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Türk Hijyen ve Deneysel Biyoloji Dergisi

Association of KIR2DL4 gene polymorphisms with obesity

KIR2DL4 gen polimorfizmlerinin obezite ile ilişkisi

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ABSTRACT

Objective: Obesity is seen at many parts of the world and has become one of the main issues of public health. Obesity is the consequence of genetic, behavioral, environmental, physiological, social and cultural variables resulting in energy imbalance and encouraging unnecessary accumulation of fat. In our country, as in other countries, the incidence of obesity is increasing day by day. It has been shown that obesity contributes to the development of many risky diseases such as cancer, cardiovascular diseases, diabetes, hypertension. Another important disease group accompanying obesity is immune system diseases. Inflammation changes and immune cell functions in obese individuals play an important role in the pathophysiological effects of obesity. Obesity is a multifactorial and complex disease. Natural killer (NK) cell is cytotoxic lymphocytes, which is essential element of innate immune system. The human killer cell immunoglobulin-like receptors (KIR) which is a class of transmembrane glycoproteins expressed on NK cell as well as a subgroup of T cell. KIR2DL4 is atypical KIR which varies by other family members in terms of cellular location, signaling, specificity of ligands, as well as protein function. The aim of the study was to investigate the relationship between KIR2DL4

ÖZET

Amaç: Obezite dünyanın pek çok yerinde görülmekte olup halk sağlığının temel sorunlarından biri haline gelmiştir. Obezite, genetik, davranışsal, çevresel, fizyolojik, sosyal ve kültürel değişkenlerin sebep olduğu enerji dengesizliği ve gereksiz yağ birikimi sonucu oluşur. Ülkemizde de diğer ülkelerde olduğu gibi obezite görülme sıklığı gün geçtikçe artmaktadır. Obezitenin, kanser, kalp-damar hastalıkları, diyabet, hipertansiyon gibi birçok riskli hastalığın gelişmesini kolaylaştırdığı gösterilmiştir. Obeziteye eşlik eden bir diğer önemli hastalık grubu da immün sistem hastalıklarıdır. Obez bireylerdeki inflamasyon değişiklikleri ve immün hücre fonksiyonları, obezitenin patofizyolojik etkilerinin oluşmasında önemli bir role sahiptir. Obezite, multifaktöryel ve kompleks bir hastalıktır. Doğal öldürücü hücreler (NK) sitotoksik lenfositlerdir ve doğal bağışıklık sisteminin önemli bir parçasıdır. Doğal öldürücü hücre immunglobulin benzeri reseptörler (KIR) hem NK hücrelerinde eksprese olan transmembran glikoproteinleridir hem de T hücrelerinin alt grubudur. KIR2DL4, hücresel konum, sinyalleşme, ligandların özgüllüğü ve protein işlevi açısından diğer KIR geni aile üyelerine göre atipiktir. Çalışmada, KIR2DL4 polimorfizmleri ile obezite arasındaki ilişkiyi

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polymorphisms and obesity.

Methods: 50 obese (BMI≥30) and 50 non-obese (BMI≤30) individuals participated in the study. After collecting blood samples, DNA isolations and PCR reaction, the relevant regions of the KIR2DL4 gene were amplified and digest with the suitable enzyme. Allele and genotype frequencies were calculated by direct counting and genotype distributions in the groups were compared by chi-square analysis.

Results: No statistically significant association was found between obesity and rs649216, rs660773 polymorphisms (p>0.05). However, a significant difference was determined as a result of the comparison of genotype frequencies of KIR2DL4 rs660437- 9769 C>A polymorphism between obese and control group (p=0.004). CC genotype frequency was found to be higher in obese group (44%) than in control group (24%).

Conclusion: rs660437 C allel frequency was significantly higher in obese group, so it might be a risk factor for the development of the disease (p=0.004).

Key Words: Obesity, KIR2DL4 gene, polymorphism

araştırmak amaçlanmıştır.

Yöntem: Çalışmaya, 50 obez (BKI≥30) ve 50 obez olmayan (BKI≤30) birey katılmıştır. Kan örnekleri toplanarak, DNA izolasyonları yapılmış PCR reaksiyonu ile KIR2DL4 geninin ilgili bölgeleri çoğaltılıp uygun enzimle kesildikten sonra genotip sayımı yapılmıştır. Allel ve genotip frekansları direk sayım yöntemi ile hesaplanmış ve gruplardaki genotip dağılımları ki-kare analizi ile karşılaştırılmıştır.

Bulgular: Obezite ve rs649216 ve rs660773 polimorfizmleri arasında istatistiksel olarak anlamlı bir ilişki bulunmamıştır (p>0.05). Fakat, KIR2DL4 rs660437-9769 C>A polimorfizminin genotip sıklıklarının hasta ve kontrol grubunda karşılaştırılması sonucunda istatistiksel olarak anlamlı bir fark gözlenmiştir (p=0.004). CC genotip sıklığı obez grupta (%44) kontrol grubuna (%24) göre daha yüksek bulunmuştur.

Sonuç: rs660437 C allel frekansı obez grupta anlamlı olarak daha yüksek bulunmuştur, bu nedenle hastalığın gelişimi için bir risk faktörü olabilir (p=0.004).

Anahtar Kelimeler: Obezite, KIR2DL4 geni, polimorfizm

INTRODUCTION

Obesity is defined as a situation related with an abnormal accumulation of fat. Its prevalence is increasing day by day especially in developed countries (1). Obesity is a multi-factorial disease such as affected from genetic, behavioral, environmental, physiological factors (2). Many diseases accompainied with obesity like type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, non-alcoholic fatty liver disease, reproductive dysfunction, respiratory abnormalities, psychiatric conditions, and it even increase the risk for certain types of cancer. Most of these diseases progressively related with body mass index (BMI) (3). Obesity is detected by kind of ways like BMI, body surface area (BSA), waist circumference (WC), waist-to-hip ratio (WHR), and waist to height ratio. BMI is below 18.5 defines the underweight. BMI ranges from 18.5 to<25 means normal weight and from 25.0 to<30 is accepted as overweight. When BMI is 30.0 or higher, this indicates presence of obesity. Obesity is often classified into 3 groups; class 1 (BMI 30 to<35), class 2 (BMI 35- 40) and class 3 (BMI 40 or higher). Obesity in Class 3 is also called as “extreme” or “severe” obesity (4).

Obesity is a multifactorial disease which has a complicated pathogenesis. Researches reported that

there is relationship between obesity and genes. Genetically there are two kinds of obesity; syndromic and nonsyndromic. Prader-Willi, Cohen, fragile X syndrome are forms of syndromic obesity and POMC, PC1, leptin, leptin gene receptor, NPY gene mutations are examples of nonsyndromic obesity (1). Energy imbalance in obesity induces matabolic defects and finally tissue stress occurs (5). Studies about obesity involving diabetes or insulin resistance exhibited that there is a strong correlation between innate immune system and consumption of too much food. One of the most characteristics of obesity is inflammation. Many researches showed that inflammation is the result of obesity and linked with energy homeostasis, damaged insulin secretion (6). The human major histocompatibility complex (MHC), also called human leukocyte antigen (HLA), distinguish the non-self molecules of immune system. HLA genes are located on choromosome 6 in three main groups; HLA class I, HLA class II, and HLA class III (7).

Natural killer (NK) cells are cytotoxic lymphocytes essential for innate immunity. NK cell activation is defined by the interplay for both inhibitory and activating signals transmitted from a range of cell surface receptors that include the immunoglobulin-like killer cell family (KIR) in humans (8).

The human killer cell immunoglobulin-like receptors (KIR) belong to immunoglobulin family. KIR genes are located in chromosome 19q13.4 in human genome, expressed on the surface of Natural Killer Cells (NK) and some subset of T cells (9). NK cell activation is adjusted by KIR signals which could be inhbitory or activating (10). There are 17 genes described, 6 of them activating, 9 of them inhibitory and 2 of them are pseudogenes interacting with HLA (9). KIR genes are polymorphic and researches have indicated that KIR genes and HLA association may lead to autoimmune diseases, cancer and infection (10).

KIR2DL4 is atypical KIR which varies from other family members in terms of cellular location, signaling, specificity of ligands, as well as protein function. The structure of KIR2DL4 is unique; domain D1 is unavailable but alternatively it still has a domain structure D0-D2. KIR2DL4 have inhibitory as well as signaling domains in a unique way (11). Many polymorphism studies related to obesity were carried out before, but there is no report for the study on the association of KIR2DL4 gene polymorphism with obesity in the literature. The aim of this study was to analyze rs660773 G>A (intron 7), rs660437 C>A (intron 7) and rs649216 C>T polymorphisms in KIR2DL4 gene in obese individuals and normal weight control group and consequently to search for an association of these polymorphisms with obesity.

MATERIAL and METHOD

This study was approved by the Gaziantep University Clinic Research Ethics Committee (Date:28.09.2020, Number: 176). Informed consent was obtained from all individual participants included in the study.

The present study included 50 adult obese patients (BMI≥30) who were recruited at the Department of General Surgery, Faculty of Medicine, SANKO University during the period September 2018 to September 2019.

The control group consist of sex-matched, unrelated 50 adult subjects with normal BMI≤30 who came to the clinic for non-obesity reasons and had no diagnosis related to obesity or associated disorders.

Peripheral blood samples from the obese and control groups were collected in EDTA tubes. Specimens were kept at +4°C until DNA isolation. Whole genomic DNA was isolated from 200 µl of the fresh blood samples by Invitrogen by Thermo Fisher Scientific Pure Link Genomic DNA mini kit. The purity and concentration of DNA samples were measured by Nanodrop spectrophotometer. DNA concentrations of obese group were ranged between 31.6 and 267 ng/µl, and the average concentration was 77.60 ng/µl. The DNA concentrations of control group were ranged between 27.56 and 399.6 ng/µl and the average

concentration was 71.46 ng/µl.

The polymorphic region of the KIR2DL4 gene were amplified by PCR using specific primer sequences and 297 bp PCR product was obtained. A total of 50 µl reaction mixture was prepared and the amplification was carried out in the following conditions: 94°C for 5 min, 35 cycles 94°C for 40 seconds, 55°C for 40 seconds, 72°C for 40 seconds and final extension 72°C for 5 min. PCR products were electrophoresed on a 2% agarose gel containing ethidium bromide for visualization and then were digested with specific restriction enzymes (EarI, BspMI, HpyCH4III) according to the manifacturer guidelines. For the analysis of rs649216-9571 C>T polymorphism, digestion was performed at 37°C for 5 hours and the products were separated by agarose gel (3%) electrophoresis at 100V for 30 minutes. The PCR samples were digested at 37°C for 16 hours and restriction products were separated by agarose gel (4%) electrophoresis at 80V for 75 minutes for the rs660437-9769 C>A polymorphism. rs660773-9797 G>A polimorphism was analysed after digestion of samples at 65°C for 3 hours and separation of fragments by agarose gel (3%) electrophoresis at 90V for 60 minutes. Restriction enzymes used in this study and digestion products are given in Table 1.

Allele and genotype frequencies were calculated by direct counting. Genotype frequencies of polymorphisms in obese group and control group were compared by chi-square (x^2) analysis. "SPSS 22.0 for Windows" program was used for statistical analysis.

Table 1. Restriction enzymes and digestion products

Polymorphism	Restriction Enzyme	Digestion products (bp)
rs649216-9571 C>T	EarI	TT 297 CT 297/253/44 CC 253/44
rs660437-9769 C>A (intron7)	BspMI	CC 297/150/147 CA 297/147/103/47 AA 147/103/47
rs660773-9797 G>A (intron7)	HpyCH4III	AA 297 GA 297/286/11 GG 286/11

RESULTS

The female/male ratio in both obese group and control group was 1:1. Twenty-five men and 25 women were included in each group. The ages of obese group mean age was 41.64 ± 10.62. The age in control group mean age was 42.14 ± 11.27. The demographaic datas are given at Table 2. The genotype frequencies of KIR2DL4 gene polymorphisms in obese and control group are given Table 3.

KIR2DL4 rs649216 – 9571 C>T polymorphism were compared between obese group and control group and no significant difference was found (p>0.05).

KIR2DL4 rs660437- 9769 C>A polymorphism were compared between obese group and control group, a significant difference was found (p=0.004). CC genotype frequency was found to be higher in obese group (44%) than in control group (24%). AA genotype was seen in 74% of the control group, however the frequency of this genotype in obese group was 40%. CA genotype frequency was significantly higher in obese group (16%) than in control group (2%) (p=0.004) (Table 3).

Table 2. Comparison of demographic factors between obese and control groups

Variables	Obese group	Control group
Age (years)	41.64 ± 10.62	42.14 ± 11.27
Weight (kg)	114.3 ± 18.68	60.46 ± 8.94
Height (m^2)	2.86 ± 0.25	2.77 ± 0.26
BMI (kg/m^2)	40.02 ± 6.6	21.7 ± 2.04

Table 3. Genotypes frequencies of KIR2DL4 gene polymorphisms in obese and control group

Genotypes	Obese (n=50) n (%)	Control (n=50) n (%)	x^2	*p*
rs649216- 9571 C>T				
TT	14 (28)	17 (34)	4.35	0.114
CT	19 (38)	25 (50)		
CC	17 (34)	8 (16)		
rs660437- 9769 C>A				
CC	22 (44)	12 (24)	**10.87**	**0.004**
CA	8 (16)	1 (2)		
AA	20 (40)	37 (74)		
rs660773 - 9797 G>A				
AA	28 (56)	18 (36)	5.01	0.082
GA	10 (20)	19 (38)		
GG	12 (24)	13 (26)		

DISCUSSION

Obesity is one of the most important ilness all over the world. The major reasons of obesity are known as energy imbalance, age, sex, poorly physical activity. Obesity lead to many diseases such as diabetes mellitus, cancers, cardiac disorders and hypertension (12-15). Researches demonsrated that genetic and other factors (nutrition, physical activity, educational level etc.) may affect obesity. Up to now many studies have been performed but it is not enough to understand the relationship between obesity and genes. Leptin gene, leptin receptors, uncoupling proteins (UCP), Fat Mass and Obesity-associated (FTO) are the most important genes which have a connection with obesity.

In a FTO gene polymorphism study, it has been reported that rs9939609e "AA" genotype is related with greater values of BMI (16). Relationship between obesity and FTO gene is verified by single-nucleotide polymorphisms (SNPs) such as rs9939609, rs17817449, rs3751812, rs1421085 (17). A research showed that FTO gene rs9939609 polymorphism could increase

the strenght of insulin resistance and obesity (18). The Uncoupling Protein 1 (UCP1) rs1800592 and rs3811791 promoter polymorphisms were found to be associated with obesity (19). IL-18 has an effect in immune response, inflammation, autoimmune and infectious diseases, and also in obesity by enhancing adipogenesis (20). In a study about IL18RAP gene, it was demonstrated that there is a relationship between high BMIs and rs7559479 AG-GG genotypes (21).

Interleukin (IL)-6 have inflammatory and anti-inflammatory effects. In a meta-analysis research in the IL-6 promoter region, -174G>C polymorphism was associated with a significantly increased risk of obesity (22).

Leptin receptor gene is crucial for obesity. In a research, three polymorphisms (K109R, Q223R, and K656N) have been investigated in obese group. There was no significant difference in allele frequencies or genotype distributions for the K109R and K656N polymorphisms, however Q223R polymorphism was reported to be an important predictor of 5% of the variation in the structure of the body (23). Another important gene for obesity is Insulin-induced gene 2 (INSIG2) in which regulates adipogenesis and lipid storage. significant association has ben found between rs12464355 polymorphism INSIG2 gene and LDL in children (24).

KIR2DL4 is a member of the Killer Cell Ig-like receptor (KIR) family and the gene is positioned on chromosome 19p13.4. KIR2DL4 has a high degree of conservation; it has a low value of polymorphism among other KIR genes. This might be a possible reason of limited number of studies on KIR2DL4 polymorphisms.

In a KIR2DL4 gene polymorphism research with asthma, it has been reported that KIR2DL4 9A/10A polymorphism has no significant relation with asthma (25).

Protein expression study with KIR 2D (L1, L3, L4, S4) and KIR 3DL1 in non-small cell lung cancer (NSCLC). High expression of KIR2D (L1, L3, L4, S4) and KIR3DL1 have been reported in non-small cell pulmonary cancer (NSCLC) tumor cells and tumor infiltrating lymphocyte (TIL). Besides, positive expression of KIR2D (L1, L3, L4, S4) and KIR3DL1 on tumor cells or TILs in NSCLC patients was linked with weak prognosis (26).

In an SNP study for KIR2DL4 gene in pre-eclampsia and normal pregnancy condition, no significant difference in genotype distributions and allele frequencies in obese group and controls was found. But, expression analysis resulted that KIR2DL4 mRNA level was significantly lower in placenta tissues with pre-eclampsia than those with normal pregnancy (27).

KIR2DL4 gene polymorphisms were studied in spontaneous miscarriage. KIR2DL4 9A/10A genotype were higher in male partners of miscariage group than the control group, but no association was found between KIR2DL4 polymorphism and spontanous abortion susceptibility in female (28).

A meta-analysis study about association between KIR polymorphisms and T1DM reported that KIR2DL1 and 2DS1 polymorphism might be a potential protective factor for T1DM in the specific ethnicity (29).

The goal of this study was to analyze rs660773 G>A (intron 7), rs660437 C>A (intron 7) and rs649216 C>T polymorphisms in KIR2DL4 gene in obese and normal weight control group and consequently to search for an association of these polymorphisms with obesity. There was no significant difference between obese and control group for rs660773 and rs649216 ($p>0.05$), however there was significant difference for rs660437 ($p=0.004$). rs660437 C allel frequency was significantly higher in obese group, so it might be a risk factor for the development of the disease. While rs660773 and rs649216 polymorphisms seem to have no effect on the development of the disorder.

This is the first study about the role of KIR2DL4 gene on obesity in literature. The major drawback for this study is the small size of the study groups. When the post-hoc power analysis was performed, the power =0.80 was obtained, the effect size (OR) =0.31, and alpha =0.05. Further comprehensive researches with increased number of samples are needed for better understanding the relationship between immune system and obesity.

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ETHICS COMITTEE APPROVAL

* This study was approved by the Gaziantep University Clinic Research Ethics Committee (Date:28.09.2020 and No:176).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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