J Surg Med. 2022;6(4):436-439.
DOI: 10.28982/josam.930690

Research article

Journal of Surgery and Medicine
e-ISSN=2602-2079

Investigation of the association between HLA-G polymorphisms and obesity

Alaa Mohammad [1], Deniz Mıhçıoğlu [2], Filiz Özbaş Gerçeker [1, 3], Başar Aksoy [4]

[1] Department of Biochemistry Science and Technology, Graduate School Of Natural & Applied Sciences, Gaziantep University, Gaziantep, Turkey
[2] Department of Nutrition and Dietetics, Faculty of Health Science, SANKO University, Gaziantep, Turkey
[3] Department of Biology, Faculty of Art and Science, Gaziantep University, Gaziantep, Turkey
Department of Biochemistry Science and Technology, Graduate School of Natural & Applied Sciences, Gaziantep University, Gaziantep, Turkey
[4] Department of General Surgery, School of Medicine, Gaziantep, Turkey

ORCID ID of the author(s)
AM: 0000-0001-6139-0178
DM: 0000-0003-2355-8026
FÖG: 0000-0003-1243-5880
BA: 0000-0002-8879-433X

Corresponding Author
Deniz Mıhçıoğlu
Gazimuhtar Paşa Bulvarı No:36 - 27090 Şehitkamil / Gaziantep, Turkey
E-mail: denizmihcioglu@hotmail.com

Ethics Committee Approval
Ethics approval for the study was obtained from Gaziantep University Ethics Committee, 01.08.2018, 2018/176.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
This study was supported by Scientific Research Projects Governing Unit of Gaziantep University (2018 - 2019 FEF.YLT.18.21)

Previous Presentation
The study is presented in "1st International Congress On Sustainable Agriculture and Technology", Gaziantep, Turkey (1-3 April 2019 P-012)

Published
2022 April 4

Abstract

Background/Aim: Obesity is a global public health problem seen worldwide, with an increasing prevalence over time. Obesity is a multifactorial disease affected by both genetic and environmental factors. It is accompanied by many other diseases, the most important of which are immune system disorders. Induction of suppression of HLA-G molecule T and B lymphocytes is associated with natural killer cells and antigen-presenting cells. The HLA-G gene shows functional polymorphisms in regulatory regions. The HLA-G gene is slightly more conserved compared to other HLA genes, both in the same population and among different populations. The aim of this research is to determine the association of HLA-G gene polymorphisms (14 bp insertion/deletion 3 'UTR (rs66554220), rs41557518, and rs1063320) with obesity.
Methods: Fifty normal (BMI ≤ 30) individuals having no obesity-related chronic disorder, and 50 obese (BMI ≥ 30) individuals were included in the study. After DNA isolation, PCR and PCR-RFLP methods were used for genotyping.
Results: The genotype frequencies of HLA-G polymorphisms (rs66554220, rs41557518, and rs1063320) in the normal and obese groups were compared, and as a result, no significant difference was found ($P > 0.05$).
Conclusion: No significant association was found between rs66554220, rs41557518, and rs1063320 polymorphisms and obesity.

Keywords: Obesity, HLA-G, Polymorphism

How to cite: Mohammad A, Mıhçıoğlu D, Gerçeker FÖ, Aksoy B. Investigation of the association between HLA-G polymorphisms and obesity. J Surg Med. 2022;6(4):436-439.

Introduction

Obesity presents significant risks to society's health, and its incidence is rising rapidly. The complicated impacts of this disease, including interactions between genetic and environmental susceptibility, are difficult to be treated and prevented [1]. Obesity is the result of chronic excess in energy intake compared to expenditure, resulting in extravagant quantities of triglycerides being stored in adipose tissue [2]. The surplus energy is collected and stored in the fat cells that will be enlarged (hyperplasia) or multiply (hypertrophy), which is obesity's pathological lesion. Enlarged fat cells cause clinical issues due to obesity, either due to the weight or mass of the excess fat or due to increased free fatty acid and peptide secretion from enlarged fat cells. In turn, obesity causes other diseases, like diabetes mellitus (DM), heart diseases, gallbladder disorder, some cancers, and osteoarthritis [3].

Body mass index (BMI) is the most frequently used measure of obesity; it is defined as the weight of an individual in kilograms (kg) divided by the height of the individual in meters squared (kg/m^2) [4]. The World Health Organization (WHO) categorizes an obese individual with a BMI $\geq$ 30 kg/m^2, and individuals having BMI $\geq$ 40 kg/m^2 are defined as extremely obese. The etiology of obesity is multifactorial; there is a complicated interaction between genetics, hormones, and the environment [5]. The genetics of obesity result from structural changes, deletions, or mutations influencing genes that encode proteins engaged in the regulation of appetite and metabolism, and are transferred under X-linked models or autosomal Mendelian traits [6]. Twin studies have shown that genetic inheritance leads to 40–75% of situations of obesity [7].

Obesity is categorized as syndromic, monogenic, or polygenic obesity. Syndromic obesity is seen together with dysmorphic characteristics, hyperphagia, other symptoms of hypothalamic impairment, cognitive delay, and organ-specific defects [8]. Pleiotropic syndromes occur when a single gene affects two or more unconnected phenotypic characteristics; for example, Bardet-Biedl syndrome and chromosomal rearrangements that involve obesity usually involve Prader-Willi, WAGR, and Sim-1 (single-minded gene) syndromes [9]. Significant genes such as the leptin gene (LEP), leptin receptor gene (LEPR), pro-opiomelanocortin gene (POMC), and melanocortin 4 receptor gene (MC4R) are associated with obesity [10]. Obesity is described as an excessive adiposity, with several associated comorbidities, particularly immunity dysfunction. Changes in the function of immune cells and inflammation in obesity play an important part in almost all obesity pathophysiological impacts [11]. Modified circulating concentrations of inflammatory cytokines, for example, tumor necrosis factor alpha (TNF-α), interlukine-6 (IL-6), and C-reactive protein (CRP), were described in both overweight and obesity in adults [12, 13].

The gene region encoding the tissue antigens of the immune system has been defined as the main tissue compatibility component, major histocompatibility complex (MHC), and also named as "human leukocyte antigen" (HLA) genes. HLA genes are members of the HLA class Ib antigens, and are located on chromosome 6p21.3 [14]. HLA-G is expressed in fewer tissues and is less polymorphic than the other molecules in class I. A few researchers have shown that in preeclampsia and recurrent spontaneous abortion cases, decreased mRNA and protein levels were seen compared to normal placentas [15, 16]. HLA-G may have different effects, either helpful in inflammatory and autoimmune diseases, or risky in some cases such as tumors and infectious diseases [17]. HLA-G genes have polymorphic locations, which present at 5' UTR (upstream regulatory region) and 3' UTR (untranslated region) compared to the protein coding region. Polymorphisms show distinct effects in these regions. Gene transcriptions are influenced in 5' UTR while mRNA processing and stability are influenced in the 3' UTR [18]. The HLA-G polymorphic 3'UTR has an essential function in arranging the expression of the HLA-G gene [19]. The 14bp ins/del (5′ ATTTGTTCATGCCT-3′) polymorphism contains a deletion (del) or insertion (ins) of 14 base pairs in the +2960 site in exon 8 [20]. The ins allele is linked with an alternative splicing where 92 bp is deleted, shifting the stability of mRNA and decreasing the concentrations of HLA-G [21]. The 14 bp ins/del (5′ ATTTGTTCATGCCT-3′) polymorphism and soluble concentrations of HLA-G are associated with diverse diseases involving autoimmune disorders, recurrent abortions, certain cancers, as well as inflammatory diseases, along with coronary heart disease (CHD) [22–24].

Obesity is a disease characterized by hormonal and metabolic differences and is associated with more than one genetic, environmental, and metabolic factor. Obesity is accompanied by many diseases, the most important of which are immune system disorders. The aim of this research is to determine the association between HLA-G gene polymorphism and obesity by analyzing the 14 bp insertion/deletion 3' UTR rs66554220, rs41557518, and rs1063320 polymorphisms in obese and healthy subjects.

Materials and methods

This study, started in September 2018 and completed in September 2019, was confirmed by The Ethics Committee of Gaziantep University on 01.08.2018 with ethical approval number 2018/176, and the study was carried out in accordance with the Declaration of Helsinki. When the post-hoc power analysis was performed, the power = 0.73 was obtained, the effect size (OR) = 0.34, and alpha = 0.05. Fifty obese individuals (BMI $\geq$30 kg/m^2) and 50 normal (BMI $\leq$30 kg/m^2) individuals participated in the study. The samples were collected in the Department of General Surgery, Faculty of Medicine, SANKO University.

DNA Isolation

Blood samples (4 ml) were collected in ethylene diamine tetra acetic acid (EDTA) tubes and stored at -20°C in a refrigerator until DNA isolation. The Thermo Fisher Scientific Pure Link Genomic DNA mini kit was used for isolating whole genomic DNA.

Amplification and Genotyping of the HLA-G Gene

The ingredients for the rs66554220, HLA-G 14 bp ins/del polymorphism in the 3'UTR, 1597 ΔC, rs41557518 the cytosine deletion at codon 130 in exon 3, tagging the HLA-G *01:05N null allele, and the rs1063320 (+3142GΔC) polymorphisms of the HLA-G gene were roughly the same (2–

2.5 µl of Buffer, 2 µl of dNTP, 0.3 µl of forward, and 0.3 µl reverse primer) and a total volume of 25 µl. Reaction conditions were 94°C for 5 min, 35 cycles at 94°C for 30 s, 55–58°C for 30 s, 72°C for 30 s, and final extension at 72°C for 5 min.

The rs66554220 polymorphism is genotyped directly after polymerase chain reaction (PCR). The rs41557518 and rs1063320 polymorphisms are genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method; The PPUMI and BaeGI enzymes were used to digest the PCR products. The sample fragments were run in agarose gel electrophoresis, and genotyping was done according to the band sizes. The sizes of the digestion products (bp) and genotypes for rs41557518 and rs1063320 polymorphisms are given in Table 1.

Table 1: Restriction products and genotypes of HLA-G gene polymorphisms

Polymorphisms	rs66554220		rs41557518		rs1063320	
Restriction Products (bp)	del/del	210 bp	HLA-G *01:05N (+/+)	504 bp	CC	406 bp
	ıns/ıns	224 bp	HLA-G *01:05N (-/-)	389 bp, 115 bp	GG	316 bp, 90 bp
	del/ıns	210/224 bp	HLA-G *01:05N (+/-)	504 bp /389 bp / 115 bp	CG	406 bp /316 bp / 90 bp

Statistical analysis

The allele and genotype frequencies were determined by direct counting. The genotype frequencies of HLA-G polymorphisms in the normal and obese groups were compared by chi-square (χ^2) test, and statistical significance was accepted at the $P<0.05$ level. The SPSS statistical software package version 22.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses.

Results

The mean age of obese patients was 41.64 (10.62) years. The obese group consisted of 25 (50%) male and 25 (50%) female patients with a mean weight of 114.568 (18.86) kg, mean height of 2.86 (0.258) m^2, and mean BMI of 40.1 (6.66). The mean age of the control group was 42.14 (11.27) years. Similar to the obese group, the control group also consisted of 25 (50%) males and 25 (50%) females. The mean weight and height of the control group were 60.46 (8.94) kg and 2.775 (0.26) m, respectively. The mean BMI for the control group was calculated as 21.7 (2.04). As expected, there is a significant difference in the mean weight of the obese group [114.568 (18.86) kg] and that of the control group [60.46 (8.94) kg]. After comparison of the genotype frequencies for rs66554220, rs1063320, and rs41557518 polymorphisms in the groups, no significant difference was found ($P > 0.05$) (Table 2).

Table 2: Genotype distribution for HLA-G gene polymorphisms

rs66554220	Control (n=50) n (%)	Obese (n=50) n (%)	χ^2 (*P*-value)
del/del	16 (32)	15 (30)	1.02 (0.60)
del/ins	26 (52)	23 (46)	
ins/ins	8 (16)	12 (24)	
rs1063320			
CC	7 (14)	7 (14)	1.19 (0.55)
CG	27 (54)	22 (44)	
GG	16 (32)	21 (42)	
rs41557518			
HLA-G*01:05N (+\+)	3 (6)	2 (4)	1.08 (0.58)
HLA-G*01:05N (+\-)	27 (54)	23 (46)	
HLA-G*01:05N (-\-)	20 (40)	25 (50)	

Discussion

Obesity is a globally important issue worldwide, being related to so many chronic diseases such as hypertension, cardiovascular diseases, and type 2 diabetes [25]. In general, obesity depends on the accumulation of fat, insufficient physical activity, defects in the endocrine system and homeostasis, and unconscious food consumption. Another important reason for improving obesity is the genetic background [26]. There are numerous research studies in the literature investigating the relationship between genetic factors and obesity. Obesity is identified as low-level systemic inflammation, especially in some tissues containing adipose tissue and the liver. Extreme fat accumulation in the body may cause changes in the functions and numbers of some cells of the immune system like mast cells, neutrophils, and T and B lymphocytes [27]. The HLA-G gene is a member of a non-classical class I, located in the human major histocompatibility complex, which plays a role in modulating the immune system [28]. Due to the relationship between obesity and the immune system, the present study aimed to investigate for the first time the association of the HLA-G gene polymorphisms (rs66554220, rs41557518, and rs1063320) with obesity.

Study groups consisted of obese and control individuals. PCR/PCR-RFLP methods were used to analyze three HLA-G polymorphisms. Statistically, no significant relation was seen between the analyzed HLA-G gene polymorphisms and obesity. The 14-bp insertion/deletion polymorphism (rs66554220) containing the 3' UTR region is crucial because of the gene expression regulation, RNA stability, and alternative splicing of HLA-G [29].

HLA-G may negatively affect the regulation of the human immune response. The results of a meta-analysis study about the relationship between many cancers and rs66554220 have shown that 14-bp ins/del polymorphism may have a role in cancer risk [30]. Rheumatoid arthritis (RA) is an autoimmune disease. HLA-G 14 bp ins/del and +3142G > C polymorphisms were detected in RA patients in an Iranian population. It is concluded that there was no statistically significant difference between 14 bp ins/del and RA; however, there were significant associations between the +3142G > C variant and a predisposition to RA [31].

Marzuillo et al. [32] studied HLA-G 14 bp in obese children and adolescents. They have reported an association between the ins/ins genotype and the homeostasis model assessment. Many case-control studies have shown inconsistent results that some of the ins alleles were significantly associated with decreased or increased risk, and additionally, some have shown no significant association [33].

Chen et al. [34] studied the relationship between HLA-G rs16375 and rs41557518 polymorphisms and esophageal cancer patients in the Kazakh and Han nationalities in Xinjiang. It is indicated that there was an increased risk of EC in patients with the HLA-G (rs16375) 14bp del genotype (−14bp/−14bp) and the HLA-G rs41557518 0105N genotype (C/C) compared with HLA-G 14bp del genotype (+14bp/+14bp) and the 0105N genotype (C/C) in the Kazakh, but in the Han, there was not.

The HLA-G gene exhibits characteristic properties. HLA class I genes present a large amount of exonic polymorphic

sites in spite of the HLA-G gene having restricted polymorphic sites and casual dispersion across the exons and introns [35].

The HLA-G gene is 4170 bp in size and consists of 8 exons. Four isoforms, HLA-G1 to -G4, are membrane attached, and three isoforms, HLA-G5 to -G7, are soluble. The HLA-G gene isoforms are moderated by alternative splicing of HLA-G mRNA [36, 37].

Limitations

Obesity is a complex disease having many causes and effects. HLA-G still may act in the development of obesity through different pathways. It should be considered that there may be differences in genotype distributions between populations.

Therefore, the association of obesity and HLA-G gene is controversial and needs to be examined by further studies with large sample sizes and in different ethnicities.

Conclusion

There is no study in the literature about the effects of the HLA-G gene on obesity. The present study aimed to determine the association between obesity and the HLA-G gene polymorphisms (14 bp insertion/deletion 3 'UTR (rs66554220), rs41557518, and rs1063320). We found no statistical association between obesity and rs66554220, rs41557518, and rs1063320 polymorphisms. Obesity is a multifactorial disease related to so many factors such as genetics, diet, and environment, among others, in addition to ethnic differences that should also be considered.

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