complicated disorder with genetic background [3]. The popularity of type2 diabetes had increased considerably since 1960, and aproximately 80 % of the people with type 2 diabetes mellitus are overweigh [15]. Leptin which is produced by adipose tissue, has an important role in obese and diabetic people [16]. Mutations in leptin and leptin receptor can affected the leptin receptor signal [17]. According to the previous studies, mutations in this gene are associated with overweight in humans. Several polymorphisms in leptin gene and its receptor have been identified, such as A19G and K109R. Due to A19G SNP, the promoter of leptin gene can affect the expression of this gene. Also the K109R polymorphism in leptin receptor gene is associated with incomplete binding of leptin to its receptor [12, 18]. The present study examined the relationship between A19G leptin gene polymorphism and K109R leptin receptor gene polymorphism in people with obese and diabe in Yazd province. In the present study, no significant correlation was found between K109R polymorphism, diabetes diseases and obesity risk. The LEPR K109R SNP (rs1137100) is an A→G substitution in codon 109 (AAG to AGG) at position 326 in exon 4 [19]. K109R SNP causes some changes in leptin receptor in conversion lysine amino acid to arginine amino acid (Lys/K to Arg/R) and these changes can affect functional consequences but its alternation in functionality is not clear. There are not reason that the polymorphisms in LEPR are related to diabetes [20]. Hancock et al reported that associations of some LEPR SNPS (which includes K109R). Environmental changes that play a key role in the metabolic phenotypes such as overweight. The researchers suggest that these SNPs such as LEPR K109R might have advantageous in the other area. Consist with them; LEPR K109R SNP is an examples to explain the correlation between genetic sensitivity to metabolic diseases and environmental factors [12].

Also no significant differences were found between A19G polymorphism and diabetes and obesity risk. This conclusion is consist with most previous studies and confirmed by several

studies including investigations on population groups [21-23]. The A19G (rs2167270) SNP is a single base substitution A→G in exon 1 of the LEP gene. Because this SNP is located within the first untranslated exon of the gene, it is not fully understood that this polymorphism how can change the protein function. Nevertheless, it is suggested that the SNP is in disequilibrium with promoter area variation and may has an effect on the gene expression. But it is not clear that how DNA sequence in the promoter area of this gene could influence promoter activity or gene function.

The serum leptin levels between the patient and control groups were different and this may be because of higher amount of adipose tissue in patient than control group. Many investigators demonstrated that leptin has a main correlation with BMI [24]. In this study, leptin levels were in correlation with BMI HBA1C is a glycosylated hemoglobin in people with diabetes and it is an important marker for control this disease [25]. We not found correlation between leptin levels and different genotype groups of A19G and K109R polymorphisms, and this result is confirmed [12].

CONCLUSIONS

The present results show that SNPs in LEP gene and LEPR gene include A19G and K109R are unlikely related to obesity and diabetes in Iranian people but our findings suggest that each of these SNPs may have synergistic effects on obesity and diabetes.

CONFLICT OF INTEREST

The authors declare no conflicts of interest

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