AUGUST 2019 M.Sc. in Biochemistry Science and Technology ALAA AMER MOHAMMAD

REPUBLIC OF TURKEY

GAZİANTEP UNIVERSITY

GRADUATE SCHOOL OF NATURAL & APPLIED SCIENCES

ASSOCIATION OF HLA-G GENE POLYMORPHISMS WITH OBESITY

M.Sc. THESIS

IN

BIOCHEMISTRY SCIENCE AND TECHNOLOGY

BY

ALAA AMER MOHAMMAD

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Supervisor

Prof. Dr. Filiz ÖZBAŞ GERÇEKER

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Asst. Prof. Dr. Deniz MIHÇIOĞLU

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Name of the Thesis : Association of HLA-G Gene Polymorphisms with Obesity

Name of the Student : Alaa AMER MOHAMMAD

Exam Date : 28.08.2019

Approval of the Graduate School of Natural and Applied Sciences

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This is to certify that we have read this thesis and that in our consensus/majority opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Alaa AMER MOHAMMAD

ABSTRACT

ASSOCIATION OF HLA-G GENE POLYMORPHISMS WITH OBESITY

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M.Sc. in Biochemistry Science and Technology
Supervisor: Prof. Dr. Filiz ÖZBAŞ GERÇEKER
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August 2019
69 Pages

Obesity is a disease that is defined by hormonal and metabolic differences and is associated with genetic, environmental and metabolic factors. Inflammation changes and immune cell functions in obese individuals have an important role in the pathophysiological effects of obesity. The human leukocyte antigen G (HLA-G) belongs to the class I antigen of non-classical HLA. HLA-G gene is found on chromosome 6 (6p21.31). HLA-G molecule is associated with natural killer cells and antigen presenting cells and induction of the suppression of T and B lymphocytes. This study aims to clarify the association of HLA-G gene polymorphisms with obesity. The study subjects were obtained from 50 normal (BMI<30) individuals and 50 obese (BMI>30) patients. In this study, three HLA-G gene polymorphisms HLA-G 14 bp insertion/ deletion polymorphism in the 3′ UTR (rs66554220), rs41557518 and rs1063320 using the PCR agarose gel electrophoresis technique were studied. The statistical analysis for HLA-G (rs66554220), HLA-G (rs41557518), and HLA-G (rs1063320) polymorphisms showed that there is no significant association with obesity ($p>0.05$).

Key Words: Obesity, HLA-G, PCR, Polymorphism

ÖZET

HLA-G GEN POLİMORFİZMLERİNİN OBEZİTE İLE İLİŞKİSİ

AMER MOHAMMAD, Alaa

Yüksek Lisans Tezi, Biyokimya Bilimi ve Teknolojisi

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Ağustos 2019

69 sayfa

Obezite, hormonal ve metabolik farklılıklar ile tanımlanan, genetik, çevresel ve metabolik olarak birden fazla faktör ile ilişkilendirilmiş bir hastalıktır. Obez bireylerdeki inflamasyon değişiklikleri ve immün hücre fonksiyonları obezitenin patofizyolojik etkilerinin oluşmasında önemli bir role sahiptir. İnsan lökosit antijeni G (HLA-G), klasik olmayan HLA sınıf I antijen grubunda yer almaktadır. HLA-G geni, 6. kromozomun kısa kolunda bulunur (6p21.31). HLA-G molekülü T ve B lenfositlerin baskılanmasının indüklenmesi ve doğal öldürücü hücreler ve antijen sunan hücreler ile ilişkilidir. Bu çalışmanın amacı HLA-G gen polimorfizmi ile obezite arasındaki ilişkinin belirlenmesidir. Çalışmaya 50 normal (BKI<30) ve 50 obez (BKI>30) birey dahil edilmiştir. Çalışmada HLA-G geni le ilgili üç polimorfizm; 14 bç insersiyon/ delesyon 3' UTR (rs66554220), rs41557518 ve rs1063320 PCR-agaroz jel elektroforez metodu ile analiz edilmiştir. Çalışmanın sonucunda, polimorfizmler ile obezite arasında istatistiksel olarak anlamlı bir ilişki bulunmamıştır.

Anahtar Kelimeler: Obezite, HLA-G, PCR, Polimorfizm

"Dedicated to my family"

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor, **Prof. Dr. Filiz ÖZBAŞ GERÇEKER** for her guidance, support, continuous encouragements and inspiration throughout this research.

I am deeply grateful to my co-supervisor, **Asst. Prof. Dr. Deniz MIHÇIOĞLU**.

I also owe my special thanks to the head of the department **Prof. Dr. Canan CAN** for her support and suggestions.

I would like to thank to my friends **Huda SHERZAD AHMAD** for her support and help, and **Ceren YILDIZ**, **Nazlı BOZMAN** for their standing and supporting me in every time.

I dedicate this thesis to my **Mother**, **Father** and all my family. Without their caring & support it would not have been possible. They taught me to trust in Allah and gave me the strength throughout this program.

This study was supported by **the Scientific Research Projects Department of Gaziantep University**.

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α	Alpha
χ^2	Chi Square
Δ	Delta
∞	Infinity
µl	Microliter

LIST OF ABBREVIATIONS

AICR	American Institute for Cancer Research
APC	Antigen Presenting Cell
BDNF	Brain Derived Neurotrophic Factor Gene
BMI	Body Mass Index
bp	Base Pairs
BPH	Benign Prostatic Hyperplasia
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DEL	Deletion
DNA	Deoxyribonucleic Acid
FFM	Fat-Free Mass
FTO	Fat Mass and Obesity-Associated Protein
GWAS	Genome-Wide Association Studies
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HLA-G	Human Leukocyte Antigen-G
HNSCC	Head and Neck Squamous Cell Carcinoma
IL-10	Interleukin-10
IL-17	Interleukin-17
IL-6	Interleukin-6
ILT2	Immunoglobulin-Like Transcript 2
INS	Insertion
KIR2DL4	Killer Cell Immunoglobulin-Like Receptor 2DL4
LEP	Leptin
LEPR	Leptin Receptor

MC4R	Melanocortin 4 Receptor
MHC	Major Histocompatibility Complex
mRNA	Messenger Ribonucleic Acid
NCD-RisC	NCD Risk Factor Collaboration
NK	Natural Killer
OA	Osteoarthritis
PCR	Polymerase Chain Reaction
POMC	Pro-Opiomelanocortin
PWS	Prader-Willi Syndrome
RBD4	Retinol Binding Protein 4
RCC	Renal Cell Carcinoma
RFLP	Restriction Fragment Length Polymorphism
SDB	Sleep-Disordered Breathing
sHLA-G	Soluble Human Leukocyte Antigen-G
SNP	Single Nucleotide Polymorphism
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TH17	T-helper 17
TNF-α	Tumor Necrosis Factor Alpha
UTR	Untranslated Region
UV	Ultra Violet
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist-to-Hip Ratio

CHAPTER 1

INTRODUCTION

1.1 Obesity

Obesity presents significant risks to society's health, and its incidence is rising fast. The complicated impacts of this disease including interactions between genetic and environmental susceptibility are difficult to be treated and prevented (Karpe and Lindgren, 2016). Obesity is the result of chronic excess in energy intake compared to expenditure, resulting in extravagant quantities of triglycerides being stored in adipose tissue (O'Rahilly, 2009). The surplus energy is collected and stored in the cells of fat, that will be enlarged (hyperplasia) or multiply (hypertrophy) which is obesity's pathologic 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 (George and Bray, 2003).

The etiology of obesity is multifactorial; there is a complicated interaction between genetic, hormone and environment (Kaila and Raman, 2008). The latest increase in obesity has been influenced by environmental factors including low physical activity and excessive eating and it is estimated that the genetic factor represents for 40-90% of the BMI population variation (Hjelmborg et al., 2008; Wardle et al., 2008).

The distribution and the amount of fat in the body either around the waist (abdominal, android or central obesity) or peripherally around the body (gynoid obesity) have significant health consequences and central distribution of fat in the body is connected with greater morbidity and mortality risk than the more peripheral distribution (Kissebah and Krakower, 1994).

Body mass index (BMI) and also waist-to-hip ratio (WHR) are most common techniques of obesity measurement (Chan et al., 2003). Body mass index (BMI) is used as the most frequently measure of obesity; it is defined as the weight of an individual in kilograms (kg) divided by the height of an individual in meters (m^2) (kg/m^2) (Xia and Grant, 2013). Overweight and obesity was categorized by the WHO in adults based on numerous cutoffs of BMI (WHO, 2000).

Table 1.1 BMI classification (WHO, 2000)

Classification	**BMI (kg/m^2)**	**Risk of comorbidities**
Underweight	<18.5	Low (other health risk)
Healthy weight	18.5-24.9	Average
Overweight	25-29.9	Increased
Obesity class I	30-34.9	Moderate
Obesity class II	35-39.9	Severe
Obesity class III	>40	Very severe

World Health Organization (WHO), categorizes an obese individual with a BMI ≥30 kg/m^2, and individuals having BMI ≥40 kg/m^2 are defined as extremely obese (WHO, 1998). Having been regarded an issue mostly in high-income countries, already overweight and obesity have been increasing dramatically in low- and medium-income nations, especially in urban environments (NG et al., 2014). Whereas the increase in BMI in high-income countries appears to have reduced, the rate is still high. In several low- and medium-income nations, the increase in obesity rate between children and adolescents proceeds to be extremely alarming (NCD-RisC, 2016; NCD-RisC, 2017).

In addition, although less frequently used WHR is a more accurate metric of body fat (abdominal fat) distribution (Brown, 2009). It can be used to classify types of body into two primary classifications: pear and apple. Apple-shaped type of body is more prevalent among males due to abdominal obesity. Women generally accumulate fat around the hip and thighs to create a pear-shaped type of body (Ashwell, 2009). Apple-shaped fat distribution is regarded more hazardous than pear-shaped fat

distribution due to the accumulation of fat around the visceral organs in the deep abdominal region.

This hidden fat may lead to metabolic disorder like diabetes type Il and increases cardiovascular risk as well. If the women's waist circumference is > 80 cm and for men is > 94 cm, insulin resistance as well as arterial hypertension may develop. Amplified hip circumference is connected with enhanced hip subcutaneous fat, total leg muscle mass and gluteal muscle (Thoma et al., 2012; Marcin et al., 2014).

Table 1.2 WHR classification (WHO, 2008)

Health risk	**Women**	**Men**
Low	0.80 or lower	0.95 or lower
Moderate	0.81–0.85	0.96–1.0
High	0.86 or higher	1.0 or higher

According to the World Health Organization (WHO), 65% of the population of the world lives in nations in which comparing to underweight, more individuals die from obesity and excess weight (Rukh, 2016). Obesity is a result of 2.6 million worldwide deaths as well 2.3% of the worldwide burden of disorder (Van Gaal et al., 2006). In 2014 World Health Organization (WHO) evaluated over 1,9 billion overweight adults globally (39%) and (13%) 600 million obese adults (WHO, 2015). Over the previous 40 years, the incidence of obesity has almost tripled globally. In 2016, 13% of adults worldwide were obese and, in several countries, up to 40% were obese (WHO, 2016; The GBD 2015 Obesity Collaborators, 2017).

1.2 Diseases Associated with Obesity

Co-morbidities related to obesity include sleep disturbance, mobility issues, respiratory difficulties, cardiovascular diseases, dyslipidemia, hypertension, type 2 diabetes mellitus, certain kinds of cancer and psychological distress (Switzer et al., 2013).

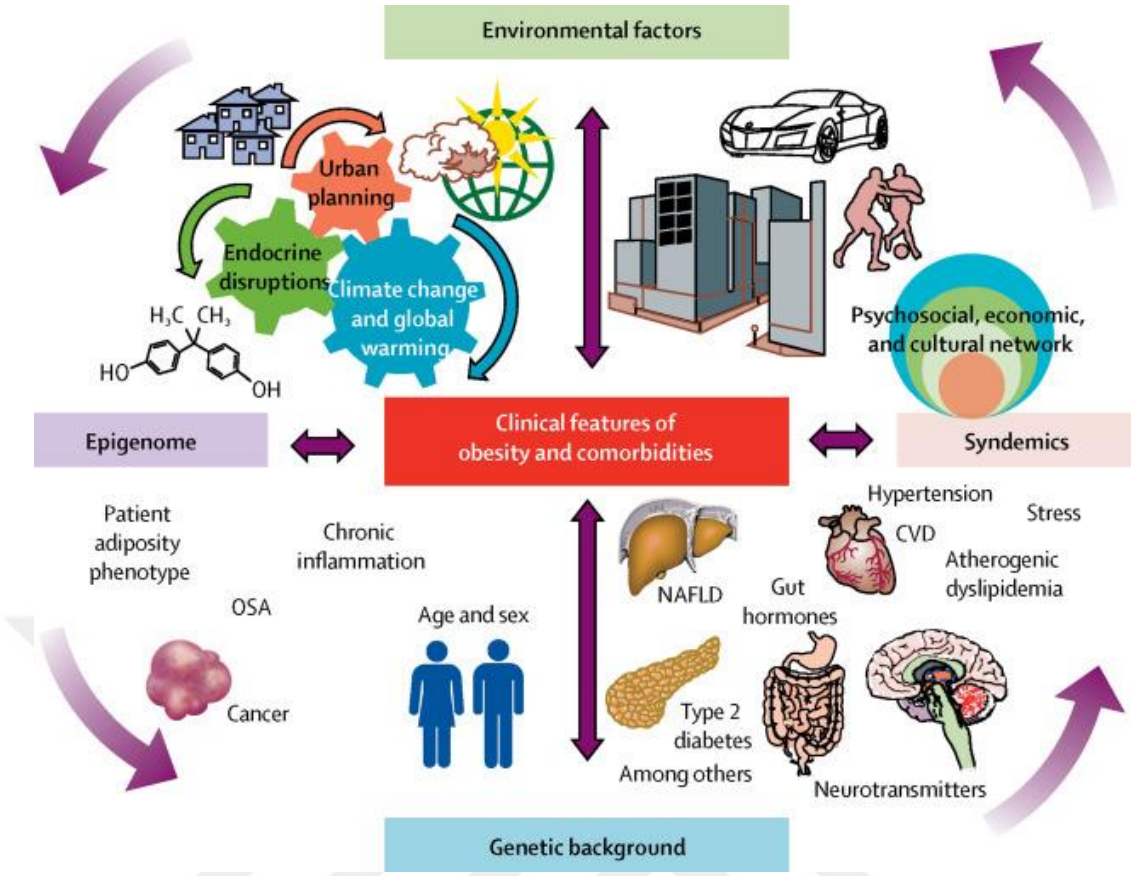

Figure 1.1 Etiology and diseases related to obesity (Frühbeck et al., 2018)

The risk of developing type 2 diabetes in obesity is determined by the site of accumulating fat and also by degree of obesity (Eckel et al., 2011). Amplified upper body fat along with visceral adiposity, which is reflected in enhanced abdominal girth or the ratio of waist-to-hip, is connected with metabolic syndromes such as type 2 diabetes, and cardiovascular disease (Björntorp, 1991). In particular, central obesity that is an accumulation of abdominal fat, as specified by an elevated the waist: hip ratio, is considered to be an independent cause for type 2 diabetes regardless of the severity of obesity (Montague and O'Rahilly S, 2000).

According to WHO, obesity as well as overweight account for 44% of incidents of diabetes, about 7-41% of some cancers and also 23% of patients with ischemic heart disorder (WHO, 2012).

Additionally, morbidity and mortality of cardiovascular disease (CVD) are said to be increased in people who are overweight, particularly with central adipose tissue deposition (van Gaal et al., 2006). The independent risk factor for CVD including

coronary heart disease, stroke, myocardial infarction, congestive heart failure, angina pectoris, fibrillation of atrial and high blood pressure is obesity (Klein et al., 2004).

Most of the obesity-related comorbidities causes increasingly elevated incidence of hypertension in the obese population (Guh et al.,2009). High blood pressure incidence in obese adults is 32.2% in females and 38.4% in males, in comparison to BMI of lower than 25 kg / m^2 the incidence of high blood pressure was 16.5% in females and 18.2% in males (Hirose et al., 1998).

Obesity is generally associated with a particular dyslipidemia profile which involves increased level of low-density lipoprotein (LDL), increased levels of triglycerides and reduced concentrations of particles of high-density lipoprotein (HDL) (Musunuru, 2010). The most important causative factor to obesity-related dyslipidemia is probably unregulated fatty acid that is released from adipose tissue, particularly visceral adipose tissue, via lipolysis, that promotes production of very-low-density lipoprotein and enhanced transfer of fatty acids to the liver (Jung and Choi, 2014).

Obesity also has a major role in reproductive diseases, especially in females. It is correlated with infertility, menstrual disorders, anovulation, reproductive difficulties, adverse outcome of pregnancy and miscarriage (Dağ and Dilbaz, 2015). Due to the increased peripheral aromatization of androgens to estrogens, the secretion of gonadotropin is impacted in obese females. In obese females, insulin resistance as well as hyperinsulinemia result in hyperandrogenemia. The growth hormone, insulin like growth factor binding, sex hormone binding globulin proteins are reduced and leptin concentrations are elevated. Consequently, the hypothalamic-pituitary-gonadal (HPG) axis that is a neuro-regulation worsens (Parihar, 2003). These changes may account for damaged ovulatory function as well as reproductive health (Dağ and Dilbaz, 2015). In many researches, the incidence of infertility in obese females is found to be three times greater than in non-obese females (Rich-Edwards et al., 1994).

A significant association was reported between BMI and cancer (colon, gastric cardia, gallbladder, liver, kidney, pancreas, and esophageal adenocarcinoma)

predisposition (Gregg and Shaw, 2017). Calle et al. (2003) suggested that the risk of cancer mortality related to obesity is 14.2% for men while it is 19.8% for women.

Furthermore, obesity is closely associated with an enhanced risk of knee osteoarthritis, but there was only a mild association with hip osteoarthritis (Reijman et al., 2007). Osteoarthritis has a significant impact on patient movement, loss of productivity, disability and early life patients may become disabled from osteoarthritis (Lievense et al., 2002).

The rise in the incidence and severity of nonalcoholic fatty liver disease was associated with increasing patterns in obesity (Li et al., 2016). Nonalcoholic fatty liver disease is accepted as an important cause of chronic liver disorders in the rapidly industrializing environment, with an estimated worldwide incidence of 25-30% globally, increasing to 90% in patients who are morbidly obese (Fazel et al., 2016).

Respiratory problems also happen in obese individuals owing to enhanced fat in the abdomen and the wall of chest, that also reduces lung volume, alter breathing patterns and reduces respiratory compliance (Pi-Sunyer and Xanvier, 1993). Gaining weight and increasing BMI are combined with lung volume reductions indicated by the more restrictive spirometric ventilation pattern. Cross-sectional and longitudinal research have shown that an increase in BMI depresses the forced expiratory volume in 1 second, functional residual capacity, forced vital capacity and the expiratory reserve volume. Also, there is modest in residual volume and total lung capacity in morbid obesity. At these extreme rates of obesity (BMI > 40 kg / m^2), functional residual capacity approaches residual volume (Wannamethee et al. ,2005 b).

Obstructive sleep apnea, sleep-related breathing disease (Peppard et al., 2013), is defined by the pharyngeal airway being periodically narrowed and obstructed during sleep. Obesity is regarded a significant risk for obstructive sleep apnea growth and progress. The incidence of obstructive sleep apnea reported to be increased 2-fold in obese or severely obese individuals compared to the normal-weight adults (Peppard et al., 2000). Obesity may worsen obstructive sleep apnea at particular locations due to fat deposition. Fat deposition in the tissues around the upper airways tends to

result in less lumen and enhanced collapse of the upper airways, affecting apnea (Shelton et al.,1993).

1.3 Genetics and Obesity

Genetics of obesity are the result of structural changes, deletions or mutations influencing genes that encode proteins engaged in regulation of appetite and metabolism and are transferred under X-linked models or Mendelian autosomal (Pigeyre et al., 2016). Twin studies have shown that the genetic inheritance leads to 40–75% of situations of obesity (Wardle et al., 2008). Monozygotic twins demonstrate a more similar gaining of body fat compared to dizygotic twins, indicating that the genotype has a major impact on an altered energy equilibrium (Hainer et al., 2001). Adoption studies also support the genetic element of obesity, since adopted children's BMI is associated to their biological parents BMI more than the adopted parents BMI (Stunkard, 1986). Depending on the hypothesis that the prevalent single nucleotide polymorphism (SNP) could describe a significant percentage of heritability in the population (Lee, 2011). Recent heritage studies have shown that 27-30% of total variance of BMI in adults and children could be attributed to prevalent SNPs (Llewellyn, 2013). If the genetic factors having role in the development of obesity are well defined, biology of energy balance would be better understood and molecules and pathways which could be targeted for therapeutic action could be identified (Fawcett and Barroso, 2010).

Obesity is categorized as syndromic obesity, monogenic and polygenic obesity.

1.3.1 Syndromic Obesity

This type of obesity seen together with dysmorphic characteristics, hyperphagia, other symptoms of hypothalamic impairment, cognitive delay and defects of organ-specific (Farooqi and O'Rahilly, 2005). Obesity is noted in 30 Mendelian diseases as a clinical characteristic (Qi and Cho, 2008). Pleiotropic syndromes occur when a single gene affects two or more unconnected phenotypic characteristics for examples Alström, Albright's hereditary osteodystrophy, Bardet-Biedl, Forssman and Lehmann, Borjeson, Cohen, Mehmo, Fragile X, Simpson-Golabi-Behmel, Wilson–Turner, Ulnar-mammary syndromes and chromosomal rearrangements that involves obesity usually involve Prader-Willi, WAGR, and Sim-1 (single-minded gene)

syndromes (Farooqi and O'Rahilly, 2006). The syndrome of Prader-Willi is the most prevalent syndromic cause of childhood and adult obesity. The Prader Willi syndrome is triggered by a lack of paternal gene expression on chromosome 15q11-q13. Either by loss of entire paternal chromosome 15 and with appearance of two maternal homologues in 22% of patients (uniparental maternal disomy) or by deletion of the critical paternal segment (75%). PWS is categorized by obesity, hyperphagia, hypotonia, hypogonadotropic hypogonadism, short stature and mental retardation (Shawky and Sadik, 2012).

1.3.2 Monogenic Obesity

Monogenic obesity refers to the presence of mutations in a single gene. They are frequently autosomal loss-of function mutations having a dominant or codominant effect resulting in haploinsufficiency. A mutated copy of the gene is adequate to develop the phenotype, heterozygous persons are obese, and in homozygous persons this condition is more severe (Muñoz et al., 2017). Throughout the leptinergic-melanocortinergic mechanism, most of the genes that are responsible of monogenic obesity are engaged. Significant genes involve leptin gene (LEP), leptin receptor gene (LEPR), pro-opiomelanocortin gene (POMC), melanocortin 4 receptor gene (MC4R) and brain derived neurotrophic factor gene (BDNF). Genetic variations in MC4R lead towards the most prevalent monogenic type of obesity, responsible for 2 to 3% of obesity situations in childhood and adulthood, while rare LEP gene and LEPR gene mutations show the highest impact sizes (Levian et al., 2014).

Congenital deficiency of leptin is a rare autosomal recessive disease (Pigeyre and Meyre, 2018). The hormone leptin is excreted via adipose tissue which is composed of 167 amino acids. Leptin works on the brain's hypothalamic areas, which regulate eating habits, and also plays a crucial role in regulating body weight by preventing food intake and enhancing energy consumption (Wasim et al., 2016). Leptin replacement therapy has had a beneficial effect in avoiding weight gain and obesity; recombinant human leptin (metreleptin) therapy has effectively normalized eating habits and caused weight loss. (Wabitsch et al., 2015).

Another autosomal recessive disease is leptin receptor deficiency, resulting in mutations deactivating LEPR (Pigeyre and Meyre, 2018). Patients with functional

deficiencies in LEPR have phenotypic resemblance to LEP-deficient patients; with serious hyperphagia and endocrine defects, they have fast weight gain during first few months of life (Le, 2013). These patients cannot advantage from recombinant leptin treatment because the receptor does not respond to its ligand; in these cases, serum leptin levels are high (Dubern and Clement, 2012).

Deficiency of POMC is a rare autosomal recessive syndrome (Pigeyre and Meyre, 2018). The pituitary gland, hypothalamus, and many peripheral tissues, along with the skin are expressing POMC gene (Muñoz et al., 2017). POMC deficiency may cause increased fetal mortality and is involved in early-onset obesity, adrenal insufficiency, neonatal adrenal crisis, and hypoglycemic seizures in the neonatal period (Mendiratta et al., 2011).

Hypothalamic neurons are expressing MCR4 and is crucial for appetite and energy expenditure regulation; its dysfunction in humans leads to impaired satiety, hyperphagia and obesity. Therefore, homozygous mutations are related with severe obesity (Hainerová and Lebl, 2013).

Mutation in MC4R once were regarded an autosomal dominant type of obesity, but not all MC4R that are heterozygous carriers are becoming obese, although homozygous mutants appear completely penetrating early-onset obesity (Pigeyre et al., 2016).

Homozygous MC4R mutations are exceptionally rare (Dubern et al., 2007). This mutation's homozygous carriers are hyperphagic and display rapid weight gain during the first few months of life (Dubern et al.,2007). Patients lacking MC4R display increased linear growth, hyperinsulinemia, and an increase in bone mass in adults and children (Farooqi et al., 2000; Garg et al., 2014). Furthermore, patients experience a rise in fat and also lean mass, which is not found in several types of monogenic obesity (Farooqi et al., 2003).

1.3.3 Polygenic Obesity

Defect in multiple gene induce polygenic obesity with small impacts that cooperate with the environment (Cummings and Schwartz, 2003). Some characteristics may occur in various genes owing to simultaneous incidence of DNA variation. Any

group of alleles at separate gene loci which controls inheritance of the quantitative phenotype or changes expression of such a qualitative trait are called ' polygenic ' variants. (Hinney et al., 2010).

In polygenic obesity, numerous genes have been analyzed by different methods; the identification of polymorphisms in candidate genes, Genome Wide Association Studies (GWAS), exome analysis, case-control and cohort investigations (Muñoz et al., 2017).

The GWAS strategy is a high-throughput approach that enables geneticists to monitor a large collection of the single nucleotide polymorphisms (SNP) producers (0.1–5 million SNPs) around the entire human genome in such an unbiased way, that use powerful statistical techniques to explore the associations between both the illness phenotype as well as representing all common variations in the population (Xia and Grant, 2013).

For the association with a particular phenotype, further than 2,000,000 gene variants could presently be evaluated in GWAS. This technique was highly effective for different phenotypes (Frayling, 2007). Stringent p-value limit of $p \leq 5 \times 10{-}08$ was founded as a gold standard shortly after the first GWAS were released. An approximate amount of 1,000,000 tested SNPs has been presumed for GWAS, then this limit leads in a Bonferroni correction (Dudbridge and Gusnanto, 2008).

The first gene correlated with polygenic obesity by GWAS was FTO, which is connected with obesity-related fat tissue, confirmed in different age groups and populations of different origin (Loos and Yeo, 2014). FTO was among the genes identified in GWAS for type 2 diabetes mellitus (T2DM). It was discovered by BMI adjustment that the association with T2DM was due to the greater BMI of diabetic patients compared to non-diabetic individuals (Frayling et al., 2007). The FTO gene in human is found on chromosome 16 (16q12.2) and its mRNA is expressed primarily in the hypothalamus, which regulate energy balance (Zhang et al., 2018). Changes in FTO gene was recognized in the very first GWAS for the early onset of obesity, conducted for Germen people in 487 highly obese adolescents and children and 442 lean individuals (case-control analysis) (Hinney et al., 2007).

1.4 Immune System and Obesity

Obesity is described as an excessive adiposity, with several comorbidities connected with obesity, particularly the dysfunction of immunity. Changes in the function of immune cell and inflammation in obesity play an important part in almost all obesity pathophysiological impacts (Odegaard and Chawla, 2008).

Modified circulating concentrations of inflammatory cytokines for example tumor necrosis factor alpha (TNF-α), interlukine-6 (IL-6) or/and C-reactive protein (CRP) were described in both overweight and obesity in adults (Festa et al., 2001; Park et al., 2005). This incident has been associated to the development of insulin resistance, greater cardiovascular risk and metabolic diseases linked to obesity (Hotamisligil, 2006). In particular, body fat measurements correspond positively with inflammatory protein serum concentrations and it is interesting to note that abdominal obesity measurements like waist circumference (WC) appear to be closer to inflammatory markers than BMI or full body fat, indicating a greater impact of central obesity on inflammation (Festa et al., 2001; Park et al., 2005). Obesity, like many situations of malnutrition, seems to negatively impact the immune function (de Heredia et al., 2012).

Recent researches have shown that the function of immune cells in obese individuals has changed in comparison to healthy weight individuals (Milner and Beck, 2012). Nieman et al. (1999) recorded significant discrepancies in the leucocyte amount as well as count of subsets and oxidative burst and phagocytic activity of the monocyte among lean and obese people.

Furthermore, mononuclear circulating cells in the obese people display pro-inflammatory in comparison to people who are healthy-weight (Ghanim et al., 2004). Impaired proliferation of lymphocytes to polyclonal stimulation was also recorded (Nieman et al., 1999). An important potential result of obesity is T2D which is correlated with damaged immune cell behavior (Geerlings and Hoepelman, 1999). People that have genetic mutation that prevent leptin hormone from being synthesized properly are becoming morbidly obese and exhibit weak defense of immunity (Farooqi et al., 2002). Strangely, obesity has been used to improve thymic growth and decrease the variety of T-cell repertoires, Consequently, immune

surveillance might be impacted (Yang et al., 2009). Studies have actually associated obesity with excessive infection risk (Falagas and Kompoti, 2006).

High concentration of inflammatory mediators like interlukin-6 as well as inflammation markers like high-sensitivity C-reactive protein (hsCRP) are associated with individuals having obesity (Rexrode et al., 2003). The concentration of the TNF-α gene and also expression of protein in adipose tissue of human as well correlates with BMI and reduces after losing of dietary weight (Kern et al.,1995). IL-6, which is excreted by macrophages and also by adipocytes like TNF-α, damages the lipoprotein lipase and contributes to the disordered storage function of fat in adipose tissue (Trujillo et al., 2004). The immune system consists of 2 distinct arms—the innate immune and adaptive immune systems (Andersen et al., 2016).

Adipose tissue of lean people has different immune cells that are anti-inflammatory. These immune cells assist to preserve insulin sensitivity and store additional energy throughout the form of triglyceride. The amount of pro-inflammatory immune cells in adipose tissue of obese individual is highly increased. The simultaneously decreased amount of anti-inflammatory immune cells accelerates the dysfunction of the adipose tissue and the response of pro-inflammatory (Choe et al., 2016).

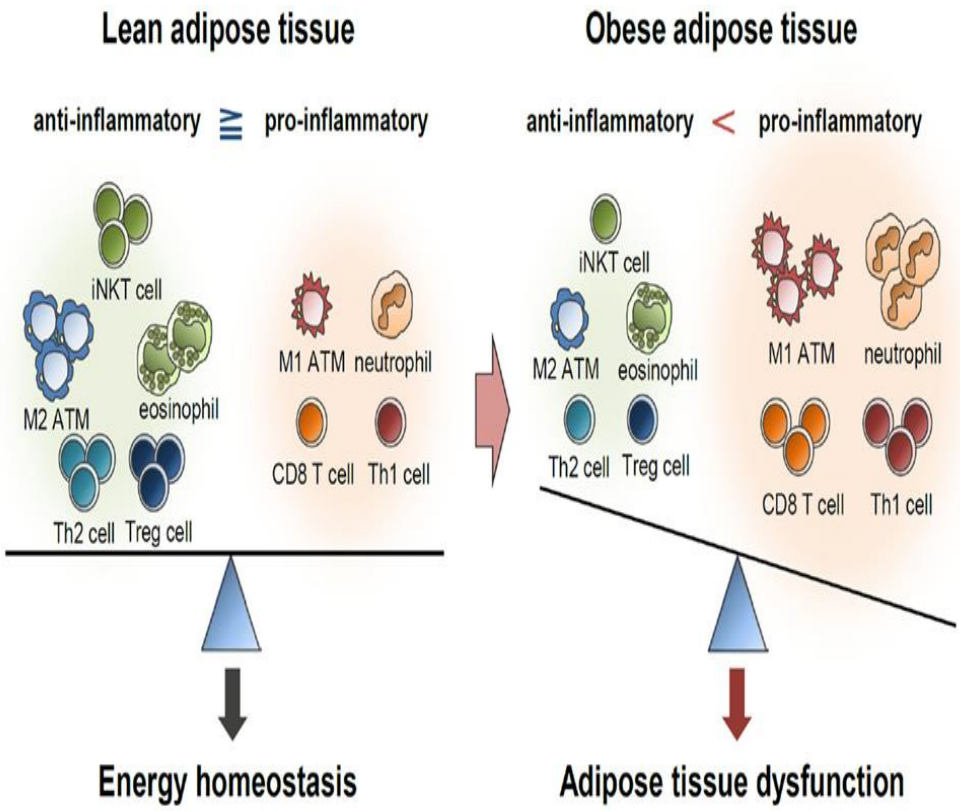

Figure 1.2 Immune response in adipose tissue in lean and obese people (Choe et al., 2016).

Much of the studies that link inflammation with metabolic illness concentrated onto the role of response of innate immunity (McLaughlin et al., 2017). Macrophages are important mediators of adipose tissue inflammation which are the most widely available immune cells that infiltrate adipose tissue in obese people (Ferrante, 2007). There is an increase in inflammatory bio-markers like CRP and neutrophilia in children who are obese and at age of 3 years (Skinner et al., 2010). This suggests that throughout childhood, several factors of obesity-induced inflammation may effectively be started. Chronic overnutrition creates pathological development of adipose tissue in which hypertrophic adipocytes do not store the excess energy effectively, leading to dysfunction of adipose tissue, dyslipidemia and insulin resistance (Mark et al., 2014).

Recently, several studies have recognized the function of apoptosis inhibitor macrophage (AIM) throughout the pathogenesis of autoimmune disorders related to obesity (Arai and Miyazaki, 2013). The T-helper 17 cells (Th17) are a newly found

type of T lymphocytes of CD4 effector. Th17 cells secrete interlukin-17 (IL-17) and have now been acknowledged in autoimmune disease pathogenesis (Noack and Miossec, 2014). It has recently been found that obesity can lead to Th17 cell induction, partially through an IL-6-dependent mechanism that exacerbates autoinflammatory disorders for example colitis and also multiple sclerosis in many mouse models (Winer et al., 2009). Obesity is now known as a small-grade systemic disease with high concentrations of inflammatory molecules including TNF-α, IL-6, as well as C-reactive protein (CRP) (Yudkin et al., 1999; Fantuzzi, 2005).

Inflammation of adipocyte outcomes from the interaction of immune cells and adipocytes. In obese people, adipocytes excrete less anti-inflammatory adiponectin and enhance expression of macrophage chemoattractant (MCP-1 and NAMPT), pro-inflammatory factors (TNFα, IL-6, RBP4, ANGPTL2), as well as leptin that cause responses to pro-inflammatory immune cell (Fernandez-Riejos et al., 2010; Ouchi et al., 2011). By activating and also binding of leptins receptor (LEPR-B) in the brain, leptin will improve energy expenditure whereas reduce consumption of food (Myers et al., 2010). It was reported that the concentration of circulating leptin (Considine et al., 1996) as well as leptin mRNA expression in adipose tissue (Kouidhi et al., 2010) are elevated in obesity, probably due to leptin resistance (Friedman and Halaas, 1998). In people who are obese and have insulin resistance, the expression of TNF-α is also enhanced and is favorably linked with insulin resistance (Hotamisligil et al., 1993).

1.5 HLA-G Gene

HLAs have been categorized as significant histocompatibility complexes (MHCs). Due to their significant position in allowing immune system to identify "self" versus "non-self" antigens (Hudson and Allen, 2016). The classical loci of HLA composed of class Ia (HLA-A, HLA-B, HLA-C) that is engaged in presentation of antigen to CD8+ T cells, and class Ib (HLA-E, HLA-F, HLA-G, and HLA-H) that is involved in presentation of natural killer cells (NK cells) , as well as class II (HLA-DR, HLA-DQ, HLA-DM, HLA-DP) that is engaged in display of $CD4^+$ T cells (Allard et al., 2014; Leddon and Sant, 2010). HLAs encoded on chromosome 6p21.3 in human, that is the most variable region in human genome (Shiina et al., 2009)

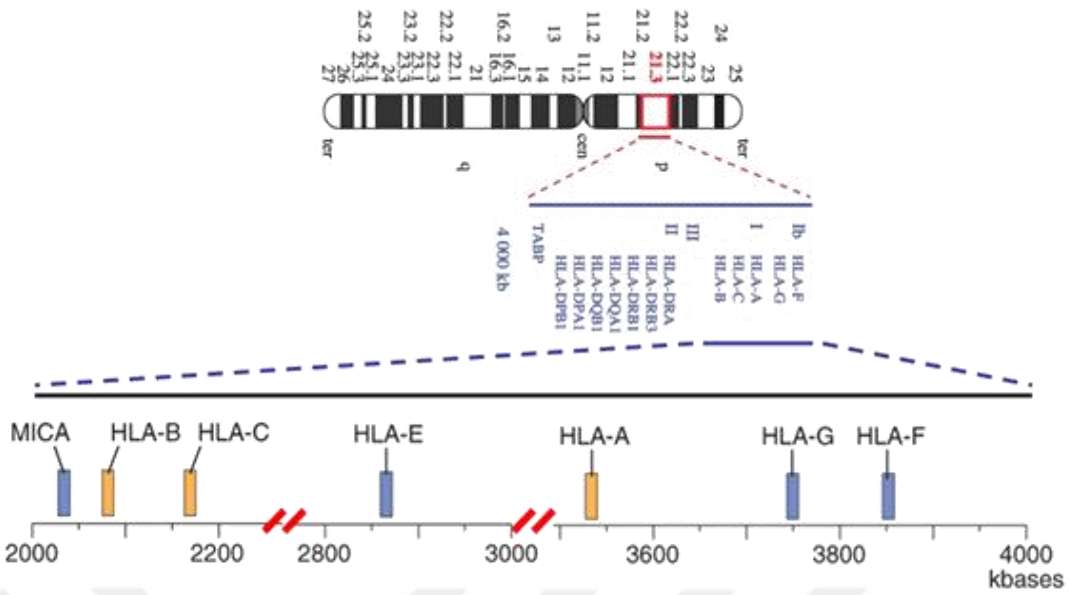

Figure 1.3 The site of HLA-G gene on chromosome (Mizuki et al., 1997).

The human leukocyte antigen G (HLA-G) is a part of the class I antigen of non-classical HLA (Verloes et al., 2017). As they are different from classical HLA- A, HLA-B and HLA-C molecules for a decreased polymorphism and a limited tissue distribution (Baricordi et al., 2008). HLA-G gene is found on chromosome 6 (6p21.31) (Hashemi et al., 2017).

HLA-G has a strong chain that is not covalently connected to β2-microglobulin. HLA-G gene also shows similarity to HLA loci, displaying 8 exons as well as 7 introns, that codes heavy chain particle and sits at chromosome 6, while chromosome 15 represents β2-microglobulin (Carosella et al., 2008). HLA-G is distinctive among Class I genes as it produces four encoding membrane-bound proteins (HLAG1-G4) and three soluble proteins (HLA-G5-G7) through alternative splicing (Hashemi et al., 2017).

HLA-G shows 7 protein isoforms (Figure 1) generated by alternative splicing of the primary transcript, four of which being membrane-bound (HLA-G1, G2, G3 and G4) and three soluble isoforms (G5, G6 and G7). HLA-G1 is the complete isoform showing a structure similar to the membrane-bound classical HLA molecule, associated with β2-microglobulin (Ishitani and Geraghty, 1992; Paul et al., 2000). Furthermore, the isoform of soluble HLA-G1 is made by the membrane HLA-G1

proteolytic cleavage (Menier et al., 2010). The HLA-G2 isoform does not have an α2 domain that is encoded by exon 3. And HLA-G3 encoded by exons three and four doesn't show α2 domain as well as α3 domain, although HLA-G4 that is encoded by exon four has no α3 domain. Soluble HLA-G5 isoforms demonstrate similar extra globular domains of the HLA-G1 as well as soluble HLA-G6 isoforms demonstrate similar extra globular domains HLA-G2, produced by intron 4 conserving transcripts blocking transmembrane domain (exon 5) translation. Intron 4 is translated from the 5′ region once a stop codon is generated, giving the isoform HLA-G5 and HLA-G6 a tail of 21 amino acids that are involved in their solubility. The isoform HLA-G7 only has the α1 domain connected to two amino acids that are encoded by intron 2, that is maintained in the corresponding transcript. There is no exon 7 for all alternate transcripts (Ishitani and Geraghty, 1992; Paul et al., 2000).

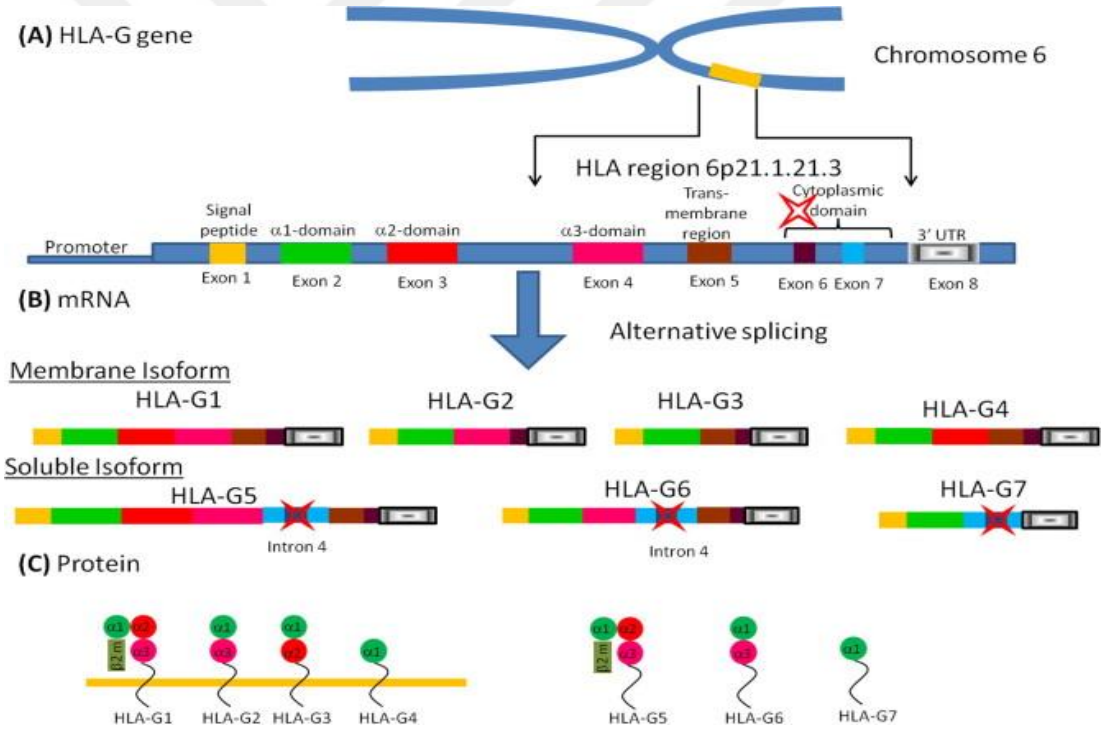

Figure 1.4 The isoforms of HLA-G gene (Catamo et al.,2014)

HLA-G1 and HLA-G5 are the primary isoforms of healthy tissue, including erythroid, thymus, trophoblast, cornea as well as endothelial precursors (Menier et al., 2008). Functionally, HLA-G is a full-power inhibitor of function of the cells engaged in graft rejection, which include NK cells, antigen-presenting cells (APC),

and CD 4^+ and CD 8^+ T lymphocytes (Carosella et al., 2000). For many illnesses including cancer, HLA-G is recognized as an immunomodulatory molecule (Yie and Hu, 2011). HLA-G functions to inhibit the cytolytic response of T and NK cells through binding to its receptors which is an inhibitory surface (ILT2 & KIR2DL4). Large HLA-G expression was detected in ovarian, breast, kidney, stomach, pulmonary and colorectal cancers (Paul et al., 2000). Expression of HLA-G could be stimulated in pathological circumstances like autoimmune, inflammatory as well as viral infectious illnesses, transplantation and cancers (Carosella et al., 2008). HLA-G inhibit the activity of cytotoxic of uterine and peripheral blood NK cells as well as CD8+ T cells, the alloproliferative response of CD4+ T cell, inhibit dendritic cell maturation as well as activating regulatory T cells. The HLA-G molecule's immunosuppressive activity is mediated primarily by its interaction with immune cell inhibitory receptors: ILT-2 receptor on B cells, APC and few T, also NK cells, ILT-4 receptor on APC and KIR2DL4 expressed by NK and few T cells (González et al., 2012).

Multiple investigations have looked at the HLA-G gene's polymorphic nature. Many polymorphic locations have been available at $5'$ UTR (upstream regulatory region) and $3'$ UTR (untranslated region) compared to protein coding region. The polymorphism in the $5'$ UTR influences the HLA-G gene transcription whereas mRNA processing and stability are influenced in the $3'$ UTR (Donadi et al., 2011). The gene HLA-G is identified by limited polymorphism, including 53 HLA-G alleles, 18 HLA-G proteins and 2 null alleles, that is recognized so far (Durmanova et al., 2017).

The HLA-G polymorphic 3'UTR plays a crucial part in regulating expression of HLA-G gene (Poomarimuthu et al., 2017). The polymorphism 14bp ins/del (5′ ATTTGTTCATGCCT-3′) involves in a deletion (del) or insertion (ins) of 14 base pairs in the +2960 site in exon 8 (García-González et al., 2014). It controls the expression of HLA-G by affecting the splicing model and stabilization of mRNA allowing many soluble and membrane isoforms to be produced less (García-González et al., 2014; Rousseau et al., 2003). The Ins allele is connected with an alternative splicing where 92 bp is deleted, which shifts the stability of mRNA and reduces the concentrations of HLA-G (Brenol et al., 2012).

This polymorphism and soluble concentrations of HLA-G are correlated with various illnesses including autoimmune disorders, recurrent abortion, certain cancer, as well as inflammatory diseases, along with coronary heart disease (CHD) (Shankarkumar et al., 2011; Boiocchi et al., 2012; Dardano et al., 2012).

The +3142 C/G polymorphism enhances micro-RNA affinity and downregulates expression of the HLA-G (Rousseau et al., 2003). The existence of G (guanine) at + 3142 improves the strength of miR-148a, -148b and -152, down-regulating expression of HLA-G by degradation of mRNA (Tan et al., 2007; Castelli et al., 2009). Both polymorphisms therefore influence the expression of HLA-G, and are linked with different inflammatory and autoimmune disorders (Larsen and Hviid, 2009).

The HLAG* 01:05N null allele defined by a single base-pair of cytosine deletion (1597ΔC) in exon 3 of HLA-G is associated with an abolished expression of the full-length HLA-G protein isoforms HLA-G1 and HLA-G5 in female homozygous for the1597ΔC mutation (Ober et al., 1998).

1.6 Diseases Associated with HLA-G Gene

The developing embryo inherits half of its father's genes; thus, its growth throughout the uterine surroundings represents a semi-allogeneic graft. Therefore, the fetus might be rejected instead of being saved by the maternal immune system (Shakhawat et al., 2010). HLA-G expression is associated with protection and implantation of semi-allogeneic embryo from the immune system of mother (Carosella et al., 2008). HLA-G is released in cells of the fetal extra-villous trophoblast (Kovats et al., 1990). An HLA-G immune suppressive mechanism is performed by contact with cells of the uterine natural killer (NK) (Rouas-Freiss et al., 1997).

High concentration of HLA-G was considerably associated with better graft acceptance in hematopoietic stem cell, kidney, heart and kidney/liver -transplanted patients (Carosella et al., 2008). NK cells and alloreactive CD4+ and CD8+ T cells play a vital role in allograft rejection, which are expressing inhibitory HLA-G receptor, and it has been demonstrated that their functions are inhibited by membrane-bound and sHLA-G (Rouas-Freissa et al., 2003).

There are immunological abnormalities in T1D and T2D that enhance insulin resistance. Higher concentrations of HLA-G in individuals with defective glucose metabolism were found to be prevalent (Solini et al., 2010). This information indicate that HLA-G antigens may be involved in the diabetic disorder. Additionally, some SNPs close to the HLA-G gene had type 1 diabetes (Eike et al., 2009). Interestingly, HLA-G has been observed on surface of islet cells that induce to insulin secretion and it's also observed in some secretory granules. Depending on this information, an inadequate expression of HLA-G in pancreatic islets could keep the activation of T-cells and the onset of diabetes. (Cirulli et al., 2006).

HLA-G appears to be involved in NK cells' viral immune departure (Tripathi and Agrawal, 2007). Human immunodeficiency virus type 1 (HIV-1) up-regulates HLA-G molecules and down-regulates classical HLA-A and -B (Lozano et al., 2002). In addition, the polymorphisms of HLA-G 14bpINS and + 3142 G influence HIV exposure (Silva et al., 2013) but not mother–child transmission in African population (Segat et al., 2014).

Enhanced HLA-G hepatocyte expression was correlated with milder levels of fibrosis and hemosiderin deposition in hepatitis C virus (HCV) of infected liver samples. (de Oliveira Crispim et al., 2012). Increased concentrations of plasma sHLA-G were linked to acute HCV inflammation and enhanced concentrations of IL-10 and IFN-g (Weng et al., 2011).

When tumor cell displays HLA-G, it inhibits the cytotoxic activity of T and NK cells, enabling the propagation of tumor cells. If reduced expression for classical HLA molecules is followed by enhanced expression of immunomodulatory molecules like HLA-G, the efficient cytotoxic immune reaction against cancer cells is greatly impoverished (Donadi et al., 2011).

Improved HLA-G expression in various tumor kinds was noted, including breast cancer, papillary thyroid carcinoma, colorectal cancer, lung cancer, esophageal cancer acute myeloid leukemia (Lefebvre et al., 2002; Ye et al., 2007; Cao et al., 2011; Guo et al., 2011; Nunes et al., 2013).

1.7 The Aim of the Study

Obesity is a disease characterized by hormonal and metabolic differences and is associated with more than one genetic, environmental and metabolic factors. Obesity is accompanied by many diseases, the most important of which are immune system disorders. The aim of this study is to analyze the 14 bp insertion/deletion 3' UTR rs66554220, rs41557518 and rs1063320 polymorphisms in the HLA-G gene region in obese and healthy subjects. The information obtained at the end of the project will contribute to the detection of genetic factors causing obesity.

CHAPTER 2

LITERATURE

Rich-Edwards et al. (1994) studied BMI in adolescent and ovulatory disease. Through this study, they indicate that high BMI at age of 18 is a major risk for ovulatory infertility, even at concentrations below those regarded obese.

Colditz et al. (1995) investigated that with a higher BMI, the probability of diabetes mellitus is risen, and even females with normal weight had a high risk relative to females with stable weight. 2204 diabetes cases have been diagnosed, and risk of diabetes increased with higher BMI.

Manson et al. (1995) investigated the relationship between women's body weight and the mortality. They discovered that body weight and mortality from all factors were directly associated within these middle-aged women. Women who had a BMI of 32.0 or higher had greater risk of death from cancer and cardiovascular disease in comparison to lean women. Among women for whom the weight used to be stable since early adulthood, the smallest mortality rate was recorded.

Trentham-Dietz et al. (1997) discovered that weight gain at 18 and 35 years was correlated with enhanced risk of postmenopausal breast cancer and reduced risk was linked with weight loss in females. These results indicated that avoiding weight gain research programs are a means of reducing the risk of postmenopausal breast cancer.

Peppard et al. (2000) evaluated the autonomous longitudinal correlation between weight gain or loss and sleep-disordered breathing (SDB), In which weight gains of 10% expected a 6-fold rise in probability of moderate to severe SDB development. They reported that programs for clinical and public health that lead in even small weight control are probable to be efficient in handling SDB and decreasing SDB onset.

Lievense et al. (2002) discovered stronger connections between obesity and hip osteoarthritis (OA). Generally, for a positive relationship between obesity and the onset of hip OA, moderate data was discovered.

Wilson et al. (2002) studied the relationship between BMI categories and risk factors for CVD. They reported that an enhanced relative and population attributable risk of hypertension and cardiovascular complications is connected with being overweight.

Calle et al. (2003) studied the effect of obesity and overweight on the threat of cancer mortality. Their study was based on the cancer-free population of over 900,000 U.S. adult (404,576 males and 495,477 females), 57,145 cancer deaths occurred throughout the 16-year follow-up. Based on the relationships found throughout this research, they predict that present trends of obesity and overweight in the U.S. would account for 14% for all cancer deaths in males and 20% in females. Higher body weight was therefore connected with enhanced mortality levels for all cancers combined and numerous specific locations for cancers.

Leong et al. (2003) investigated the relationship between birth weight and adult obesity. They suggested that small and heavy birth weights are correlated with greater adult BMI and aid a hypothesis which experience of fetal might affect adult obesity with future implications for multiple significant cancers risk.

Wannamethee et al. (2005a) studied the impacts of BMI background on significant CVD and diabetes diseases. There had been 1989 significant CVD incidents and 449 cases of diabetes throughout 20-year follow-up of 7,176 British males. Weight gain has been correlated with enhanced risk of CVD and diabetes during 15-year follow-up. Weight loss had been correlated with reduced diabetes risk, irrespective to original weight.

Wannamethee et al. (2005b) studied the impacts of fat distribution and the composition of body on function of lung and searched for a relationship in elderly males. They discovered that lung function is negatively correlated to central adiposity as well as total body fat. However, enhanced fat-free mass (FFM) representing muscle mass rises is correlated with enhanced lung function and reduced probability of FEV1: FVC (low forced expiratory volume in 1: forced vital capacity) in the elderly.

The results of the study by Martínez et al. (2006) about the severity and mortality in acute pancreatitis in obesity indicated significantly higher frequency of severe acute pancreatitis in obese patients. In addition, these patients created considerably further systemic complications, particularly local ones, and mortality was quite higher in obese patients.

Pischon et al. (2006) investigated the relationship of anthropometric measures, which include the waist-hip circumference and renal cell carcinoma (RCC) risk among 348,550 men and women that were free of cancer at baseline throughout 6 years of follow-up. They reported that obesity is associated with enhanced threat of RCC regardless of women's fat distribution, while low hip circumference is associated with enhanced men's risk of RCC.

Flegal et al. (2007) studied the association of the excess deaths with underweight, overweight, and obesity. They suggest that the combination of overweight and obesity was correlated with enhanced diabetes and kidney disease death and reduced mortality from other non-cancer non-CVD conditions.

Fujimoto et al. (2007) investigated the effect of the body size and central adipocity on the risk of type 2 diabetes. The Diabetes Prevention Program hired and randomized people with damaged glucose tolerance with placebo, metformin, or lifestyle modification therapy, in which 758 participants were involved in this study. They concluded that reduced diabetes risk through lifestyle interference was correlated with reduced BMI. Body weight and distribution of central body fat following age adjustment and ethnicity, as well as, lifestyle action might have decreased the threat of diabetes by impacts on both total body fat and central body fat, however metformin seemed independent of body fat.

Maheshwari et al. (2007) investigated the impact of obesity and overweight on assisted reproductive technology. In comparison to women with a BMI of 25 kg/m^2 or less, women with a BMI $\geq$ 25 kg/m^2 had a reduced opportunity of pregnancy following in vitro fertilization.

The correlation between BMI and the prevalence and improvement of radiological knee and hip osteoarthritis was investigated by Reijman et al. (2007). Based on the accessibility of baseline and follow-up radiographs, 3585 individuals aged ≥ 55 years were chosen from Rotterdam Study. As a result, they discovered that high BMI at baseline was correlated with osteoarthritis knee incidence, though not with osteoarthritis hip incidence.

In the Cancer Prevention Study II Nutrition Cohort, BMI and weight change was studied in comparison to occurrence of prostate cancer by disease level and diagnostic grade among 69,991 males. Rodriguez et al. (2007) found that obesity improves the risk of further aggressive form of cancer of a prostate and might reduce the incidence or probability of less aggressive tumors being diagnoses and they suggest that men who lose weight could lower their prostate cancer risk.

Thygesen et al. (2008) studied the effect on weight changes on the risk of colon cancer. They included 46,349 males between the ages of 40 and 75. 29.5% percent of all situations of colon cancer were reported to be attributable to BMI higher than 22.5. Their findings give evidence that overweight and obesity among males are modifiable risk factors for colon cancer and indicate that even in future lives, weight does have a significant impact on the risk of colon cancer.

Tukker et al. (2009) investigated the association of overweight with the health problems in the lower extremities including osteoarthritis, disability and pain. A random sample aged >25 years (n:3664) was evaluated from the Dutch population They found that overweight and obesity are strongly associated with health issues of the lower extremities like osteoarthritis, pain and disability.

A research on the association between soluble HLA-G with metabolic and inflammatory form of T2DM and/or obesity was done by Solini et al. In their study, 144 individuals were found to be sHLA-G positive, it was much more common in subjects with T2DM or defective glucose tolerance than in healthy glucose tolerance. sHLA-G-positive people had greater concentrations of BMI, systolic blood pressure, and cholesterol; lower concentrations of insulin sensitivity; and nearly 2-fold greater concentrations of IL-6, insulin sensitivity-related cytokine, while IL-10 had been similar. They suggested that a frequent expression of sHLA-G, associated with a

classic insulin resistance biomarker such as IL-6, appears to categorize individuals with reduced glucose metabolism (Solini et al., 2010).

Silva et al. (2013) studied the possible impact of two HLA-G polymorphisms on the 3'UTR on predisposition to cervical cancer in Brazilian patients. Polymorphism (14bp In /Del and +3142C/G) had been analysed in a total of 105 cervical specimens. 50 samples were without lesions and 55 were with lesions as well as 33 samples with invasive cervical cancer and 22 samples with high grade squamous intraepithelial lesion. In summary, they reported that 3 ‘UTR of HLA-G is connected with an enhanced threat of developing cervical cancer, particularly in smokers.

White et al. (2014) investigated the interaction of soluble HLA-G with acute rejection and early development of lung transplantation bronchiolitis obliterans. They discovered that levels of lung soluble HLA-G were straightly associated with class A rejection though not with lymphocytic bronchiolitis. They revealed that soluble levels of HLA-G in bronchoalveolar lavage though not in serum correlate with number of acute episodes of rejection during the first 12 months after lung transplantation and can therefore be a reactive indicator of rejection.

A study about HLA-G polymorphism influence on Type I Diabetes Mellitus and age of onset was done by Gerasimou et al. (2016). They analyzed 14-bp deletion (del) polymorphism (rs371194629) at the 3’UTR of HLA-G in the T1DM patients in Cypriot. This research showed a strong connection between HLA-G 14-bp polymorphism and T1DM age of onset.

Shobeiri et al. (2016) discovered that in females with gestational diabetes, HLA-G concentrations were lowered compared to the control subjects. They therefore proposed that HLA-G estimation in pregnant females could be regarded as a marker in gestational diabetes diagnosis.

Zambra et al. (2016) studied the role of 8 polymorphisms in the 3'UTR of HLA-G gene on development of prostate cancer (PCa) as well as benign prostatic hyperplasia (BPH). They analyzed totally 468 patient DNA samples (152, BPH; 187 PCa), and 129 healthy controls. The result of the study indicated that there is a significant impact of HLA-G 3'UTR polymorphisms on PCa predisposition.

Zidi et al. (2016a) studied human leucocyte antigen (HLA)-G, HLA-E and HLA-F genetic polymorphisms in Tunisian patients with coronary artery disease (CAD). They revealed that the HLA-G + 3142 G allele was significantly associated with enhanced risk of CAD. There was no association with the other investigated polymorphisms.

Zidi et al. (2016b) investigated the relationship between HLA-G polymorphisms with breast cancer in Tunisian population. a significant connection was found between the G allele in + 3142 C>G polymorphism and breast cancer. There was no association of 14-bp Ins/Del polymorphism with breast cancer.

Agnihotri et al. (2017) studied the involvement of HLA-G polymorphism on the Head and Neck Squamous Cell Carcinoma (HNSCC). HLA-G polymorphism at 3′UTR 14bp INDEL (rs371194629) and + 3142G / C (rs1063320) were analyzed in 383 HNSCC individuals and 383 healthy individuals. They found that the genotype of C / C, Del / Ins and Ins / Ins as well as C and Ins allele might be significant risk factors with a powerful effect of tobacco on HNSCC the population of North Indian.

A study on the level of soluble HLA-G in obese pregnancy was done by Beneventi et al. (2017). It was found that sHLA-G levels are greater in females with normotensive overweight / obese and their infants; and smaller in females with preeclamptic overweight / obese and their cord (Beneventi et al., 2017).

Emmery et al. (2017) studied the role of HLA-G 14bp insertion/deletion gene polymorphism in newborn birthweight as well as placental weight. Interestingly, in homozygous 14 bp Del / Del newborns, they found the greatest average birth weight as well as placental weight, and lowest in 14 bp Ins/Ins newborns. The 14 bp Del/Del genotype was also connected with large expression of HLA-G in membrane of trophoblast.

Hashemi et al. (2017) found that HLA-G gene polymorphisms considerably improved the likelihood of recurrent spontaneous abortion in a sample of an Iranian population.

Frankenthal et al. (2019) studied the impact of pre-pregnancy BMI and improper gestational weight gain on adverse obstetric results among females receiving treatment with assisted reproductive technology comparable to spontaneously conceived pregnancies. Based on the result of their study they suggested that pregnancy obesity and improper gestational weight gain in assisted reproductive technology and spontaneously conceived pregnancies were correlated with negative obstetric results.

León-Aguilar et al. (2019) investigated the contribution of maternal obesity to long-term changes in concentration of plasma lipid. They take samples from normal weight, overweight, and obese pregnant women and their children for their study. Maternal obesity leads to long-lasting modifications in the offspring's plasma ceramides indicating that such lipids could be used as earlier predictors of risk of metabolic disease owing to maternal obesity.

CHAPTER 3

MATERIAL AND METHODS

3.1 Material

Fifty obese individuals (BMI>30 kg/m^2) and 50 normal (BMI<30 kg/m^2) individuals participated to the study. The samples were collected from Department of General Surgery, Faculty of Medicine, SANKO University. 4 ml blood samples were collected in EDTA tubes and stored at -20 °C in refrigerator until DNA isolation. The study was approved by the Ethics Committee of Gaziantep University and supported by the Scientific Research Projects Governing Unit of Gaziantep University.

3.2 Methods

Pure Link Genomic DNA Kit was used to isolate DNA from the samples.

Table 3.1 Pure Link Genomic DNA isolation mini kit contents

Contents	**K1820-01**	**K1820-02**
Collection tube	50 each	5 × 50 each
Spin column with collection tube	100	5 ×100
Genomic Lysis/Binding Buffer	10 ml	50 ml
Genomic Digestion Buffer	9 ml	45 ml
Genomic Wash Buffer 1	10 ml	50 ml
Genomic Wash Buffer 2	7.5 ml	37.5 ml
Genomic Elution Buffer	10 ml	50 ml
RNAse A	1 ml	5 ml
Protinase K	1 ml	5 ml

3.2.1 Amplification of HLA-G Gene by Polymerase Chain Reaction

In this study three polymorphisms were studied in both obesity and control groups.

- rs66554220, HLA-G 14 bp ins/del polymorphism in the 3'UTR
- 1597ΔC, rs41557518 the cytosine deletion at codon 130 in exon 3, tagging HLA-G *01:05N null allele
- rs1063320 (+3142G>C)

Table 3.2 Primers used for PCR amplification of HLA-G gene

	Primer sequences	**PCR Sample Size**
HLA-G_rs66554220		
Forward	5' GTGATGGGCTGTTTAAAGTGTCACC '3	224 bp or 210 bp
Reverse	5' GGAAGGAATGCAGTTCAGCATGA '3	
HLA-G_ rs41557518		
Forward	5' CAGGTTCTCACACCCTCCAG '3	504 bp
Reverse	5' CCTCCACTCCCTCAGAGACTTCATC '3	
HLA-G_ rs1063320		
Forward	5' CATGCTGAACTGCATTCCTTCC '3	406 bp
Reverse	5' CTGGTGGGACAAGGTTCTACTG '3	

The ingredients for the three polymorphisms of HLA-G gene were roughly the same except the concentration of DNA was higher in HLA-G_ rs41557518 and HLA-G_ rs1063320 polymorphisms in contrast to HLA-G_rs66554220.

Table 3.3 Standard ingredients for PCR of HLA-G gene

PCR Mix Components	**Volume (µl)**
Buffer	2-2.5 (µl)
dNTP	2 (µl)
Primer Forward	0.3 (µl)
Primer Reverse	0.3 (µl)
DNA	0.5-4 (µl)
Taq Polymerase	0.1 (µl)
H_2O	15-19 (µl)
Total Volume	**25 (µl)**

Table 3.4 Program used for PCR of HLA-G gene

Stage	**Steps**	**Temperature**	**Time**	**Cycle**
1	Initial Denaturation	94°C	5 min	35
	Denaturation	94°C	30 sec	
2	Annealing	57-58°C	30 sec	
	Extension	72°C	30 sec	
3	Final Extension	72°C	5 min	
4	Hold	4°C	∞	

3.2.2 Preparation of Agarose Gel

- 2 g of agarose was weighed on precision scale.
- 100 ml 1X TBE was added.
- The solution was shaken gently and kept in oven for 5-10 minutes until agarose had dissolved.

- The agarose solution was cooled under water for 1 min.
- Redsafe dye was added to the solution.
- The agarose was putted into a gel tray with the well comb in place.

Loading Samples and Running an Agarose Gel:

- The agarose gel is placed into the gel box (electrophoresis unit)
- 5µl loading buffer dye was added to 8µl of each of PCR sample and mixed. (Thermo Fisher 6X loading dye were used)
- Carefully loaded a molecular weight ladder (50 bp Gene Ruler) into the first lane of the gel.
- Samples were carefully loaded into the other wells of the gel.
- The gel was run at 120 volt for 25 minutes for checking PCR results, and at 100 volt for 40 min for RFLP analyses.
- UV light was used to visualize DNA fragments. The fragments of DNA were usually referred to as 'bands' due to their appearance on the gel.

3.2.3 Analysis of HLA-G Gene Polymorphisms

Restriction Fragment Length Polymorphism (RFLP) is a molecular genetic analysis technique that enables people to be recognized in particular DNA areas based on distinctive models of restriction enzyme slicing. Also referred to as RFLP Analysis, the method uses polymorphisms in the genetic codes of individuals. Although all species members have fundamentally the same genetic structure, such small differences compensate for phenotype variations between people, like appearance or metabolism.

HLA-G_rs66554220 polymorphism was genotyped by the polymerase chain reaction method. The sample fragments were run in agarose gel electrophoresis for 30 min at 100 V to separate the samples on the bases of length.

The polymorphism of HLA-G_rs41557518 was genotyped with PCR-RFLP technique. 0.3 µl of PPUMI enzyme (restriction enzyme) with 6.5 µl of nuclease free water and 1 µl of 10x buffer G was added to 8 µl of PCR sample. Then the samples were incubated at 37°C on heat block for 4 hours, and then the sample fragments

were running in agarose gel electrophoresis for 30 min at 100 V to separate the samples on the bases of length.

The polymorphism HLA-G_rs1063320 was genotyped by PCR-RFLP technique. 0.3 µl of BaeGI enzyme (restriction enzyme) with 6.5 µl of nuclease free water and 1 µl of 10x buffer G was added to 8 µl of PCR sample. Then the samples were incubated at 55°C on heat block for 4 hours, and then running the sample fragments in agarose gel electrophoresis for 30 min at 100 V to separate the samples on the bases of length.

Table 3.5 Genotyping for HLA-G gene polymorphisms

Polymorphism	Band Size
HLA-G_rs66554220 Genotyping	
Deletion (+/+)	210 bp
Deletion (-/-)	224 bp
Deletion (+/-)	210/224 bp
HLA-G_rs41557518 Genotyping (the presence of HLA-G *01:05N allele)	
+/+	504 bp
-/-	389 bp, 115 bp
+/-	504 bp /389 bp / 115 bp
HLA-G_rs1063320 Genotyping	
CC	406 bp
GG	316 bp, 90 bp
CG	406 bp /316 bp / 90 bp

3.3 Data Analysis

The PCR-RFLP technique was used to analyze the genotyping of the polymorphisms. The allele and genotype frequencies were determined by direct counting. The genotype frequencies of the HLA-G gene polymorphisms were compared between obese and control group by using the chi-square (χ^2) test. "SPSS 22.0 for Windows" program was used for the statistical analyses. p value < 0.05 were considered as statistically significant.

CHAPTER 4

RESULTS

4.1 Demographic and Clinical Data

The analyses were performed on 50 patients with obesity and 50 non obese individuals. The patients were diagnosed in Department of General Surgery, Faculty of Medicine, SANKO University. Data including age, gender, height, weight and BMI about individuals were obtained from the medical records and are given in the Table 4.1. and Table 4.2.

The age of obese patients was between 18-68 years, while the mean age is 42.1 ± 10.6. Among 50 patients, 31 were 40 years old and above, while 19 out of 50 were younger than 40 years.

Obese group consist of 25 (%50) male and 25 (50%) female patient.

The mean weight of obese group was 114.568 ± 18.86, the mean height was 2.86 ± 0.258, and the mean BMI was 40.1 ± 6.66 (Table 4.1).

The age of control group was between (18-60) years, while the mean age was 42.14 ± 11.27. Among 50 individuals 34 was 40 years old and above, while 16 control individuals were younger than 40 years.

Similar to the obese group, control group also consist of 25 (%50) male and 25 (50%) female.

Table 4.1 Demographic and clinical data of obese group

Number	Age	Gender	Weight\kg	Height/m^2	BMI kg\m^2
1	32	F	87	2.62	33.2
2	60	F	82	2.56	32.03
3	20	M	90	2.99	30.1
4	45	F	88	2.65	33.2
5	28	F	86	2.72	31.61
6	32	F	88	2.75	32
7	39	M	120	3.38	35.5
8	23	F	80	2.65	30.18
9	33	M	90	2.99	30.1
10	38	M	92	2.89	31.83
11	68	M	95	2.82	33.6
12	35	M	120	3.24	37
13	37	F	112	2.65	42.26
14	47	F	92	2.4	38.33
15	18	M	105	3.42	30.7
16	20	F	110	2.56	42.96
17	47	F	76	2.4	31.6
18	38	F	123	2.89	42.56
19	46	F	103	3.42	30.11
20	33	F	114	2.56	44.53
21	42	F	154	2.4	64.16
22	48	M	105	2.59	40.54
23	38	M	125.9	3.02	41.68
24	41	M	136	3.13	43.45
25	42	M	119	2.92	40.75
26	44	M	133	3.2	41.56
27	56	M	125	3	41.66
28	47	F	111.6	2.68	41.64
29	48	F	120	2.85	42.1
30	46	F	141.5	2.68	52.79
31	58	F	113.7	2.82	40.31
32	40	F	113	2.65	42.64
33	38	M	119	2.92	40.75
34	44	F	121	2.89	41.86
35	44	M	136.2	3.2	42.5
36	44	F	124.8	3.09	40.38
37	26	F	144	2.85	50.52
38	38	F	121	2.7	44.81
39	38	M	125	2.8	44.64
40	51	F	127	3.1	40.96
41	58	M	145	2.85	50.87
42	45	M	120	2.9	41.37
43	45	M	122	3.02	40.39
44	44	F	133	3.2	41.5
45	58	M	114	2.8	40.71
46	48	M	125	3.1	40.32
47	45	M	139	3.09	44.98
48	58	M	116	2.8	41.42
49	48	M	111.7	2.52	44.3
50	44	M	134	2.9	46.2

Table 4.2 Demographic and clinical data of control group

Number	Age	Gender	Weight kg	Height m^2	BMI kg\m^2
1	18	F	47.43	2.49	19
2	32	M	62.65	3.13	20
3	36	F	49.92	2.49	20
4	32	M	60.87	3.2	19
5	44	F	58.56	2.78	21
6	20	F	49.86	2.62	19
7	19	M	59.91	2.49	24
8	32	F	46.83	2.46	19
9	38	M	60.55	3.02	20
10	47	F	60.62	2.75	22
11	32	F	55.61	2.52	22
12	20	M	64.14	2.78	23
13	46	F	65.69	2.85	23
14	42	F	58.88	2.56	23
15	40	F	58.56	2.78	21
16	59	F	50.48	2.65	19
17	34	F	54.22	2.46	22
18	22	M	54.49	3.02	18
19	19	F	44.93	2.49	18
20	40	F	55.25	2.4	23
21	44	F	65.84	2.99	22
22	50	M	55.77	2.7	20
23	34	M	46.81	2.34	20
24	49	M	66.47	2.89	23
25	45	M	65.84	2.99	22
26	44	M	88.98	3.42	26
27	56	M	73.96	2.95	25
28	52	F	51.2	2.56	20
29	42	F	64.91	2.82	23
30	49	F	53.79	2.68	20
31	60	F	63.2	2.52	25
32	45	F	66.47	2.89	23
33	38	M	54.92	2.49	22
34	50	M	60.2	3.16	19
35	42	F	71.4	2.85	25
36	48	F	48.67	2.43	20
37	32	F	63.58	2.89	22
38	42	F	69.36	2.89	24
39	43	M	66.6	3.02	22
40	49	F	59.89	2.72	22
41	60	M	66.6	3.02	22
42	48	M	54.43	2.59	21
43	48	M	77.44	3.09	25
44	49	M	78.62	3.27	24
45	58	M	55.77	2.78	20
46	52	M	64	2.56	25
47	48	M	65.79	3.13	21
48	58	M	59.61	2.59	23
49	48	M	70.43	3.06	23
50	52	M	53.09	2.52	21

The mean weight of control group was 60.46 ± 8.94 and the mean height was 2.775 ± 0.26, and the mean BMI was 21.7±2.04 (Table 4.2).

Table 4.3 Comparison of demographic data of obese and control group

Variables	Obese Group	Control Group
Age*	42.1 ± 10.608	42.14 ± 11.27
Weight	114.568 ± 18.86	60.46 ± 8.94
Height (m^2)	2.8646 ± 0.258	2.775 ± 0.262
BMI	40.1 ± 6.66	21.7 ± 2.04

*: mean ± standard deviation

The mean age of obese patients (42.1 ± 10.6) and control group (42.14 ± 11.27) was very close.

As expected, there is a significant difference in the mean weight of obese group (114.568 ± 18.86) and control group (60.46 ± 8.94).

The mean height (m^2) was 2.86 ± 0.258 for obese patients, while it was 2.775 ± 0.26 for control group.

The mean BMI was 40.1 ± 6.66 for obese patients, while it was 1.7±2.04 for control subjects.

After DNA isolation, samples were measured by Nanodrop. DNA concentrations of obese group were ranged between 31.6 and 337.88 ng/µl, and the average concentration was 85.24 ng/µl. While, the concentration of control group were ranged between 27.56 and 399.6 ng/µl, and the average concentration was 71.46 ng/µl.

4.2 Genotyping of HLA-G Polymorphisms

HLA-G gene polymorphisms listed below were investigated by using PCR and PCR-RFLP method.

- rs66554220, HLA-G 14 bp ins/del polymorphism in the 3'UTR
- 1597ΔC, rs41557518 the cytosine deletion at codon 130 in exon 3, tagging HLA-G *01:05N null allele
- rs1063320 (+3142G>C)

The association of HLA-G polymorphisms with obesity have been evaluated by χ^2 analysis and p value <0.05 is accepted as statistically significant.

4.2.1 Genotyping of HLA-G_rs66554220 Polymorphism

Genotyping of HLA-G_rs66554220 was performed by PCR method. The products were electrophoresed on 3% agarose and visualized under UV light (Figure 4.1).

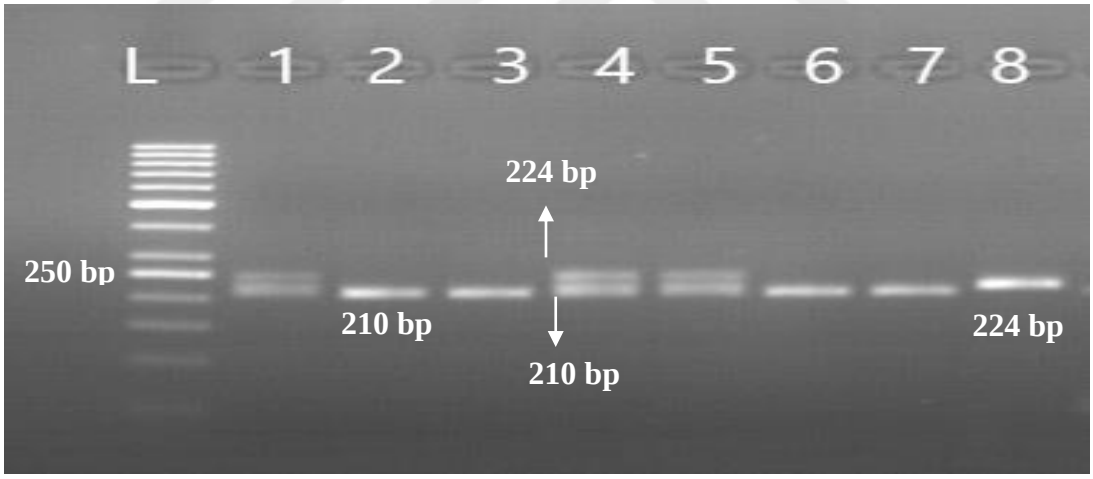

Figure 4.1 Genotyping of HLA-G rs66554220 polymorphism
Lane L: 50 bp DNA Ladder
Lane 1,4, 5: samples with heterozygotes genotype (+/-)
Lane 2, 3, 6, 7: samples with homozygotes genotype (+/+)
Lane 8: samples with homozygotes genotype (-/-)

The genotype frequencies for HLA-G rs66554220 polymorphism was compared between patient and control group and no significant difference was observed (p>0.05) (Table 4.4).

Table 4.4 Genotype distribution for HLA-G rs66554220 polymorphism

Genotype	Control (n=50) n (%)	Patients (n=50) n (%)	χ^2 (p*)
Deletion (+/+)	16 (32)	15 (30)	1.02 (0.60)
Deletion (+/-)	26 (52)	23 (46)	
Deletion (-/-)	8 (16)	12 (24)	

4.2.2 Genotyping of HLA-G_rs41557518 Polymorphism

For HLA-G_rs41557518 polymorphism, PCR-RFLP technique was used. PCR product (504 bp) of HLA-G gene (rs41557518) was digested with PPUMI restriction enzyme. The products of digestion were electrophoresed on 3% agarose and directly visualized under UV light (Figure 4.2).

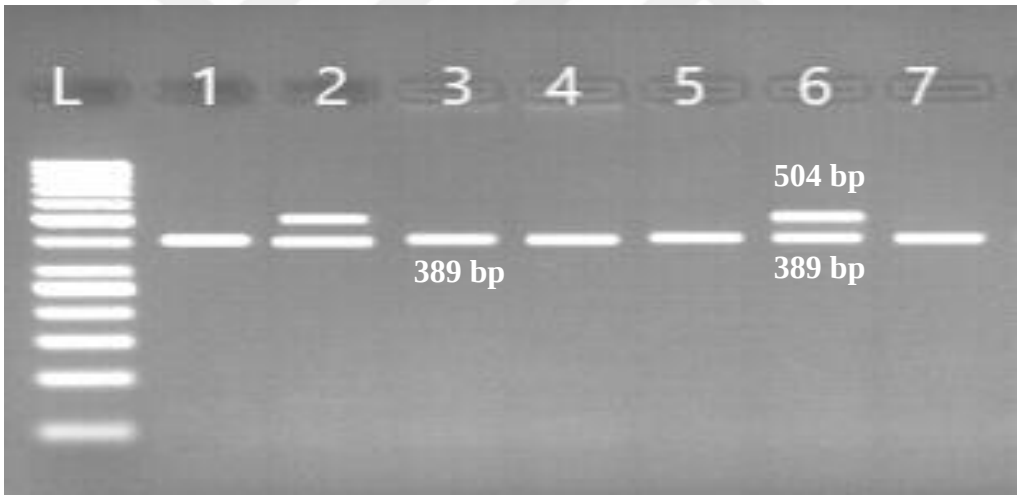

Figure 4.2 Genotyping of HLA-G_rs41557518 polymorphism
Lane L: 50 bp DNA Ladder
Lane 1,3,4,5,7: samples with homozygotes (-/-) for the absence of HLA-G*01:05N allele
Lane 2,6: samples with heterozygotes (+/-)

The genotype frequencies for HLA-G rs41557518 polymorphism was compared between patient and control group and no significant difference was observed ($p>0.05$) (Table 4.5).

Table 4.5 Genotype distribution of HLA-G_rs41557518 polymorphism

Genotype	Control (n=50) n (%)	Patients (n=50) n (%)	χ^2 (p*)
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)	

4.2.3 Genotyping of HLA-G_ rs1063320 Polymorphism

PCR-RFLP technique was used for genotyping of HLA-G_rs1063320 polymorphism.

PCR product (406 bp) of HLA-G gene (rs1063320) was digested with BaeGI restriction enzyme. The products of digestion were electrophoresed on 3% agarose and directly visualized under UV light (Figure 4.3).

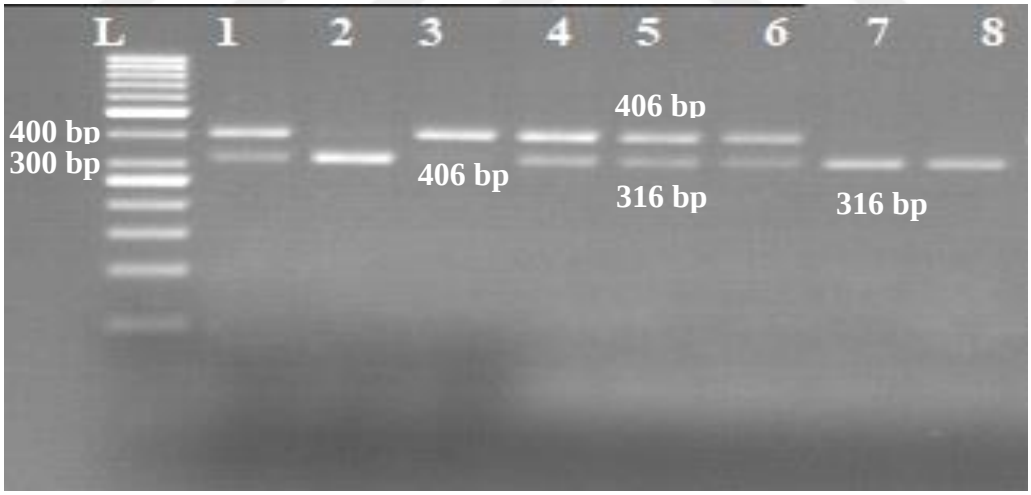

Figure 4.3 Genotyping of HLA-G_rs1063320 polymorphism
Lane L: 50 bp DNA Ladder
Lane 1,4,5,6: samples with heterozygote genotype (GC)
Lane 2,7,8: samples with homozygote (GG)
Lane 3: sample with homozygotes (CC)

Table 4.6 Genotype distribution for HLA-G_rs1063320 polymorphism

Genotype	**Control (n=50) n (%)**	**Patients (n=50) n (%)**	**χ^2 (p*)**
CC	7 (14)	7 (14)	1.19 (0.55)
CG	27 (54)	22 (44)	
GG	16 (32)	21 (42)	

The genotype frequencies for HLA-G_rs1063320 polymorphism was compared between patient and control group and no significant difference was observed ($p>0.05$) (Table 4.6).

CHAPTER 5

DISCUSSION

The fast-increasing incidence of obesity in the general population in latest decades was linked to an "obesogenic" setting that provides easy access to high-calorie food but limits physical activity possibilities. Obesity is a major public health issue since obesity-related co-morbidities include sleep disturbance, mobility issues, respiratory difficulties, type 2 diabetes mellitus, dyslipidemia, hypertension, diseases of cardiovascular and certain types of cancer, psychological distress (Switzer et al., 2013). The most significant one might be the disease of the immune system, and it is also a significant risk factor for influencing mortality causes. Obesity results from an imbalance among daily consumption of energy and energy expenditure arising in increased weight gain. Obesity is caused by multiple factors which can be genetic, cultural and societal. Other causes of obesity include reduced physical activity, disorders of endocrine system, insomnia, wrong food habits, medications, food advertisements and impaired energy metabolism (Panuganti and Gossman, 2019). Ultimately, investigating hereditary susceptibility to disease is an attempt to associate disease phenotype with the fundamental genotype.

Genetic factors perform an essential part in the susceptibility of a person to obesity (Chesi and Grant, 2015). The heritage of the BMI difference varied from 30% to 70% (Herbert et al., 2006). Several studies have been done to understand the role of epigenetic factors in obesity. Seven SNPs of zinc finger homeobox 3 (ZFHX3) gene were associated with obesity in Korean population (Yang, 2017). Two recent studies by Wang et al. (2016) and Meng et al. (2014) verified some loci recognized by GWA studies to be connected with obesity in Chinese children, and recognized ethnic variations. Obesity, like other malnutrition cases, affects immune function (Heredia et al., 2012).

The association of obesity with chronic inflammation is also well defined (Xu et al., 2003). The result of the study done by Todendi et al. (2015) in Brazil, indicated that there is an association between CRP rs1205 gene and hypercholesterolemia, and IL-6 rs2069845 gene is related to the obesity in children and teenagers.

The polymorphisms of leptin receptor (LEPR Gln223Arg), uncoupling protein 2 (UCP2 G 866 A) as well as the insulin receptor gene (INSR exon 17) were found to be associated with obesity in the Egyptian community (Hassan et al., 2018).

In Saudi Arabia, it was reported that IL-6 polymorphism which is significantly related to high BMI, lipid profile and total bilirubin differs considerably in men and women (Alharbi et al., 2014).

Chida et al. (2006) confirmed that combined deficiencies of IL-6 and IL-1 cause serious early-onset obesity in mouse.

Latest findings have given additional confirmation of HLA-G's role in the suppression of responses of immunity and long-term impacts on immune tolerance or escape (LeMaoult et al., 2004). HLA-G proteins are much more commonly expressed in inflammatory infiltrated tumor lesions than non-inflammatory proteins, suggesting that inflammation may induce HLA-G expression (Carosella et al.,1999).

HLA-G could minimize cells sensitivity from numerous types of lymphoproliferative and solid tumors diseases to cytolysis by NK cells and by cytotoxic T lymphocytes, that allow immunosurveillance escape (LeMaoult et al., 2004).

A significant indicator of inflammation of adipose tissue is the rise in IL-6. Solini et al., (2010) revealed high sHLA-G expression is associated with insulin resistance biomarkers such as IL-6, which seems to categorize individuals with reduced glucose metabolism. HLA-G is directly linked to IL-6, a cytokine that is implicated in the inflammatory subclinical status described throughout obesity and T2DM (solini et al., 2010)

A strong correlation among obesity, T2DM and HLA-G can be chronic local inflammation in adipose tissue, in which cells of the innate immune system, especially macrophages, are critically engaged. (Odegaard and Chawla, 2008)

Beneventi et al revealed that obesity affects levels of maternal and infant sHLA-G throughout pregnancy to improve reproductive success, whereas preeclampsia affects the anti-inflammatory reaction of mother-offspring (Beneventi et al., 2017).

Although HLA-G has been connected with diseases of autoimmune such as multiple scleroses (Wiendl et al., 2005), systemic lupus erythematosus (Rizzo et al., 2008), rheumatoid arthritis (Verbruggen et al., 2006), and psoriasis (Borghi et al., 2008), its involvement in the obesity is currently not known.

A study done by Marzuillo et al. (2018) revealed a significant connection between HLA-G 14 bp ins / ins genotype and insulin resistance in obese children and teenagers.

The result of another study (Hedström et al., 2014) demonstrated a remarkable relationship between the BMI level and the HLA genotype with respect to multiple sclerosis risk. They hypothesized that by enhancing the release of proinflammatory cytokines and encouraging Th1 reactions and reducing the amount of cellular T cells, obesity might boost the possibility of development of auto-aggressive CD4 + cells targeting CNS autoantigens. Obesity in the presence of HLA risk genes can also improve the possibility of auto-aggressive immunity leading to multiple sclerosis.

In the present study, it was aimed to investigate the association of HLA-G gene polymorphisms (rs66554220, rs41557518, and rs1063320) with obesity. To our knowledge, this was the first study evaluating this relationship.

The 14-bp ins/del (rs66554220) polymorphism in the 3′ UTR is the most studied HLA-G gene polymorphism. This polymorphism has been shown to influence HLA-G mRNA transcript stability and size (Rousseau et al., 2003). As a result of this study, no significant difference was found between the genotype distribution of this polymorphism in the patient and control group. Therefore, it can be concluded that this polymorphism is not associated with obesity.

The HLAG* 01:05N null allele is categorized by a single base pair deletion cytosine in exon 3 (1597ΔC), which inhibits production of full-length HLA-G protein isoform HLA-G1 and soluble HLA-G5. However, this null allele maintains its capacity to translate membrane – bound HLA-G2 and HLA-G3 and soluble HLA-G6 as well as

HLA-G7 isoforms (Ober et al.,1998). Since, no significant difference was observed in patient and control groups genotype distribution in this study, it can be stated that this polymorphism is not associated with obesity.

The +3142 C/G polymorphism (rs1063320) enhances the affinity for micro RNAs and downregulate the expression of HLA-G (Rousseau et al., 2003). This polymorphism was not found to be associated to obesity in this study, because there was no significant difference in the genotype distribution in the patient and control group.

The results of this study suggested that there is no association between the analyzed HLA-G gene polymorphisms and obesity. Still, HLA-G might have role in the development of obesity through different mechanisms. The main throwback of this study is the size of the study groups. It should also be noted that the genotype distributions might be different in different populations. Therefore, the association of obesity and HLA-G gene needs to be clarified by future studies with large sample size and different ethnicities.

CHAPTER 6

CONCLUSION

In this study, the relationship between HLA-G gene polymorphisms and obesity has been investigated to understand the possible role of this gene in disease pathogenesis. DNA samples from obese and control individuals were used to analyze three HLA-G polymorphisms by PCR / PCR-RFLP method. No significant association was found between the three polymorphisms of HLA-G gene and obesity. This data suggests that despite having immunosuppressive function, HLA-G gene seems to have no role in the development of obesity.

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