INVESTIGATION OF DRD2, LEPR, AND OPRM1 GENE VARIANTS IN OBESE SUBJECTS WITH BARIATRIC SURGERY

by

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INVESTIGATION OF DRD2, LEPR, AND OPRM1 GENE VARIANTS IN OBESE SUBJECTS WITH BARIATRIC SURGERY

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ABSTRACT

INVESTIGATION OF DRD2, LEPR AND OPRM1 GENE VARIANTS IN OBESE SUBJECTS WITH BARIATRIC SURGERY

Our study aimed to examine the gene polymorphisms (SNPs) of DRD2 (rs1800497), LEPR (rs1137100, rs1137101, and rs1805094), and OPRM1 (rs1799971) and determine the relationship between them and weight regain in obese patients who underwent bariatric surgery.

Five hundred and forty-six patients (466 women, 100 men, BMI 35–40 kg/m2, BMI ≥40 kg/m2) who were followed up clinically at Istanbul Fatih Sultan Mehmet Training and Research Hospital enrolled in the study. One hundred and seventy-nine healthy individuals (100 women and 79 men, 18.5>BMI) have taken as a control group. Seven hundred and twenty-five blood samples total have been processed.

After the nucleic acid extraction from blood samples, SNP genotyping was performed by using high-resolution melting curve analysis (HRMA). The obtained data were subjected to DNA sequence analysis.

There was no significant difference between C and T allele frequencies of the DRD2 TaqI A1 polymorphism (p=0.1, OR=0.54, 95% CI=0.24-1.21). Allele distribution did not differ statistically for the LEPR K656N (p=0.51, OR=0.75, 95% CI=0.14-3.93), LEPR K109R (p=0.78, OR=0.77 95% CI=0.35-1.72) and LEPR K109R polymorphisms (p=0.32, OR=0.64 95% CI=0.37-1.12). The distribution of A and G alleles between the patient and control groups was statistically significant for the OPRM1 A118G gene variation (p=0.001, OR=2.99 (95% G.A.=1.45-6.15)

It has determined that OPRM1 G allele carriers showed a 3-fold increased risk for obesity than the A allele carriers. Although it has been observed that the T allele of the DRD2 TaqI A1 (p=0.1) polymorphism and the G allele of the LEPR Q223R (p=0.08) variant decreases the risk for developing obesity, the results were not statistically significant.

ÖZET

BARİATRİK CERRAHİ SONRASI OBEZ HASTALARDA DRD2, LEPR VE OPRM1 GEN VARYANTLARININ ARAŞTIRILMASI

Çalışmadakı amacımız bariyatrik cerrahi operasyonu geçiren obezite hastalarında DRD2 (rs1800497), LEPR (rs1137100, rs1137101 ve rs1805094) ve OPRM1 (rs1799971) gen polimorfizmlerini (SNPs) incelemek ve tekrar kilo alımı ile aralarındakı ilişkiyi belirlemek idi.

Çalışmaya İstanbul Fatih Sultan Mehmet Eğitim ve Araştırma Hastanesinde klinik takipte olan 546 hasta (466 kadın, 100 erkek, BMI 35–40 kg/m2, BMI ≥40 kg/m2) dahil edildi. Kontrol grubu olarak 179 sağlıklı birey (100 kadın, 79 erkek, 18.5>BMI) alındı. Toplam 725 kan örneği çalışıldı.

Örneklerden nükleik asit ekstraksiyonu işlemleri tamamlandıktan sonra yüksek çözünürlüklü erime eğrisi analizi (HRMA) ile SNP tespiti yapıldı. Elde edilen veriler DNA dizi analizine tabi tutuldu.

DRD2 TaqI A1 polimorfizmi için C ve T allel sıklıkları arasında önemli bir fark bulunmadı (p=0,1, OR=0,54, %95 G.A.=0,24-1,21). LEPR K656N (p=0,51, OR=0,75, %95 G.A.=0,14-3,93), LEPR K109R (p=0,32, OR=0,77 %95 G.A.=0,35-1,72) ve LEPR Q223R (p=0,08, OR=0,64 %95 G.A.=0,37-1,12) polimorfizmleri açısından allel dağılımlarında istatiksel anlamda fark tespit edilmedi. OPRM1 A118G varyasyonu için (p=0,001, OR=2,99 (%95 G.A.=1,45-6,15) A ve G allelerinin hasta ve kontrol grubu arasındakı dağılımı istatiksel olarak anlamlı bulundu.

OPRM1 A118G polimorfizmi için G alleli taşıyan bireylerde obezite gelişme riski olasılığının A alleline göre neredeyse 3 kat fazla olduğu saptandı. DRD2 TaqI A1 polimorfizmi için T alleli (p=0,1) ve LEPR Q223R polimorfizmi için G allelinin (p=0,08) obezite gelişme riskini azalttığı gözlemlense de istatiksel anlamda fark saptanmadı.

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LIST OF SYMBOLS/ABBREVIATIONS

WHO	The World Health Organization
NCDs	Non-communicable diseases
BMI	Body Mass Index
VFA	Visceral Fat Area
MC4R	Melanocortin-4 receptor
POMC	Proopiomelanocortin
NTRK1	Neurotrophic receptor tyrosine kinase 2
SIM1	Single minded homolog 1
BDNF1	Brain-derived neurotrophic factor
GWAS	Genome-wide association studies
SNP	Single nucleotide polymorphism
CNV	Copy number variation
In-dels	Insertions/deletions
FAT	Fat mass obesity-associated
PWS	Prader-Willi syndrome
HIF3A	Hypoxia inducible factor 3 subunit alpha
SLC6A4	Solute carrier family 6 member 4
FTO	Fat mass and obesity-associated gene
ARC	Arcuate nucleus
LHA	Lateral hypothalamus
VTA	Ventral tegmental area
NAc	Nucleus accumbens
mSH	Medial shell
VP	Ventral pallidum
CNS	Central nervous system
LEPR	Leptin receptor
DRD2	Dopamine receptor D2
kDA	kilodalton
WAT	White adipose tissue
IL-1	Interleukin-1
TNF	Tumor necrosis factor

PPAR-γ	Peroxisome Proliferator-activated Receptor-γ
CRH	Cytokine homologous domain
FN3	Fibronectin type 3
BBB	Blood-brain barrier
Jak/STAT)	Janus kinase/signal transducers and activators of transcription
STAT3	Signal transducer and activator of transcription 3
PTPN1	Protein tyrosine phosphatase non-receptor type1
SHP-2	Src-homology 2 domain-containing phosphatase 2
SOCS3	Suppressor of cytokine signaling 3
NPY	Neuropeptide Y
AgRP	Agouti related neuropeptide
POMC	Proopiomelanocortin
CART	Cocaine-amphetamine-regulated transcript
αMSH	Alpha melanocortin stimulating hormone
WMAT2	Vesicular monoamine transporter 2
DAT	Dopamine transporter
MAO-B	Monoamine oxidase B
COMT	Catechol-o-methyl transferase
GPCR	G protein-coupled receptor
AC	Adenylyl cyclase
cAMP	Cyclic adenosine monophosphate
PKA	Protein kinase A
DARPP-32	Dopamine and cAMP-regulated phosphoprotein 32-kDa
Cdk5	Cyclin-dependent kinase 5
CaN	Calcineurin
MAPK	Mitogen-activated protein kinase
PKC	Protein kinase C
MSNs	Medium spiny neurons
ADHD	Attention-deficit/hyperactivity disorder
RYGB	Roux-en-Y Gastric Bypass
ANKK1	Ankyrin repeat and kinase domain containing 1
MOR	mu opioid receptor (μ)
DOR	delta opioid receptor (δ)

KOR	kappa opioid receptor (κ)
NOP	Non-opioid receptor nociceptin
PI 3-kinase	Phosphatidylinositol-3 kinase
T2DM	Type 2 diabetes mellitus
PPARG	Peroxisome Proliferator Activated Receptor Gamma
IRS1	Insulin receptor substrate 1
GRB14	Growth Factor Receptor Bound Protein 14
MCR4	Melanocortin 4 receptor
HRM	High Resolution Melting
Tris	Tris(hydroxymethyl)aminomethane
DTAB	Dodecyltrimethylammonium
CTAB	Hexadecyltrimethylammonium bromide
EDTA	Ethylenediaminetetraacetic acid
NaCl	Sodium chloride
µl	microliter
%EWL	Excess weight loss
OR	Odds ratio

1. INTRODUCTION

1.1. OBESITY

Throughout the world, obesity poses a significant health threat. According to the data from Non-communicable diseases (NCD) Risk Factor Collaboration, the rate of obesity has tripled since 1975. Due to the records, in 2016, it has determined that 671 million adults were obese, and 2 billion adults were overweight. It also was reported that the rates of obesity in high-income countries began to decline, however in low-income and middle-income countries it still rises. If this trend continues, studies have estimated that the number of obese adults will reach 1 billion in 2025 (1). All this makes obesity a '21st-century epidemic', according to World Health Organization (WHO) (2).

WHO has identified obesity as excessive or abnormal fat deposition that could negatively affect the whole body's functioning. Fats have higher energy efficiency and energy density than other macronutrients such as proteins and carbohydrates. Thus, 92-98% of endogenously stored energy consists of fats. However, the amount of energy stored in the protein and carbohydrate form is just 5-10% and 20-30% after absorption, processing, and storage (3).

Obesity also increases the risks of cardiovascular, metabolic, and musculoskeletal diseases, cancer, Alzheimer's disease, and depression (4). According to the reports, obesity was responsible for 5.7% of death associated with non-communicable diseases (NCDs) in 1990. However, this rate reached 11.1% in 2019 (5). In addition, obesity has a relationship with reduced life expectancy, quality, lower productivity, unemployment, and social disadvantages. Also, rising health care costs make obesity a heavy social burden (6).

In clinically, Body Mass Index (BMI) is a measurement that has typically used to estimate body fatness (general obesity). It is calculated as weight in kilograms divided by height squared in meters (kg/m^2). For those classified as overweight BMI, is calculated as≥ 25 kg/m2, and for those considered obese BMI, is ≥30 kg/m2. Studies have revealed that the role of abdominal obesity, defined as visceral fat area (VFA), is more in the development of cardiovascular diseases, hypertension, type 2 diabetes than BMI (7, 8).

Classification	BMI Category (kg/m^2)	Risk of Developing Health Problems
Underweight	<18.5	Increased
Normal Weight	18.5-24.9	Least
Overweight	25.0-29.9	Increased
Obese		
Class I	30.0-34.9	High
Class II	35.0-39.9	Very High
Class III	≥40.0	Extremely High

Figure 1.1. BMI categories

1.1.1. Risk Factors of Obesity

Obesity has a multifactorial, complex etiology, and is a largely preventable disease. Numerous mechanisms have a role in increasing the rate of obesity, such as genetic susceptibility, sedentary lifestyle, changes in dietary patterns, and epigenetic modifications caused by gene-environment interactions (9, 10, 11). But the interaction between these factors and how they contribute to energy homeostasis that causes fat accumulation is not fully understood; still, there is a considerable gap. Recently the possible relationship between gut microbiota and obesity has gotten more attention. This complex, interactive community of bacteria responds to environmental and lifestyle changes and causes alteration on hypothalamic appetite-regulating signaling (12, 13).

Obesity has defined by increased motivation for preference for highly palatable foods and overeating (14). High impulsivity, increased sensation-seeking behaviors, and lack of premeditation and perseverance have a crucial role in the development of obesity (15, 16). People often lead to consuming highly palatable foods to combat stress-called, 'comforting eating" in the modern lifestyle.

1.1.2. Genetic Predispositions of Obesity

Hereditary studies demonstrated that combinations of multiple environmental and genetic factors have a crucial role in the prevalence of common obesity. Individuals who might possess obesity-promoting genetic variants are impressed more by the current environment (1, 17). The heritability rate in obesity had determined at 40 to 70 percent in family and twin studies (1). Evidence is mounting that the causes and effects of adult obesity are rooted in early development and continuity across generations (18). Herewith, genetic approaches can give relevant information about physiology and molecular mechanisms that underlie obesity.

Generally, obesity is evaluated in two broad categories: monogenic and polygenic (also known as a common obesity). Monogenic obesity displays a Mendelian pattern of inheritance. This form of obesity is uncommon and early-onset. Generally, single gene defects or small or large chromosomal deletions are the major causes of monogenic obesity (10). Monogenic obesity has three categories. The first one is the genes that regulate leptin-melanocortin pathway. Studies unraveled that mutations in the leptin receptor, leptin, proopiomelanocortin (POMC), and melanocortin-4 receptor (MC4R) genes increase the risk for obesity.

Genes belonging to the second category are known as crucial for the development of the hypothalamus. Single-minded homolog 1 (SIM1), neurotrophic receptor tyrosine kinase 2 (NTRK2), and brain-derived neurotrophic factor (BDNF) are one of them, also, their role in obesity is not yet understood.

The third category contains a complex syndrome that is caused by hundreds of gene variants. Chromosomal rearrangements (Prader-Willi, WAGR Syndrome, etc.) and pleiotropic (Cohen Syndrome, Fragile X Syndrome, BBS Syndrome, etc.) effects are responsible for this type of obesity which is also called syndromic obesity. Although, the relationship between these genes and obesity remains fully unexplored (19, 20).

Identifying the genes that influence polygenic obesity started with genome-wide association studies (GWAS). Candidate genes are chosen for their involvement in energy regulation according to animal studies or human physiology or have a role in monogenic/extreme obesity. For this purpose, genetic variations in these genes had identified and evaluated for their association with BMI and obesity risks. Copy number variants (CNV), small

insertions/deletions (in-dels), single nucleotide polymorphisms (SNPs), and microsatellites are most common genetic variants that using candidate gene studies. Between them, SNPs are account for 90% of the genetic variation, and non-synonymous variants (the variants that change protein sequence) have shown the most robust relationship in these studies. Some studies reported that among the hundreds of genes studied, only variants of 6 (BDNF, MC4R, ADRB3, CNR1, PCSK1, and PPARG) exhibited reproducible association with obesity outcomes (1). The results could be distinct for each population because of the differences between ethnic groups and methods used to determine polymorphisms (21).

GWAS led to breakthroughs and advancements for the determination of genes that are responsible for common obesity. More than 80 GWAS studies identified >300 chromosomal loci linked with obesity, few of them are replicated, but none of them map. FTO (fat mass and obesity-associated) gene is the first identified obesity-related locus (1, 20). The association between the risk being obese or overweight and FTO SNPs has been confirmed in different studies (22). According to the research individuals who have homozygous risk alleles gain an average of 3 kg more than individuals has homozygous protective allele (23).

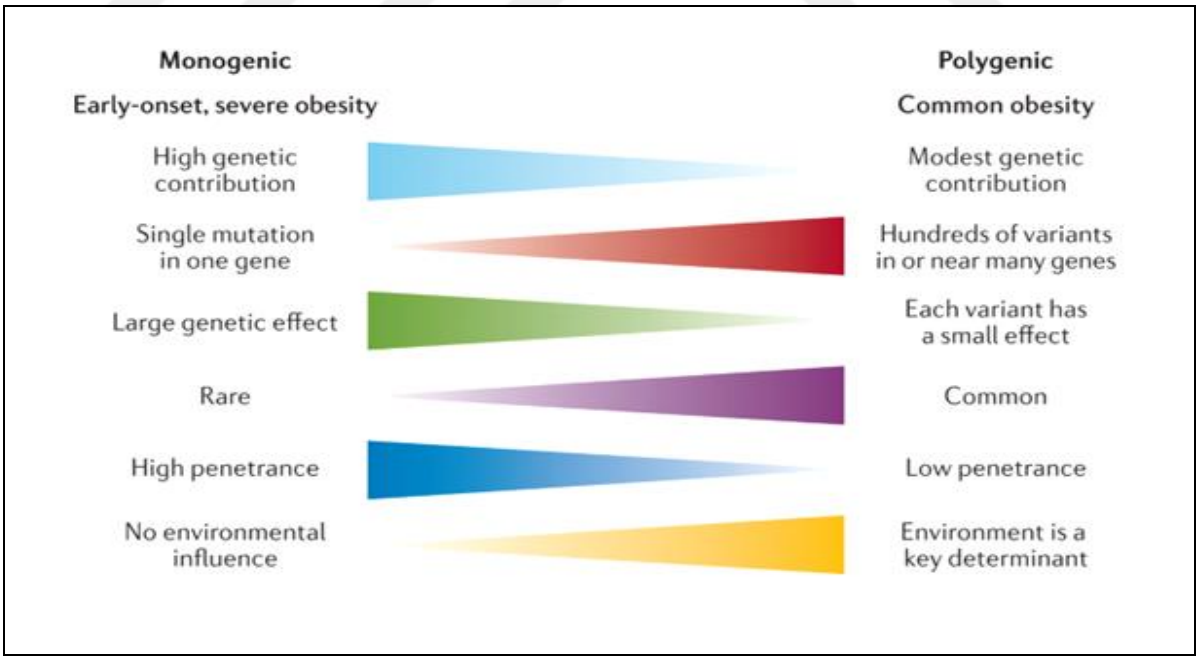

Figure 1.2. The differences between monogenic and polygenic obesity

1.1.3. Epigenetic in obesity

For the understanding of heritability in obesity, epigenetic also must be considered. Epigenetics is known as a heritable change in gene function, although there is no difference in the DNA sequence. Numerous studies retrieved the role of epiobesigenic genes in adipogenesis, appetite, glucose tolerance, and inflammation (24).

Epigenetic marks are preserved through generations, including DNA methylation and histone modifications, which are responsible for mediated biological processes like imprinting. The expression of maternal or paternal alleles is determined by genomic imprinting. Failures in imprinting, like lack of the paternal allele at chromosome 15, lead to extended appetite and obesity in Prader–Willi syndrome (PWS).

Approximately 4 million CpG sites were investigated in 74 individuals and reported that methylated regions are predictive of diseases (25, 26). Dick et al. have reported that together with the high BMI, the methylation of the HIF3A gene has also increased in adipose tissue and blood cells. According to the twin studies, serotonin transporter SLC6A4 promoter hypermethylated in blood leukocytes and BMI was related (27). Studies also have shown there are differences in DNA methylation in young identical twins than in older twins (28, 29).

1.1.4. Brain and energy balance

The majority of the genes that are determined as obesity-related genes have located in the brain. Regulation of energy homeostasis by the brain involves continually accepting neural and chemical inputs about the body's energy stores and making appropriate responses to maintain energy balance. Energy homeostasis and feeding behavior are highly complex and regulated by homeostatic and hedonic pathways. Homeostatic pathways control peripheral energy stores throughout the body and make the brain knowledgeable about them. The main purpose is to sustain maintenance energy balance and motivate for eating in depletion of energy stores. These circulating hormones are known as leptin and ghrelin. Leptin is a satiety factor and increases energy expenditure by decreasing food intake. However, ghrelin increases feeding to compensate for a negative energy balance (30, 31).

Recent studies showed that leptin and ghrelin also regulate energy homeostasis by mesolimbic dopamine signaling. Activation of the mesolimbic dopamine pathway regulates hedonic hunger. A suppressive effect of leptin on hedonic eating appears as the result of reducing dopamine release in the nucleus accumbens (NAc). (32). The role of ghrelin in reward processes such as food, drug, alcohol, and other substance abuse through dopamine and opioid signaling is also detected. This result led to hypothesize ghrelin affects the regulation of impulsivity (33, 34).

Leptin regulates energy metabolism by binding to leptin receptors. Leptin receptor (LEPR) gene variants had linked with obesity progress in some studies (35, 36).

The word "addiction" is usually using for the drug of abuse such as cocaine and alcohol. But these days term "food addiction" has become more popular (31). Food addiction comprises eating behavior, motivation, hedonic hunger, and food choice preference. Evidence supports that drug abuse and food addiction converge on the mesolimbic dopamine pathway because consumption of high palatable foods over a long-period results in neuroadaptations in these reward pathways, leading to behavioral and neurochemical alterations like in drug abuse (37, 38). The ventral striatum plays a crucial role in rewarding by regulating "wanting" and "liking" behaviors. Wanting and liking to seem almost interchangeable, but wanting is neurally and psychologically distinct. Studies also found, wanting and liking to seem to be processed by anatomically separate nucleus accumbens (NAc) regions and involve different neurotransmitters (39). Incentive salience or wanting has regulated by the mesolimbic dopamine pathway (40, 41). Dopamine is the most important mediator of reward circuitry, and released into the synapsis makes the sensation of pleasure and increases arousal, psychomotor activation, and conditioned learning. Also, it has been confirmed suppressing dopamine release reduces wanting behavior towards a stimulus but does not affect liking behavior.

Eating provides nutrients that we need and induces feelings of pleasure and gratification and increases extracellular dopamine concentrations in the NAc.

In reports of some studies, dopamine receptor D2 (DRD2) gene variants had correlated with an increased risk for obesity (42, 43). In obese individuals have a lower DRD2 availability like individuals addicted to various types of drugs. Thus, it could postulate that decreased

DRD2 density in obese individuals caused overeating to compensate for dopamine increases. An alternative explanation is that lower DRD2 carriers may be prone to addictive behaviors, like food consumption.

The endogenous opioid system is involved in energy homeostasis by modulating reward circuitry. When palatable foods had consumed, opioid receptor activation increases pleasurable sensations in both the nucleus accumbens (NAc) and the ventral pallidum (VP) (40, 44). Especially μ-opioids receptor (MOR) activation increases consumption of fatty and sweet foods.

1.1.5. Single Nucleotide Polymorphisms

The role of DNA variations in common diseases like obesity has already been known. The simplest form of DNA variation is single nucleotide polymorphism (SNP). SNPs are an alteration of one base with another and occur once in every 1,000 nucleotides throughout the genome. According to the reports, SNPs were categorized into the SNPs in the coding region and non-coding region. SNPs in the coding regions are divided into synonymous SNPs and nonsynonymous SNPs (Figure 3).

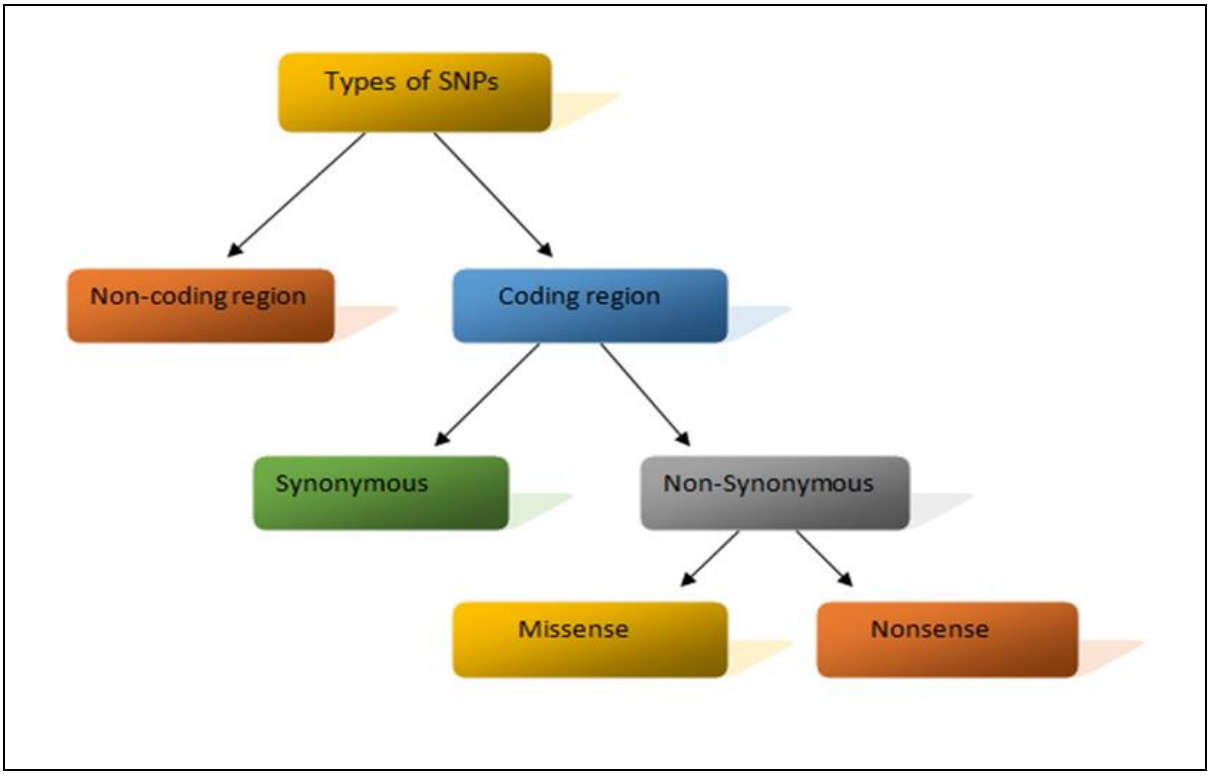

Figure 1.3. Types of single nucleotide polymorphisms (SNPs)

Synonymous SNPs do not change amino acid sequences, while nonsynonymous SNPs alter encoded amino acids and may cause changes in protein structure and functions.

High-throughput technologies for SNP genotyping have become widely used due to the possible association of SNPs with diseases or traits. These approaches also might provide answers for researchers about complex diseases, which occur due to the sum of the rare variants (17).

SNPs in various genes are related to obesity due to the GWAS data. Antonio et al. (45) reported SNP (rs1421085) in the FTO gene has a predictive role in higher body fat mass and body fat percentage. LEPR (rs1137101 and rs1045895), GRB14 (rs10195252 and rs3923113), ADIPOQ (rs1501299 and rs17300539), IRS2 (rs1805092), and PPARG (rs1801282) gene variants and overweight have found related in patients with Type 2 diabetes mellitus (T2DM) (46). Xi et al. (47) determined that the MC4R rs17782313 variant affects obesity. The role of PPARG rs1801282 on body composition and higher BMI have revealed (48). The LEPR gene polymorphisms such as Q223R (rs1137101), K109R (rs1137100), and K656N (rs1805094), dopamine D2 receptor TaqI (rs1800497) polymorphism, and OPRM1 rs1799971 polymorphism have been previously studied to explore their roles in overeating and obesity (49, 50).

1.2. LEPTIN

In 1994, Zhang et al. first discovered leptin by using positional cloning in ob/ob mice. Leptin means thin and had derived from the Greek word lepos. The ob gene (obese, LEP) encodes this hormone, composed of 167 amino acids. In humans, ob gene is mapped on chromosome 7q31.3 with three exons and two introns. Leptin is an approximately 16kDA protein with disulfide bond that is required for bioactivity, and responsible for energy homeostasis through the hypothalamus. Mutations occurring in the LEP gene result in the inabilities of leptin production, like in ob/ob mice, which results in extreme obesity, diabetes, hyperphagia, infertility, and neuroendocrine abnormalities (51, 52, 53). Studies have reported that LEP gene mutations increase the risks of obesity in animal models and humans, but the frequency of mutations is low. Homozygous mutations cause a rare autosomal recessive disease called congenital leptin deficiency. Congenital leptin deficiency is related to low leptin levels and severe early-onset obesity. The administration of leptin in these

individuals is a part of the treatment. This condition has been reported in only 12 individuals worldwide, three of whom are from Turkey (19).

Following the exploration of the LEP gene, the leptin receptor gene (LEPR) was isolated using expression cloning by Denver et al. (54). After that, studies confirmed db/db (diabetic) mouse has a mutation (C57BL/Ks) in the LEPR gene. Having a defective receptor is characterized by leptin resistance, leading to hyperphagia and decreased energy expenditure. That's why leptin injections reduce food consumption and weight in ob mice but do not affect db mice (55).

The genetically obese rats, also known as the Zucker (fa/fa) rats, are the most widely used animal model of genetic obesity. A missense mutation occurs in an extracellular domain of the leptin receptor and results in increased leptin levels and obesity in the Zucker (fa/fa) rats (51). In mice homozygous for the fa allele, obesity develops at 3 to 4 weeks of age, and %40 of their body composition makes up lipids by 14 weeks (56, 57).

Leptin is mainly secreted by differentiated white adipose tissue (WAT) and its circulating level is directly proportional to the amount of fat mass (51, 58). Leptin is secreted in different tissues, such as the ovary, placenta, stomach, lymphoid tissue, and skeletal muscle (55). Some factors, such as insulin, glucose, estrogens, interleukin-1 (IL-1), TNF-alpha induce leptin production (59). By the way, visceral and subcutaneous fat distribution and adipocyte size are also relevant to determine leptin concentrations. Higher leptin levels in women than men may be related to sex steroids or differential body-fat distribution (60, 61). In human blood, leptin circulates in a bound or free form to leptin-binding proteins. It has been shown that the free form of leptin is higher in obese subjects (62). Like other hormones, leptin levels are higher in the early morning and late evening hours (63). Leptin regulates energy homeostasis by giving information to the brain about body energy stores (54). Besides energy regulation, leptin regulates insulin sensitivity, glucose homeostasis, reproduction, osteogenesis, hematopoiesis, and inflammation (53).

1.2.1. Leptin Receptors

Leptin binds to leptin receptors (LEPR), encoded by a LEPR (db-diabetes) gene. This gene is located on chromosome 1p31 in humans (64). Leptin receptors are mainly found in the hypothalamus. Also, expressed in a variety of peripheral tissues, such as pancreatic β-cells,

immune cells, liver, and adipose tissue. Known as one of the classes I cytokine receptors, LEPR is composed of six different isoforms (LEPRa, LEPRb, LEPRc, LEPRd, LEPRe, and LEPRf) through alternate mRNA splicing. All isoforms have identical N-terminal extracellular domain but differ at the C-terminus (65, 66, 67).

Leptin receptors consist of two cytokine homologous domains (CRH1 and CRH2), two fibronectin type 3 domain (FN3), an Ig-like domain, a transmembrane domain, and a C-terminal cytoplasmic domain in the long isoform. Multiple shorter isoforms (LEPRa,c,d,f) had found with some of these domains missing. For instance, LEPRe lacks both the transmembrane domain and intercellular domain. This receptor is thought to be a crucial soluble leptin-binding protein, perhaps regulating free leptin levels (68, 69). The remaining isoforms have either short cytoplasmic extensions (LEPRa,c,d,f) (approximately 30-40 cytoplasmic residues) or have 302 amino acids intracellular tail (LEPRb). LEPRa is a highly expressed short isoform that has a role in the transportation of leptin through the blood-brain barrier (BBB). Three intracellular domains (Box1-3) in the long isoform of LEPRb are crucial for the Janus Kinase/Signal Transducers and Activators of the Transcription (Jak/STAT) pathway (70). Only one intracellular domain (Box1) in the short isoforms results in limited signaling capability. LEPRb mediates intracellular signaling of leptin, and db/db mice have severe obesity problems due to premature stop codon insertion in the cytoplasmic domain of LEPRb mRNA transcripts (71). The role of remaining isoforms is not yet so well known.

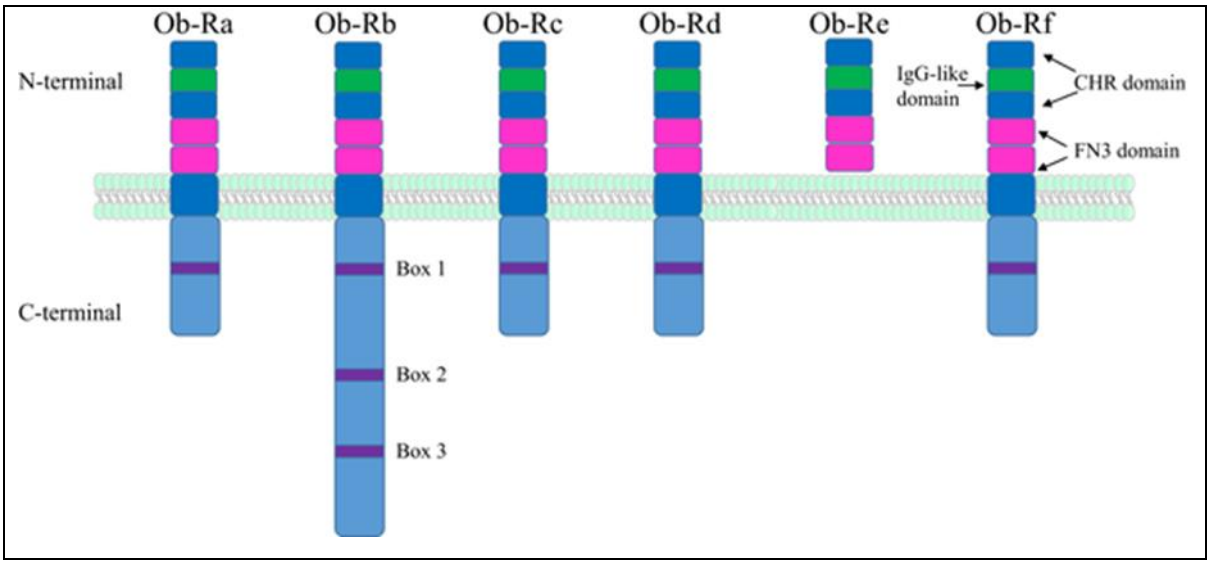

Figure 1.4. Topology of leptin receptor isoforms

1.2.2. Leptin signaling pathway

Upon binding of leptin, LEPRb homodimerizes and undergoes conformational changes that enable JAK2 activation and then phosphorylation of multiple tyrosine residues which includes Tyr1138, Tyr1077 and Tyr985 in the cytoplasmic domain (69). Signal transducer and activator of transcription 3 (STAT3) are recruited by phosphorylated Tyr1138, while ERK signaling cascades are initiated by phosphorylation of Tyr985 and protein tyrosine phosphatase SHP-2 (or protein tyrosine phosphatase non-receptor type 1 PTPN1). SHP-2 mutations observed in obese mice confirmed its role in negative feedback in leptin signaling (72). Phosphorylation of Tyr1077 induces STAT5 phosphorylation affecting transcription of target genes. STAT5 deletion in mice increases food intake and risk of obesity, but it is less severe compared to STAT3 deletion (73). JAK2/STAT3 signaling pathway regulates energy homeostasis and neuroendocrine function. Phosphorylated STAT3 (pSTAT3) homodimerizes, then translocate to the nucleus and binds as a regulatory sequence in various genes and stimulates their transcription, including suppressor of cytokine signaling 3 (SOCS3) (70, 74).

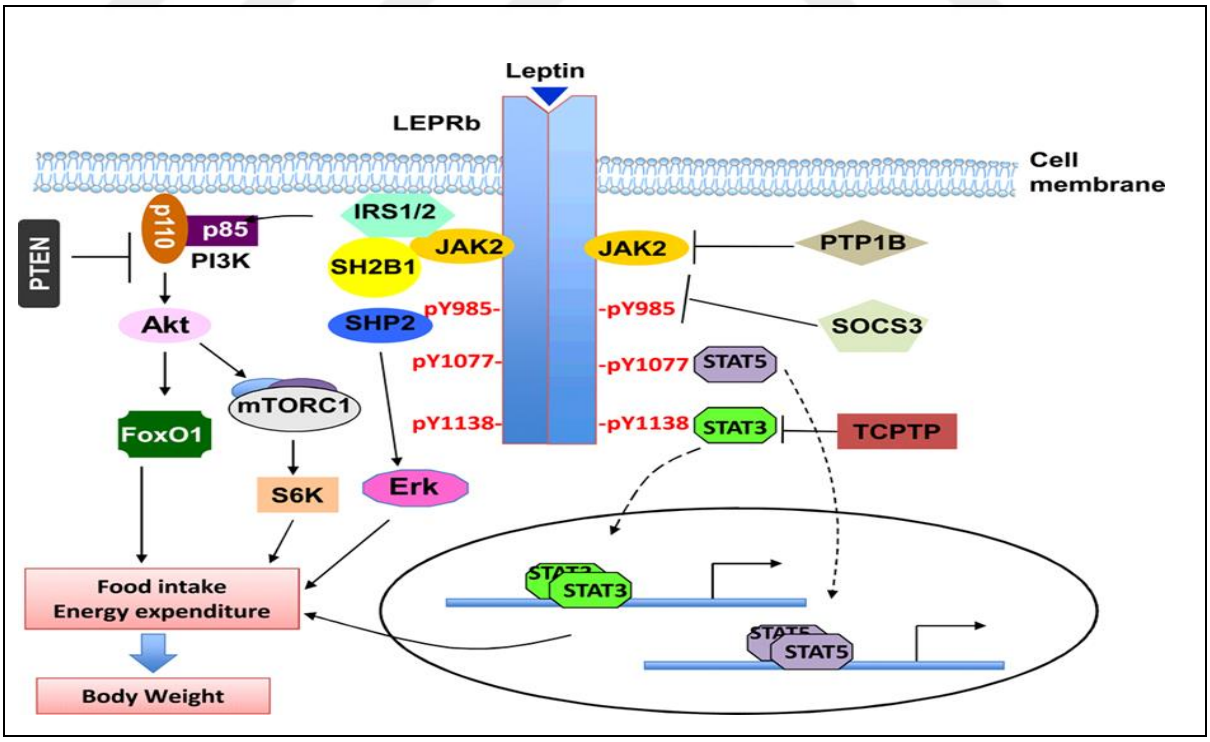

Figure 1.5. Leptin signaling pathways.

LEPRb signaling induces SOCS3 and PTP1B expression that inhibits leptin signaling (75, 76). SOCS3 bind to Tyr985 for initiating the inhibition of LEPRb/STAT3 signaling (77).

1.2.3. Interaction between leptin and hypothalamus

Although molecules that have a role in leptin transport remain unknown, therewithal LEPRa has been thought to regulate leptin transportation (78). Higher LEPRb expression occurs in different hypothalamic areas (79). Two neuronal groups in the arcuate nucleus (ARC) are related to harboring LEPRb. Regulation of agouti-related peptide (AgRP) and neuropeptide Y (NPY) expression is controlled by first-group neurons. NPY is appetite-stimulating (orexigenic) hormone in the central and peripheral nervous system. AgRP provides melanocortin-3 (MC3R) and melanocortin-4 (MC4R) receptor signaling inhibition. The second neuronal group is responsible for the proopiomelanocortin (POMC) and cocaine-amphetamine-regulated transcript (CART) expression. POMC neurons project to second-order neurons in the PVN, DMN, LH and VM. The second-order neurons process the received information and provide a projection of multiple neuro circuits in the extra hypothalamic area. After all of these processes, integrated response on food consumption develops. Destructions of the PVN and DMN produce hyperphagia and obesity, whereas deletion of the WMN and LH result in hypophagia and lean phenotype (80).

POMC is cleaved to anorexigenic peptides and alpha melanocortin stimulating hormone (αMSH). αMSH decrease appetite by activating the receptors MC3R and MC4R (77, 78).

1.2.4. Leptin receptor gene variants and obesity

LEPR Q223R (rs1137101), K109R (rs1137100), and K656N (rs1805094, formerly rs8179183) gene polymorphisms are the most common variants in obesity research. Three missense (non-synonymous) SNPs are located in exons 6, 4, and 14, respectively (81). The The K109R (A→G) transition occurs in exon four and results in the conversion of lysine to arginine (Lys/K to Arg/R) (51).

Q223R variant located in exon 6, results from an A → G substitution at position 668. This transition changes a neutral amino acid (glutamine) to a positive charge amino acid (arginine)(Gln/Q to Arg/R) (51). The LEPR Q223R gene variant occurs in the extracellular domain of the leptin receptor and could affect leptin signaling. Studies have associated this

polymorphism with higher leptin levels in obese subjects (82, 83). In obese subjects, the percentage of homozygous for the R223 allele was found higher in a Greek population. The association between human adiposity and Q223R polymorphism had observed among Caucasians. In particular, higher BMI, %FM (percent fat mass) and plasma leptin levels were reported in middle-aged males who were carried R223 allele (84). Yiannakouris et al. (83) reported the effect of the Q223R gene variant on leptin resistance.

The LEPR K656N SNP is a G→C transition (AAG→AAC) in exon 14 and causes a non-conservative change: lysine (K) to an asparagine (N). Numerous studies have examined the possible role of LEPR K656N, Q223R, and K109R gene variants in obesity and reported contradictory conclusions. Rosmond et al. (85) reported the association between lower BMI and K109K homozygotes in the Swedish population. In the Dutch population, the link between Arg109 or Arg223 alleles and high leptin levels and weight gain have reported (86). Quinton et al. (87) determined that 223Q carriers of British women have higher BMI and fat mass. In the Afro-Caribbean population, K656N polymorphism had determined as a risk factor for the emergence of obesity.

1.3. DOPAMINE

Dopamine (3-hydroxytyramine; DA) is a neurotransmitter first identified by Arvid Carlsson and his team in 1957. Together with epinephrine (adrenalin) and norepinephrine(noradrenaline), dopamine belongs to the family known as catecholamines (88). Dopamine is also necessary for adrenalin and noradrenalin synthesis as a precursor (89).

Dopamine stimulates motivation, reward and pleasure, attention, memory, lactation, nausea, and sexual behaviors in humans (90, 91, 92). Dysfunction of dopamine is related with a various psychiatric and neurological disorders (93) including bipolar disorder, schizophrenia, Parkinson's diseases, binge eating disorder, and addiction (94, 95).

Production of dopamine occurs in different brain regions, including the substantia nigra and the ventral tegmental area (VTA). In the periphery, this neurotransmitter is synthesized in the adrenal glands and has a role in the fight-or-flight response (96, 97, 98) Therewithal,

Meiser et al. (99) reported that dopamine also produces in the pancreas, spleen, and gastrointestinal tract. The synthesis of dopamine begins with the amino acid tyrosine within catecholaminergic neurons. Tyrosine is a rate-limiting enzyme produced by the liver and transported to the brain. Conversion of tyrosine to L-dopa is occurred by adding a hydroxyl group to the tyrosine. Following this, dopa decarboxylase removes a carboxyl group from L-dopa, converting it into dopamine (100). Then vesicular monoamine transporter 2 (VMAT2) regulates dopamine packaging and release (101, 102). VMAT2 is also responsible for the amount of dopamine in the cytosol to protect cells from their neurotransmitter toxicity and degeneration (103). Dopamine transporter (DAT) is a membrane protein and regulates both extracellular and intracellular dopamine concentrations and the termination of DA neurotransmission (104). Recaptured dopamine is stored into the synaptic vesicles again by VMAT2 or degraded enzymatically by catechol-o-methyl transferase (COMT) and monoamine oxidase B (MAO-B) (105).

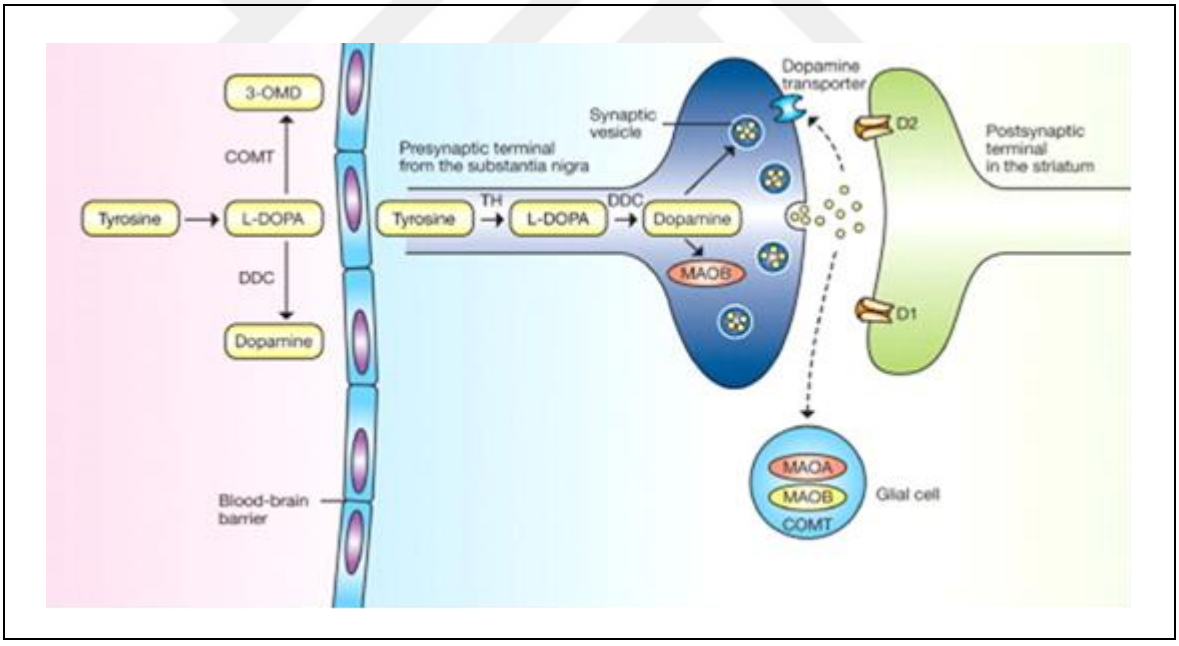

Figure 1.6. Dopamine metabolism pathways

1.3.1. Dopamine receptors

Dopamine receptors is a member of G protein-coupled receptors (GPCRs) and divide into D1-like (short i3, long C-terminal) (D1, D5 (D1B)) and D2-like (long i3, short C-terminal) (D2, D3, and D4) subfamilies according to sequence similarities and G protein coupling

specificity (106, 107). Meanwhile, studies have shown two pseudogenes that encode truncated nonfunctional receptor forms for the D5 receptor (108, 109).

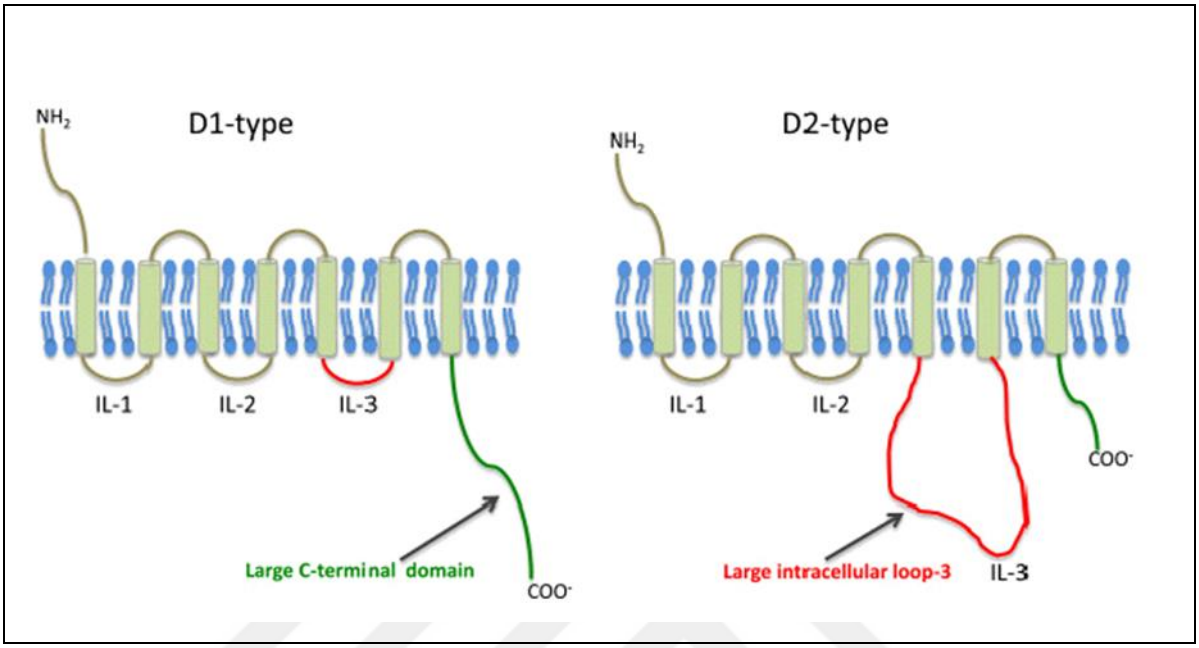

Figure 1.7. Comparative differences between D1-and D2-type receptors

Dopamine receptors show high-level sequence similarities in the transmembrane region and have different pharmacological properties. D1 and D5 receptors share 80% sequence homology. D2 and D4 receptors show 54% sequence homology, whereas the homology between D2 and D3 is 76%. D1 and D2 receptor display only 44% sequence homology (110).

Dopamine D1 and D2 receptors differ in their coding sequences due to the intron absence or presence. Dopamine D1 and D5 receptor-coding genes do not have introns, while genes coding D2-like receptors include several introns, hence having the potential to be alternatively spliced (111, 112). For example, through alternative splicing, the D2 receptor generates two distinct isoforms, the long receptor form (D2L) (444 amino acids) and the short receptor form (D2S) (415 amino acids). The difference between the D2 receptor's long and short forms, long forms have an extra 29 amino acids encoded by exon 6 (113, 114, 115).

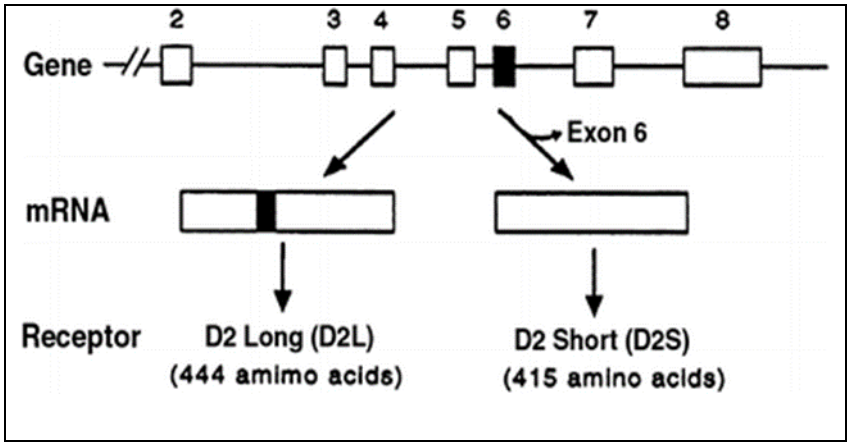

Figure 1.8. Structure of D2LR and D2SR genes and mRNAs

1.3.2. Dopamine signaling pathways

Dopamine D1-like receptors stimulate the heterotrimeric Gαs/olf family of G proteins, induce adenylyl cyclase (AC) activity, second messenger cyclic adenosine monophosphate (cAMP) production, and protein kinase A (PKA) activation. Activation of PKA increases DARPP-32 (dopamine and cAMP-regulated phosphoprotein, 32-kDa) phosphorylation in the threonine-34 residue (Thr34), which resulting protein phosphatase-1 (PP1) inhibition (116). Meanwhile, DARPP-32 phosphorylation in the threonine-75 residue by cyclin-dependent kinase 5 (Cdk5) causes inhibition of PKA. D2-like receptors activate Gαi/o proteins and inhibit PKA activity by decreasing cAMP concentration. Dephosphorylation of DARPP-32 regulates by the protein phosphatase Calcineurin (CaN), activated by D2-class receptors (117). Stimulation of the MAPK (mitogen-activated protein kinase) signaling pathway by D1, and D2-like receptors demands different surface proteins. D1-like receptors mediated MAPK signaling pathway by interacting with the NMDA glutamate receptor. Following that induce ERK activation and increase intracellular calcium levels. D1 and D5 receptors increase intracellular Ca2+ levels by interacting with D1-like receptor-interacting (calcyon) protein. Defective calcyon proteins have been found related to attention-deficit/hyperactivity disorder (ADHD) and schizophrenia (118, 119). MAPK activation through D2-like receptor regulated by protein kinase C (PKC) and tyrosine kinases phosphorylation. D2-like receptors also regulate the Akt and GSK3 signaling pathways.

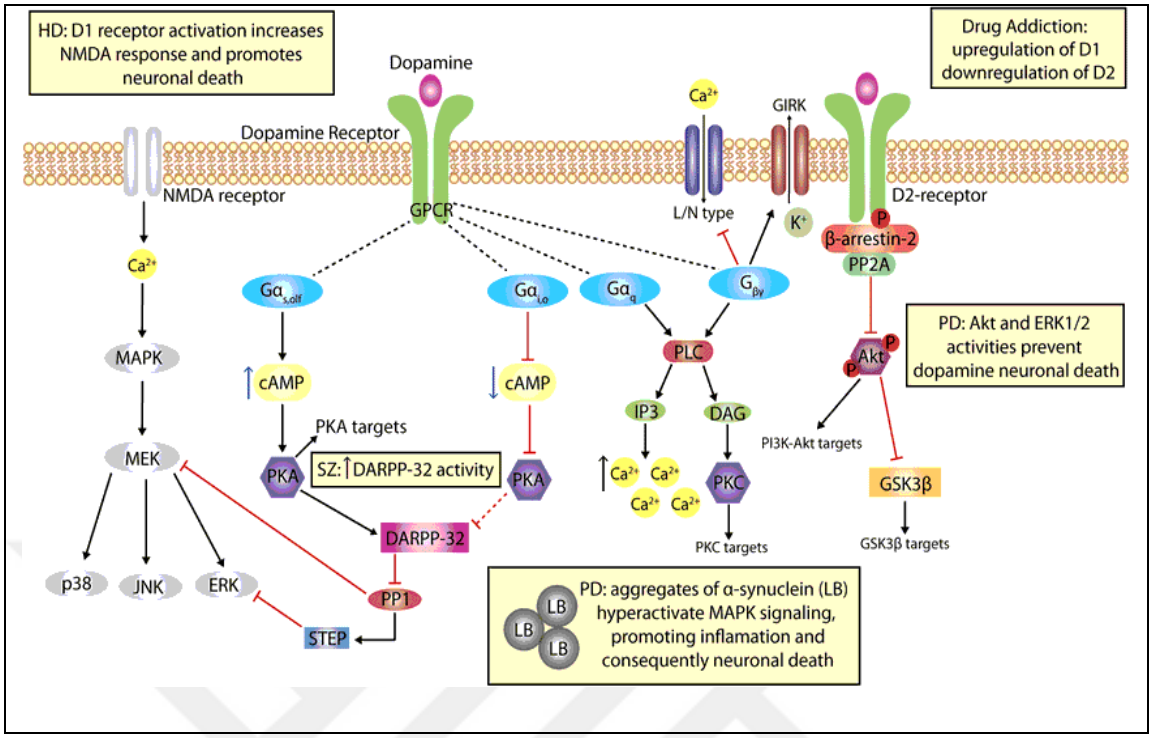

Figure 1.9. Dopamine receptor signaling pathways

The four main dopaminergic pathways are mesolimbic, mesocortical, nigrostriatal, and tuberoinfundibular. The mesocortical pathway regulates dopaminergic projections from the VTA to the septohippocampal regions and prefrontal cortex and is relevant to cognitive and emotional behaviors. In particular, increasing dopamine availability in the prefrontal cortex is related to memory and attention. Originating from VTA, the mesolimbic pathway spread to the amygdala and nucleus accumbens (NAc). Dopamine regulates addictive behaviors through this pathway. The nigrostriatal pathway is related to coordination movement and give us motivation to seek basic needs. Dopamine projections originate from the substantia nigra and reach the putamen and caudate. The tuberoinfundibular pathway which originates in the arcuate nucleus and travels to infundibular region of the hypothalamus. Dopamine connects to the pituitary gland and inhibits prolactin release through this pathway.

Medium spiny neurons (MSNs) in the NAc contain dopamine receptors (both D1 and D2) and play a role in food consumption (120).

1.3.3. Dopamine D2 receptor and obesity

Studies showed that mice fed a high-fat diet had fewer dopamine receptors than mice fed a control diet. However, expression levels were back to normal following bariatric surgery (121, 122). The effect of bariatric surgery on D2 receptor availability is controversial. Clinical studies have examined the density of the D2 receptors following bariatric surgery and have found an increase in D2 availability (121, 123), a decrease in D2 availability (124), and no changes (125).

Wang et al. (126) investigated D2 receptor density in morbidly obese subjects using positron emission tomography (PET). Like in animal models, results showed decreased dopamine D2 receptor activity in the striatum (124, 127). Dunn et al. (124) observed that brain regions associated with eating behaviors have lower D2 density after bariatric surgery. Also, it had suggested that increased dopamine levels contribute to improved eating behaviors after surgery and positively influence the reward.

Numerous studies demonstrated that polymorphisms of the DRD2 gene, especially the TaqI A1 polymorphism (the risk allele is T, also known as the A1 allele) associated with eating disorders, including obesity (50, 126, 128, 129, 130, 131, 132).

TaqI A1 polymorphism occurs in Ankyrin repeat and kinase domain containing 1 gene (ANKK1) and causes a glutamic acid to lysine substitution in serine/threonine kinase (131, 133). The risk allele of TaqI A1 polymorphism is linked to low dopamine D2 receptor activity in the brain. Individuals who carried the A1 allele were found less sensitive to dopamine reward-based circuitry (42, 130). rs1800497 T allele related to increased BMI in some studies, while other studies hadn't shown association. Roth et al. demonstrated that homo or heterozygous T allele carriers were prone to binge eating than C allele carriers. By the way T allele are the risk for the lifestyle interventions to weight loss in these individuals. In adolescent girls, it had reported that the T allele and the probability of being obese are associated (130). Cardel et al. (132) reported that total body fat and the risk for cardiometabolic diseases increased in children with two T alleles than in one or no risk alleles. The data obtained from studies determined that the T allele is a key risk factor for early-onset obesity, adolescent or adult-onset obesity, and high carbohydrate consumption (134). However, Davis et al. found that C allele carriers show a higher tendency to binge

eating, emotional eating, and hedonic eating compared to T allele carriers. Carpenter et al. (50) reported that carriers of one or more A1 alleles have a higher BMI compared to non-carriers. Studies also demonstrated the frequency of the T allele shows a difference between ethnic/racial groups.

1.4. OPIOIDS

Opioids and their derivatives were used for centuries in pain management and related diseases, whereas the discovery of opioid receptors and opioid-like peptides lasted until the 1970s. In 1973, the existence of opioid receptors was reported independently by three laboratories (135, 136, 137). In 1979, Holtzman et al. (138) first reported the role of endogenous opioids in appetitive behaviors (139).

1.4.1. Opioid receptors

There are four groups of opioid receptors: mu, (MOR, μ), delta (DOR, δ), kappa (KOR, κ), and the non-opioid receptor nociceptin (NOP). Also, a family of endogenous peptides, especially enkephalins, β-endorphins, and dynorphins, have been defined that show different affinities to opioid receptors (140,141).

As a member of the G-protein-coupled receptors (GPCR) family, opioid receptors consist of seven transmembrane helices (7TM), an extracellular N-terminus, and an intracytoplasmic C-terminus tail. Seven transmembrane domains (TM1-TM7) are linked by three intracellular loops (i1-i3) and three extracellular loops (e1-e3) (142, 143).

Opioid receptors activate Gi/Go proteins and trigger the regulation of the MAP kinase and the phosphatidylinositol-3 kinase (PI 3-kinase) pathways, cAMP, Ca2+, and K+ channels (144).

MOR, KOR, and DOR receptors are encoded by OPRM1, OPRK1 and OPRD1 genes (145). According to the amino acid sequences, the δ and μ receptor genes show 62% homology, the δ and κ receptor genes show 61% homology, and the μ and κ receptor genes show 57% homology (145). Transmembrane domains are highly preserved between opioid receptors

(except TM4 and 3 intracellular loops). However, N-terminus, C-terminus, and extracellular loops show a considerable difference in sequences.

Opioid receptors are generally located in the hypothalamus (regulating homeostatic signal) and in the mesolimbic dopaminergic pathway (modulating hedonic signal). It first was reported that blocked opioid receptors cause decreasing food intake by using opioid antagonist naloxone (138). Since then, several studies have demonstrated that opioid receptors antagonists reduce body weight by suppressing appetite in rodents, including diet-induced obese and Zucker (fa/fa) rats (146, 147, 148). Naltrexone and naloxone are both opioid antagonists and help reduce weight in the short term in human studies. However, using a long-term period cause showing upside effects and limited weight loss (149). Reward circuitry of food is regulated by the mesolimbic dopamine pathway that is projected from the VTA to the NAc. Endogenous opioids also modulate dopamine signaling both at the VTA and the NAc. Interaction with GABAergic neurons cause elevated dopamine levels (150). Thus, overeating, and concomitant opioid release could result in the downregulation of opioid receptors and the development of obesity (151).

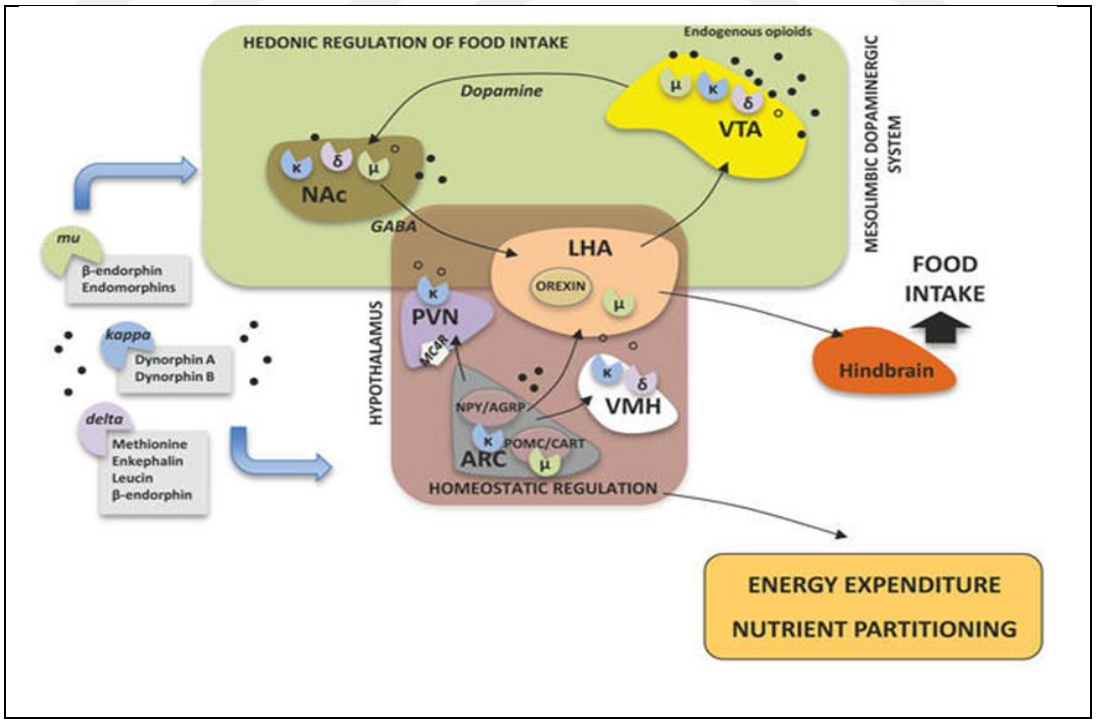

Figure 1.10. Effects of the opioid system in energy balance

1.4.2. μ -opioid receptor

μ-opioid receptors (MOR) are encoded by the opioid receptor μ 1 (OPRM1) gene and has multiple isoforms. These isoforms could differ in their structures or be truncated. Exon 1 (E1) and exon 11 (E11), two distinct promoters of the OPRM1 gene, are well-conserved in both humans and rodents. According to their receptor structure, these promoters generate multiple variants: 1) Full-length 7-transmembrane (7-TM) domains C-terminal variants (E1) 2) Truncated variants containing 6 TM domains (E11); and 3) Truncated variants containing a single TM domain (152, 153, 154, 155).

The expression of MOR receptors had been found highly, particularly in NAc, VTA, and the hypothalamus (156). It has demonstrated that naloxone injection into the VTA, NAc, and LH reduces food consumption in mice.

Opioid receptors regulate their functions through GABA neurons in several brain regions. Mu opioid receptors increase the activation of dopamine receptors by inhibiting GABA neurons (157, 158). Numerous studies have confirmed long-term high intake of palatable food cause reduced MOR availability in the mesolimbic dopamine pathway. Comparing high-fat diet-fed mice with low-fat diet (standard) controls, found out MORs mRNA levels decreased in the VTA and NAc in the first group (159, 160). Khajawa et.al. determined the downregulation of μ-opioid receptors in genetically obese mice (ob/ob) compared with lean mice. Opioid receptor antagonist naltrexone had shown to suppress feeding in mice and human studies, but using naltrexone provides minimal or no significant weight loss. These results demonstrate that the effectiveness of naltrexone is affected by the mu-opioid receptor changes.

Other studies reported that the modulation of orexin neurons causes stimulation of MORs in the NAc and increases consumption of palatable foods (161, 162).

1.4.3. OPRM1 gene polymorphism and obesity

Numerous studies supported that μ-opioid receptors activation in the ventral striatum and amygdala increases appetite for the consumption of palatable foods (156, 163). Nummenmaa et al. (48) suggested that individuals could show different feeding behaviors depending on MOR density in the amygdala. μ-opioid receptor density and opioid system activation had

related to an increased tendency to high sweet and high-fat foods. So, MOR availability could affect weight loss following bariatric surgery (164). Studies have shown that enhanced μ-opioid signaling results in high palatable food intake before and during pregnancy and maternal obesity (165).

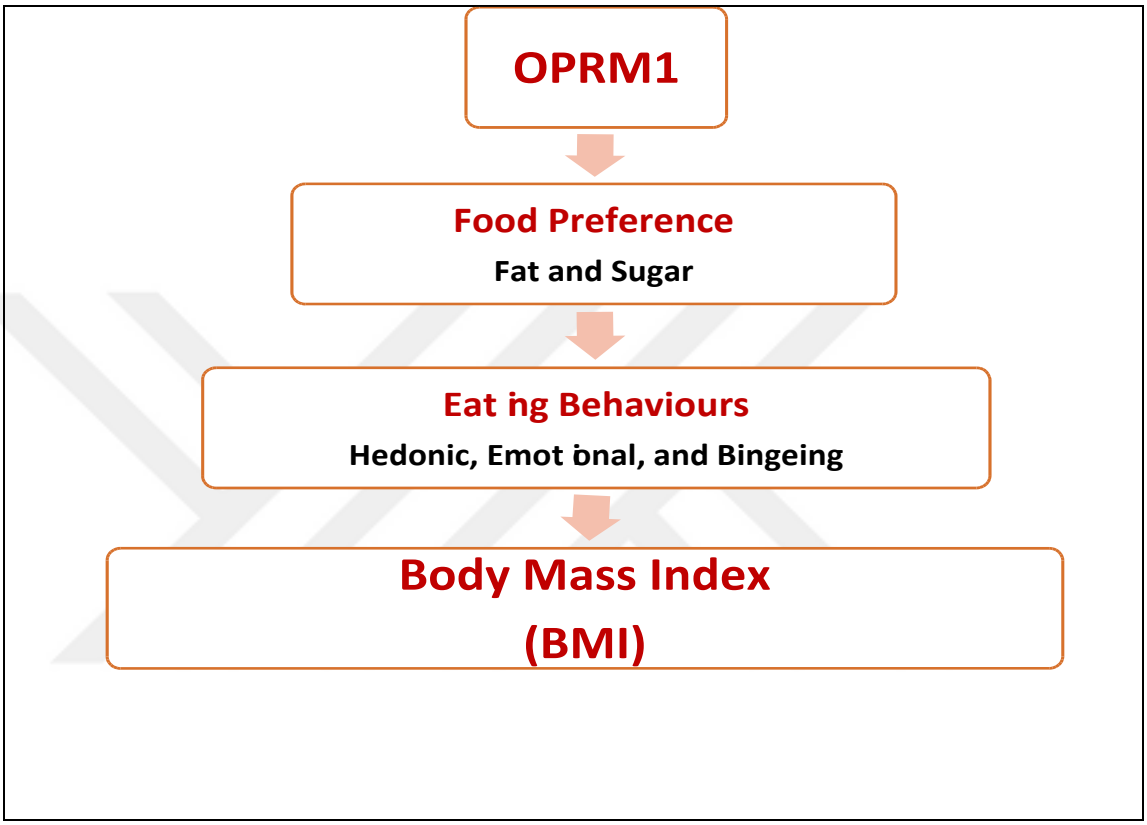

Figure 1.11. Sugar and fat food preference results in overeating and high body weight and is associated with the OPRM1 gene.

However, Papaleo et al. (140) demonstrated that MOR-knockout mice showed less motivation to eat both chow and sucrose pellets than the wild-type mice.

According to the whole genome sequencing, 3,324 SNPs have identified in the OPRM1 gene. Between them, in humans, non-synonymous SNP (rs1799971) is the most extensively investigated. This SNP located on exon 1, which results in the replacement of A to G (A118G), causing asparagine to aspartic acid (N40D) exchanges in the N terminal of the receptor. The A118G gene variant is also responsible for opioid dependence and MOR receptor density. According to the in vitro study, the G allele carriers show increased binding affinity for morphine and ß-Endorphin three times (166). However, subsequent studies

reported a lower receptor binding site availability in the variant G allele. Thus, this variant is linked with loss of function and decreased mRNA and protein expression. Such as, Klepstad et al. (167) have reported decreased MOR and mRNA expression in the human brain.

Allelic frequencies and genotype distribution of the SNP rs1799971 are different between ethnic groups (167, 168). In African Americans, G allele frequency had found 2.2%, however, this rate was 27%-48% in the Asian population and 11%-17% in Caucasians (169).

Numerous studies demonstrated the association between the G "gain of function" allele and high hedonic responsiveness to palatable foods. Davis et al. determined that among obese individuals, A1 allele carriers were higher compared with the binge eating disorder (BED) group. However, the frequency of the A118G gene variant was high in the BED patients. Due to the results, the A1-/G+ group is described as a hedonic-enhanced genotype and A+/G- group as a hedonic-diminished genotype. Another study determined that the GG homozygotes exhibited a strong desire to consume sweet and fatty foods than the GA and AA groups (170).

Extensive evidence on the MOR receptors in feeding makes them one of the therapeutic targets in obesity treatment.

1.5. BARIATRIC SURGERY

Recently, bariatric surgery is using to alleviate the negative consequences of obesity. It helps remission of obesity-related comorbidities, sustains weight loss, and improves the quality of life (171). Bariatric procedures like gastric banding, sleeve gastrectomy, and Roux-en-Y gastric bypass (RYGB) are used to curb obesity (172). The major issue is weight gain observed from 2 to 5 years after surgery (173). Buchwald et al. determined that effective weight loss is achieved in morbidly obese subjects and observed 56.7 and 66.5% loss of excess weight within 24 months after surgery. Another study has been shown weight regain observed in 30% of patients between 18-24 months (174). According to the studies comparing results after 5 and 7 years of bariatric surgery, failure rates range from 5 to 7% (175, 176, 177). However, super-obese subjects showed high failure rates from 20 to 33% in this term (174, 178).

Patients show different patterns in losing weight after surgery, and several factors are responsible for this situation. Genetic factors are one of them and have been found relevant to bariatric surgery outcomes. Bandstein et al. (179) demonstrated that SNPs in obesity-related genes significantly affect weight loss following RYGB surgery. They observed that carriers of none or a small number of risk alleles lose weight more efficiently. The effect of distinct SNPs after surgery is interesting, and due to these genetic variabilities, post-surgery weight loss between individuals can be different. So, surgery type, methods used to evaluate weight loss, and genetic variant analyses may be beneficial in obesity treatment (180, 181).

We aimed to explore polymorphisms that could play a role in eating disorders in patients who underwent bariatric surgery. Consequently, the identification of polymorphisms that are possibly linked to weight gain after surgery can provide a different perspective on additional treatment options in obese patients who are followed up clinically. DRD2 TaqI A1, LEPR K109R, K656N, and Q223R and OPRM1 A118G gene polymorphisms could be predictive in weight regain after bariatric surgery.

DRD2 TaqI A1 gene variant has been proven related to decreased D2 receptor density in the brain, which results in insufficient dopamine activity. Studies have shown that A1 allele carriers tend to overeat to compensate for this insufficiency. So, TaqI A1 polymorphism could be associated with eating behaviors and obesity (182, 183, 184, 185).

Among the polymorphisms in the OPRM1 gene, A118G polymorphism is the most common variant linked with BED. Studies have determined the relationship between the G allele and high sweet and fatty food preferences, binge eating disorders, and obesity (167, 170, 186).

Leptin is known as one of the regulators of body energy resources, and BMI. LEPR gene variants like K656N, K109R, and Q223R are risk factors for developing obesity. According to studies, these polymorphisms lead to leptin resistance by affecting receptor function and altering signaling mechanisms. Therefore, it has hypothesized that these variants could affect the pathophysiology of obesity (62, 82, 87, 187).

2. MATERIAL AND METHODS

2.1. COLLECTING SAMPLES

Five hundred and sixty-six obese subjects (466 women and 100 men) (BMI 35–40 kg/m2, BMI ≥40 kg/m2) had screened for the study. Bariatric surgery had performed on patients with a BMI >40 kg/m2 and patients with a BMI >35 kg/m2 and one or more pivotal health problems, including type II diabetes (T2DM), hypertension, and non-alcoholic fatty liver disease. The control group consisted of 179 randomly chosen, healthy subjects (100 women and 79 men) (18.5>BMI). Ethylenediaminetetraacetic acid (EDTA) tubes had used for collecting blood samples at SBÜ Fatih Sultan Mehmet hospital. Following brought to the Yeditepe University Genetic Diagnosis Center for subsequent DNA isolation and PCR analysis. DTAB-CTAB method was used for DNA isolation. Before using, genomic DNA was stocked at -20ºC.

2.2. DNA ISOLATION

2.2.1. The preparation of Dodecyltrimethylammonium (DTAB) buffer

1. 4 g DTAB (8%)
2. 0.605 g Tris (0.1 M)
3. 3.5 g of 1.2 M NaCL
4. 0.930 g EDTA (0.05 M)
5. 50 mL ddH_2O

The pH was adjusted to 8 by adding HCL.

2.2.2. The preparation of Hexadecyltrimethylammonium bromide (CTAB) buffer

1. 1.16 g NaCL (400 mM)

2. 2.5 g CTAB (5%)
3. 50 mL ddH_2O.

2.2.3. The preparation of NaCL:

1. 7.01 g NaCI (1.2M)
2. 50 mL ddH_2O

2.2.4. 70% ethanol solution

30 mL ddH_2O was added to 70 mL ethanol to prepare 100 mL 70% ethanol

2.2.5. 1X TE buffer contain:

1. Tris-HCI 10 mM, pH 8.0
2. EDTA 0.1 mM

2.2.6. The steps of DNA isolation by using DTAB and CTAB:

- 400 µl of DTAB buffer was added to 300 µl of blood samples and then was incubated at 65°C for 5 minutes.
- Immediately adding 500 µl of chloroform into the tubes, they were shaken rapidly at room temperature for at least 5 minutes.
- After centrifuging at 13.000 rpm for five minutes, the tubes were removed.
- A new tube was used to collect the aqueous layer. After that, 100 ml of CTAB buffer and 900 ml of deionized water (or nuclease-free) was added.
- An incubation of 10 minutes at -20 °C was followed by centrifugation of samples for 5 minutes at 10.000 rpm to remove the supernatant.

- To form the pellet, 200 µl of 1.2 NaCl and 300 µl of cold 100% ethanol had added. Following centrifugated at 10.000 rpm for 5 minutes, the supernatant was discarded.

- 700 µl of 70% ethanol was used to remove residues from the pellet. After that dried for ethanol evaporation.

Resuspension of the pellet has done by using TE buffer or nuclease-free water (60µl).

2.3. PURITY MEASUREMENT OF DNA

Following DNA extraction, the purity and concentration of DNA had checked by the spectrophotometer (NanoPhotometer, İmplen, Germany). Adsorption of light at 260 nm wavelength by nucleic acids allows us to determine the concentration according to the photodetector measurements. Determination of protein contamination occurs at 280 nm wavelength. Three microlitres (3 µl) from each sample were used. The generally accepted purity ratio ranges for pure DNA are 1.8–2.0 in the 260/280. Storage of samples at − 20 °C had done until use.

2.4. HIGH RESOLUTION MELTING CURVE ANALYSIS (HRM)

Primers were designed using Primer-BLAST and synthesized standard molecular biology quality (Table 1).

Table 2.1. Primers for SNP analysis

SNP	Primer	Amplicon size
DRD2, rs1800497	5’-CTCTAGGAAGGACATGATGCCC-3’ -F 5’- GCAACACAGCCATCCTCAAAG-3’-R	128bp
OPRM1, rs1799971	5’-GTAGAGGGCCATGATCGTGAT-3’-F 5’ -GCTTGGAACCCGAAAAGTCT-3’-R	301bp
LEPR, rs1137100	5’-AACTTTCCACTGTTGCTTTCG-3’-F 5’-CATTGTTCAATACATTTAAAACATAGC-3’ -R	153bp
LEPR, rs 1137101	5’-ACAGCCAAACTCAACGACAC-3’- F 5’-AGCAAAGTGAGATAAGCTAGCA-3’-R	202bp
LEPR, rs1805094	5’-TGTGTGCTTCAAATATGGCTGA-3’-F 5’- AACAGGATTATGGACCATGAAGT-3’-R	227bp

Five polymorphic loci were taken from the National Center for Biotechnology Information (NCBI) database (Table 2.)

Table 2.2. The substitution of the SNPs

SNP ID	Sequence
rs1800497	TGGACGTCCAGCTGGGCGCCTGCCT[C/T]GACCAGCACTTTGAGGATGGCTGTG
rs1137100	TTATGTGCAGACAACATTGAAGGAA[A/G/T]GACATTTGTTTCAACAGTAAATT
rs1137101	ATCACATCTGGTGGAGTAATTTTCC[A/G/T]GTCACCTCTAATGTCAGTTCAGC
rs1805094	AATGGAGATACTATGAAAAA[G/C/T]GAGAAAAATGTCACTTTAC
rs1799971	GGTCAACTTGTCCCACTTAGATGGC[A/G]ACCTGTCCGACCCATGCGGTCCGAA

HRM analysis consists of PCR amplification, melting phase, and data analysis by gene scanning. The PCR mixture was containing:10 µl Fast Evagreen qPCR Master Mix (Biotinum, California, USA), 2 µl template DNA (100 ng), 0.5 µl forward primer (10 pmol), 0.5 µl reverse primer (10 pmol) and 7 µl dH2O. Amplification was carried out using the following program: preincubation for 2 min at 95°C and amplification for 40 cycles consisted of denaturation at 95°C for 10 s, annealing at 60°C for 30 s and extension at 72°C for 30 s, after which a high resolution melting curve was generate using the following protocol: denaturation at 95°C for 1 min, annealing at 40°C for 1 min, elongation 60°C for 1 min, and cooling at 40°C for 10 s.

Data were analyzed by using the Light Cycler 4800 Gene Scanning Software. Initially, the data were normalized by selecting linear regions before (100% fluorescence) and after (0% fluorescence) the melting transition, then temperature shifts were conducted by moving the curves along the x-axis, and then difference plots were generated.

HRM analysis results had confirmed by selecting at least three samples from the wild type, homozygous mutant, and heterozygous and using DNA sequencing.

2.5. PCR-DIRECT SEQUENCE ANALYSIS.

Sequencing of PCR products was analyzed at the ABI Genetic Analyzer 3500 (Applied Biosystems, Foster City, USA) to confirm the nucleotide polymorphisms.

Cycling sequencing was performed using the Applied Biosystems Bigdye v5.1 (Applied Biosystems, Foster City, USA) kit. For this purpose, a mixture was prepared (Table 3) and subjected to cycle sequencing using the program in Table 4.

Table 2.3. The reaction components for the DNA Sequence Analysis

Reaction components	**Volume**	**Concentration**
Big Dye Ready Reaction Mix	2.59 µl	1x
5x Sequencing Buffer	1.87 µl	1x
Reverse or Forward primer	1.0 µl	10 pmol
dH2O	5.0 µl	
PCR Product	1.5 µl	
Total Volume	11.96 µl	

Table 2.4. Cycle sequencing set up

Step	Temperature	Time	Cycles
Initial denaturation	94 °C	2 min	
Denaturation	94 °C	30 s	
Annealing	50 °C	45 s	30 cycles
Extension	60 °C	4 min	
Hold	04 °C	∞	

After PCR amplification, ExoSapI (Exonuclease I) was used to remove unconsumed primers and dNTPs remainings from the PCR product mix. For this purpose, 1 µl ExoSap and 1 µl ALP (Alkaline Phosphatase) were added to 3 µl PCR product and incubated 20 min at 37°C, and then 20 min at 80°C. Sequences were analysed and alignments were performed using DNA Baser v5.15.0 software.

2.6. STATISTICAL ANALYSIS

Comparison between groups was determined by using Pearson's chi-squared test. Risk estimation and Odds ratio calculation performed. Statistical analyses had done using SPSS 22.0 software.

3. RESULTS

We could evaluate 546 patients out of 566 in obese group and 176 subjects from control group in the study. Females outnumbered males in both obese and control groups (Figure 3.1).

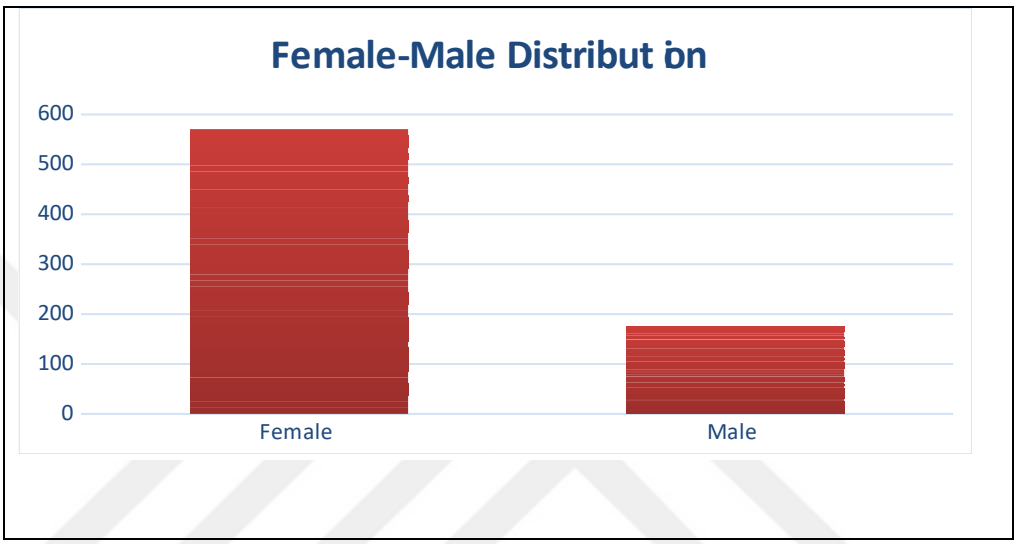

Figure 3.1. Distribution of female and male participants in the study

HRM analysis had used for the identification of five SNP polymorphisms. The melting curve of heterozygous DNA samples differs from one for homozygous DNA samples due to the formation of heteroduplexes during the synthesis process. The genotyping of homozygous samples had determined by evaluating melting temperature (Tm) shifts. The curve of melting profiles of normalized data and direct sequencing results of DRD2 TaqI A1 (Figure 3.2), LEPR K656N (Figure 3.3), K109R (Figure 3.4) and Q223R (Figure 3.5) and OPRM1 A118G (Figure 3.6) have shown below.

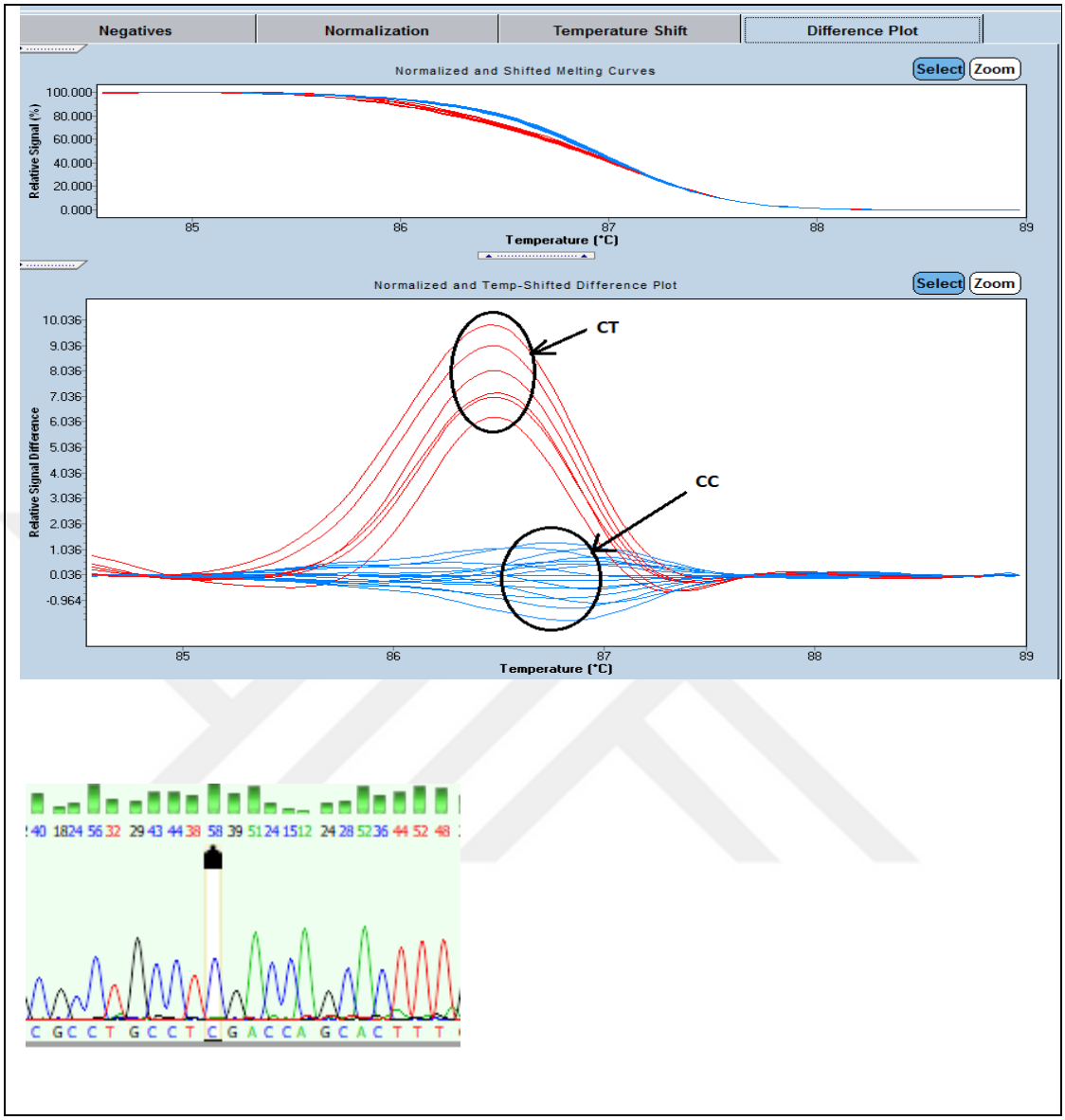

Figure 3.2. HRM analysis of the DRD2 rs1800497 polymorphism.

The two groups were well distinguished: homozygous (wild type) in blue and heterozygous CT in red.

Figure 3.3. The genotyping of OPRM1 rs179971.

Three variants were determined: mutant (GG) in grey, wild type (AA) in blue, and heterozygous (AG) in red.

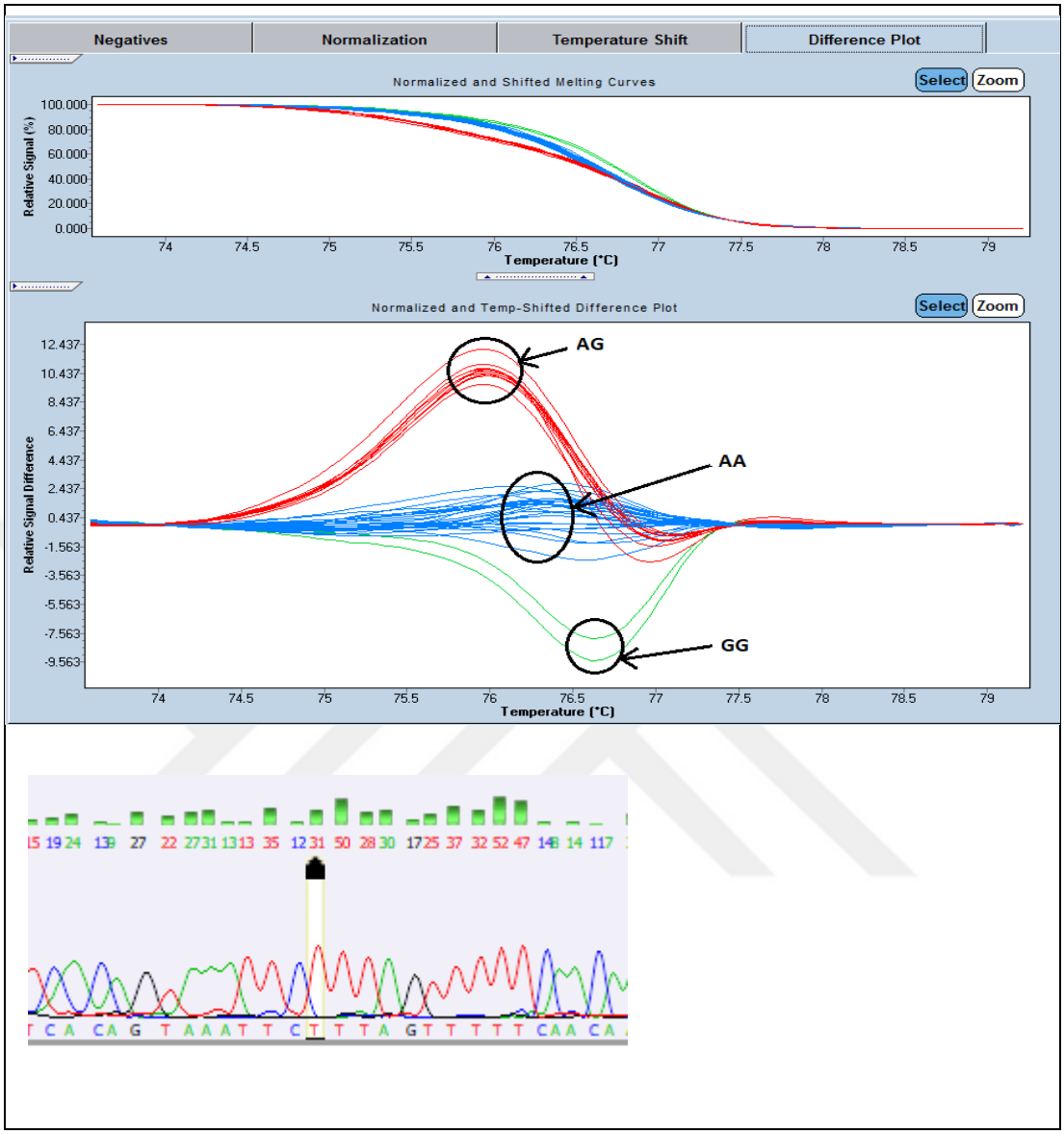

Figure 3.4. The genotyping of LEPR rs1137100.

The three groups were distinguished: mutant GG in green, homozygous (wild type) AA in blue and heterozygous AG in red.

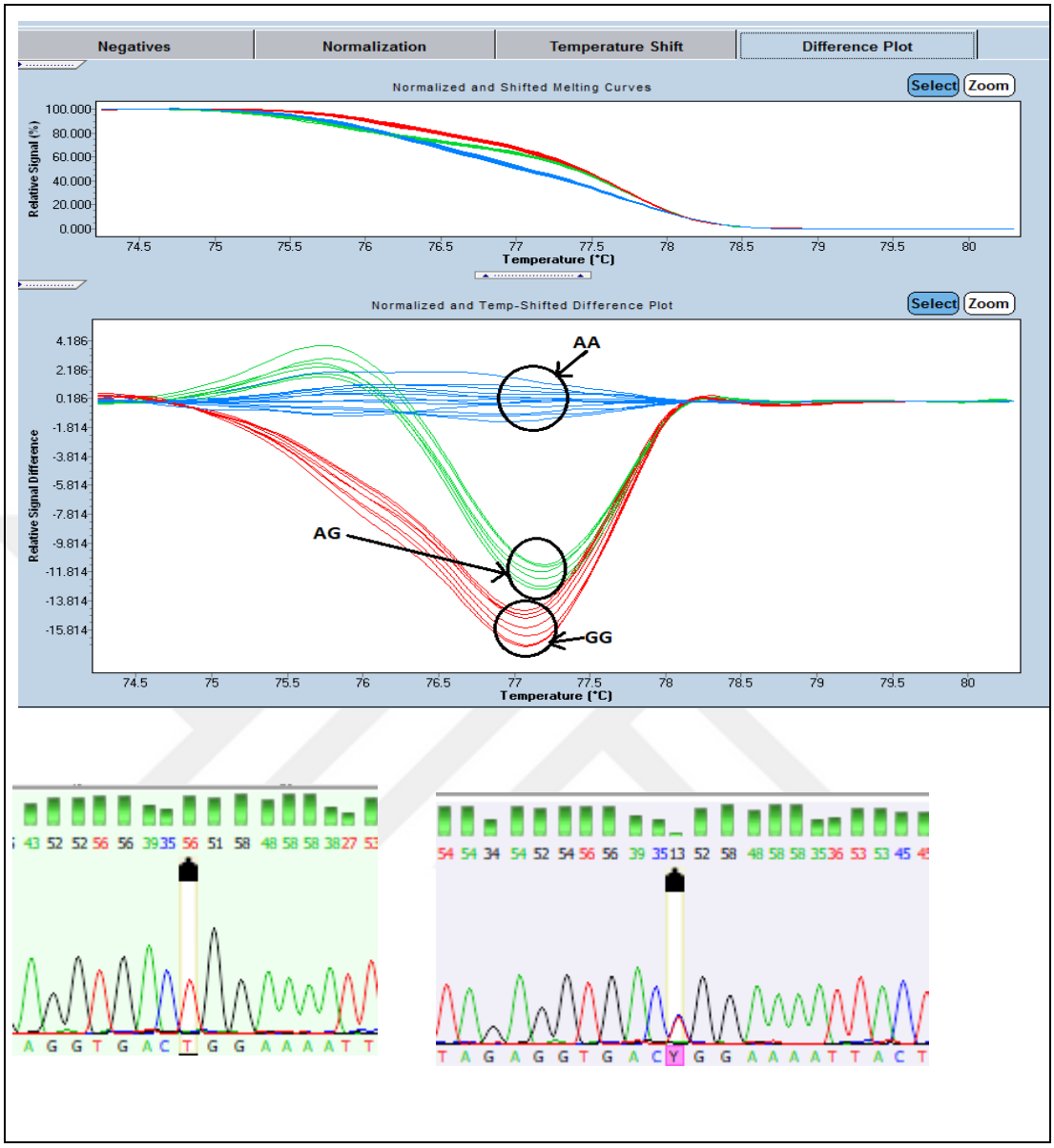

Figure 3.5. The genotyping of LEPR rs1137101.

Three variants: homozygous (wild type) AA in blue, mutant GG in red, and heterozygous AG in green had determined.

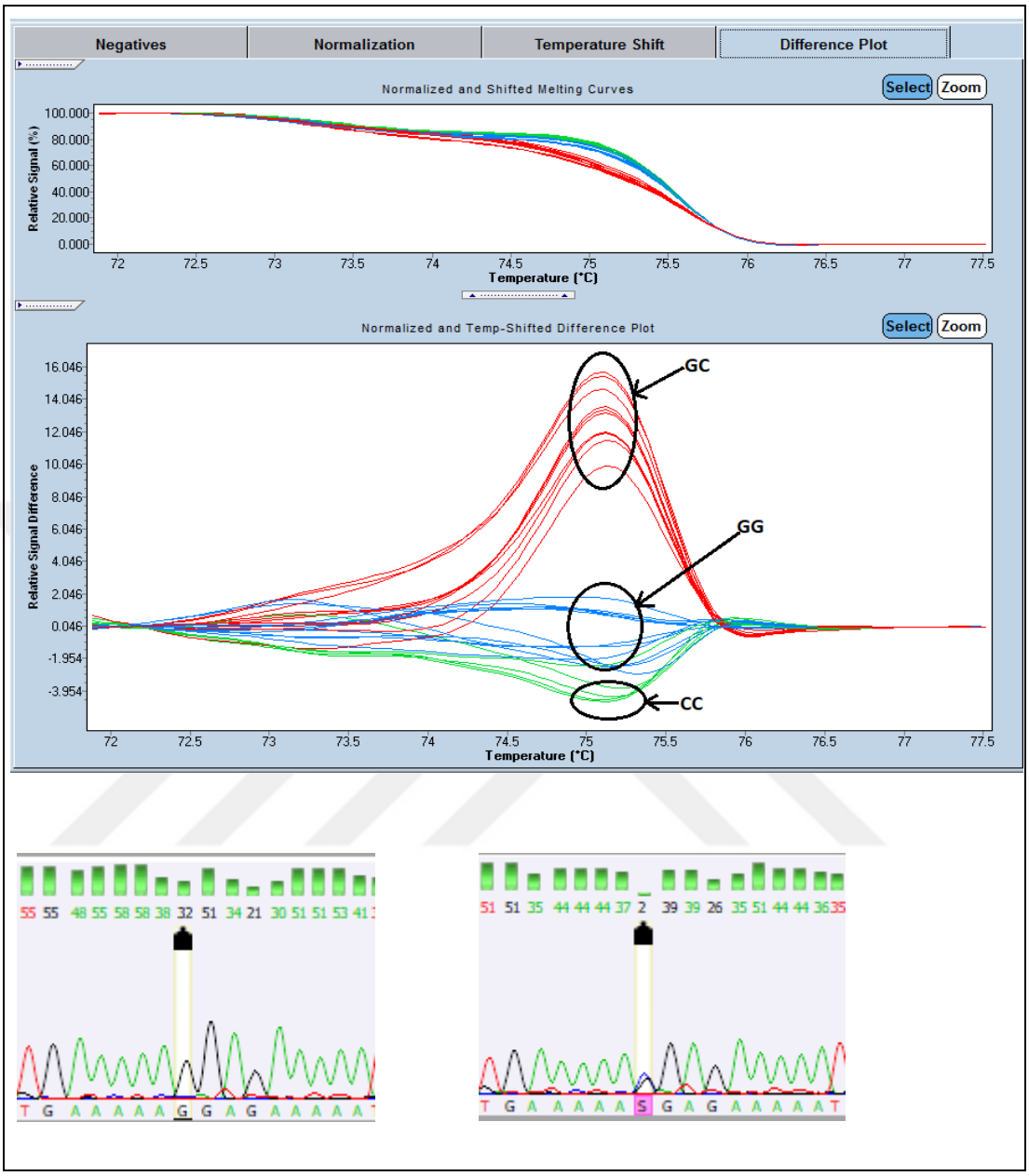

Figure 3.6. The genotyping of LEPR rs1805094.

The three groups were distinguished: mutant CC in green, homozygous (wild type) GG in blue and heterozygous GC in red.

Table 3.1. Allele frequencies of rs1800497

Allele frequency n (%)			**χ^2**	**p**
DRD2, TaqI A1, C>T	C	T		
Patients	880 (81.9)	194 (18.1)	1.385	0.1
Control group	273 (78.4)	75 (27.47)		

The obese and control groups did not show a significant difference in allele distribution (p=0.1).

Table 3.2. Distribution of rs1800497 genotypes

		Genotypes frequency, n (%)					
DRD2, TaqI *A1*		**CC**	**CT**	**TT**	**Total**	**χ^2**	**p**
Patients	n	361	158	18	546		
	%	67.2	29.4	3.4	100		
Control	n	109	55	10	176	2.781	0.427
	%	62.6	31.6	5.8	100		
Total	n	470	213	28	722		
	%	66.1	30	3.9	100		

Genotype distribution between obese and control was not substantial (p= 0.427).

Table 3.3. Allele frequencies of rs1799971

Allele frequency, n (%)			**χ^2**	**p**
OPRM1, A118G, A>G	A	G		
Patients	788 (73.23)	288 (26.77)	5.983	0.001
Control group	273 (79.82)	69 (20.18)		

Statistical significance was determined in the distribution of A and G alleles (p=0.001).

Table 3.4. Distribution of rs1799971 genotypes

		Genotypes frequency, n (%)					
OPRM1, A118G		**AA**	**AG**	**GG**	**Total**	**χ^2**	**p**
Patients	n	330	128	80	46		
	%	61.3%	23.8%	14.9%	100%		
Control	n	111	51	9	176	13.017	0.005
	%	64.9%	29.8%	5.3%	100%		
Total	n	441	179	89	722		
	%	62.2%	25.2%	12.6%	100%		

The GG genotype was found most frequent among obese patients (14.9%) than among control groups (5.3%).

Table 3.5. Allele frequencies of rs1805094

Allele frequency, n (%)			**χ^2**	**p**
LEPR, K656N, G>C	G	C		
Patients	839(80)	209 (20)	0.432	0.511
Control group	263 (77.81)	75 (22.19)		

In the obese and the control group, the LEPR94 gene polymorphism allelic distribution was not different (p=0.511).

Table 3.6. Distribution of rs1805094 genotypes

		Genotype frequency, n (%)				**χ^2**	**p**
LEPR, K656N		**GG**	**GC**	**CC**	**Total**		
Patients	n	320	199	5	546		
	%	61.1%	38%	0.9%	100%		
Control	n	96	71	2	176	0.989	0.804
	%	56.8%	42%	1.2%	100%		
Total	n	416	270	7	722		
	%	60%	39%	1%	100%		

The results obtained from the study did not show any substantial difference in the genotype distribution (p= 0.804).

Table 3.7. Allele frequencies of rs1137100

Allele frequency, n (%)			**χ^2**	**p**
LEPR, K109R, A>G	A	G		
Patients	882 (81.7)	198 (18.3)	0.073	0.32
Control group	280 (80.45)	68 (19.55)		

The allele frequency did not show a substantial difference in obese and control groups.

Table 3.8. Distribution of rs1137100 genotypes

LEPR, K109R		**Genotypes frequency, n (%)**				**χ^2**	**p**
		AA	**AG**	**GG**	**Total**		
Patients	n	364	154	22	546		
	%	67.4%	28.5%	4.1%	100%		
Control	n	115	50	9	176	0.403	0.94
	%	66.1%	28.7%	5.2%	100%		
Total	n	479	204	31	722		
	%	67.1%	28.6%	4.3%	100%		

Obese and control groups did not have substantial differences in genotype distribution (p=0.940).

Table 3.9. Allele frequencies of rs1137101

Allele frequency, n (%)			**χ^2**	**p**
LEPR, Q223R, A>G	A	G		
Patients	759 (70.4)	319 (29.6)	4.077	0.08
Control group	239 (69.48)	105 (30.52)		

Based on the analysis allele frequencies were substantial between obese and control groups.

Table 3.10. Distribution of rs1137101 genotypes

		Genotype frequency, n (%)					
LEPR, Q223R		**AA**	**AG**	**GG**	**Total**	**χ^2**	**p**
Patients	n	263	233	43	546		
	%	48.8%	43.2%	8%	100%		
Control	n	90	59	23	176	7.779	0.05
	%	52.3%	34.3%	13.4%	100%		
Total	n	353	292	66	722		
	%	49.6%	41.1%	9.3%	100%		

The distribution of the LEPR Q223R genotypes between obese individuals and the control group was not statistically significant (p= 0.05). GG homozygosity for LEPR Q223R was observed less frequently in obese subjects (8%) compared with the control group (13.4%).

Table 3.11. Odds ratios and confidence intervals

	Odds ratio	Lower limit	Upper limit
DRD2 (C/T)	0.54	0.24	1.21
OPRM1 (A/G)	2.99	1.45	6.15
LEPR094 (G/C)	0.75	0.14	3.93
LEPR100 (A/G)	0.77	0.35	1.72
LEPR101 (A/G)	0.64	0.37	1.12

The association between five gene variants and obesity had analyzed by calculating the odds ratio (ORs). According to the results, OPRM1 G allele carriers showed a 3-fold increased risk for obesity (p=0.001).

Interestingly, carrying the A1 allele of the DRD2 TaqI A1 gene variant and the G allele of the LEPR Q223R variant could decrease the risk for the development of obesity however the results were not substantial (p=0.1 for DRD2, p=0.08 for LEPR Q223R).

No statistical significance had observed in allele distribution in both LEPR K656N and LEPR Q223R genes (p=0.51 for LEPR K656N, p=0.32 for LEPR K109R).

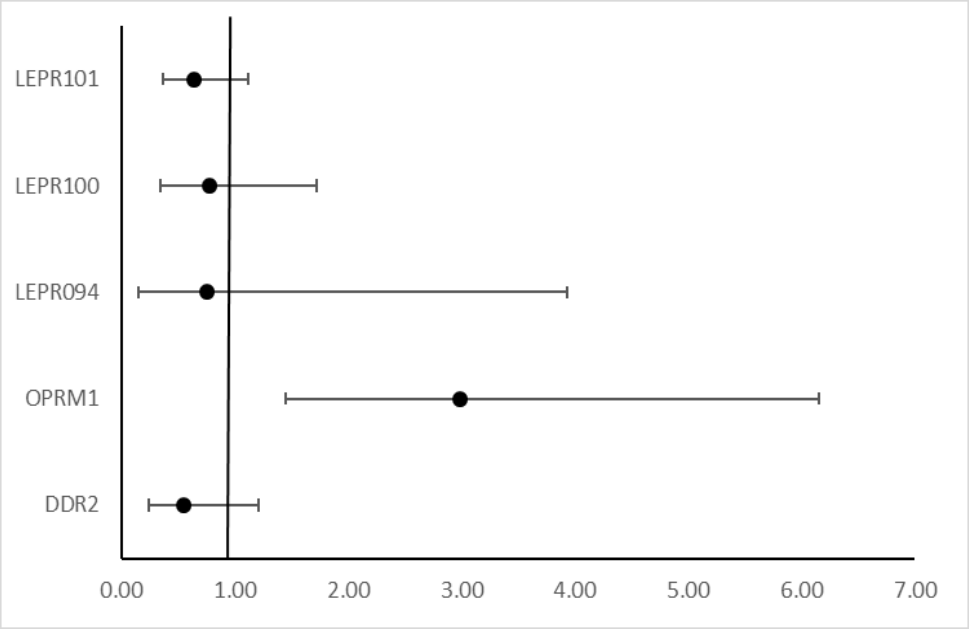

Figure 3.7. Distribution of ORs between five SNPs and obesity.

4. DISCUSSION

Since conventional weight-loss treatments do not produce substantial and sustained weight loss, bariatric surgery is the most effective treatment for severe obesity. After surgery generally, maximum weight loss is observed while most patients start to gain weight over several years. Although numerous studies have tried to identify risk factors for weight gain following bariatric surgery, there is no consensus yet. Lancaster et al. determined variability in weight loss ranging from +13.6 to -96.3 kg in patients 13-15 years after surgery. They hypothesized that genetic predisposition was probably responsible for long-term weight loss. Athanasiadis et al., (188) identified 11 risk factors for weight regain and categorized them into five groups: genetic, psychiatric, anatomical, temporal, and dietary. Ciaudin et al. (189) examined 57 SNPs to detect their role in weight gain. They observed patients who gain weight have at least one of these SNPs compared with subjects who have not gained weight. Nicoletti et al. classified obese patients as normo-responders, hypo-responders, or hyper-responders due to their phenotypic response to surgical or diet treatment. These results have underlined the role of genetic variations in weight loss.

LEPR (Q223R, K109R, and K656N), DRD2 (TaqI A1), and OPRM1(A118G) gene variants had evaluated for their role in weight gain in numerous studies. However, the results obtained from the studies are still controversial.

Leptin regulates energy expenditure, satiety, and neuroendocrine functions in the whole body. High leptin levels observed in obese subjects have been interpreted as leptin resistance caused by genetic variations at the LEPR gene or post-receptor defects. However, some studies failed to identify the role of LEPR gene variants in the development of obesity. Therefore, it had hypothesized that there might be other genetic factors or means to compensate for the effect of amino acid substitutions.

K656N, Q223R, and K109R variants of the LEPR gene are the most frequently used variants in obesity research. Hastuti et al. (190) showed a correlation between rs1137100 (K109R) and rs1137101 (Q223R) polymorphisms and obesity in Yogyakarta population. Wauters et al. (191) studied Q223R, K109R, and K656N variants in pre-and postmenopausal women. No association was determined between these polymorphisms and BMI or body fat mass. However, the Q223R variant had found associated with abdominal obesity. Besides that, the

role of K656N polymorphism in total abdominal and subcutaneous fat and hip circumference had observed in postmenopausal women. Also, K109K homozygous subjects showed significantly higher leptin levels, despite a lower body weight, fat mass and BMI. In conclusion, it had suggested that LEPR gene polymorphisms could predict the risks for developing abdominal obesity in postmenopausal women. Quinton et al. (192) reported the relationship between R allele in Q223R polymorphism and high BMI, serum leptin levels, and fat mass in postmenopausal Caucasian women. In addition, they also determined higher leptin-binding activity (LBA) in R allele carriers than the Q allele carriers. Becer et al. (193) investigated the role Q223R gene variant in obese postmenopausal Turkish women. They didn't observe any relationship between the Q223R gene variant and obesity. However, higher leptin levels had observed in the homozygous RR group compared to the QQ and QR groups. Portales et al. (194) investigated Q223R polymorphism in Spanish 303 obese and 606 non-obese subjects. They reported that the R allele carriers show a lower risk for obesity. Guizar-Mendoza et al. (195) detected the linkage between the Q223 allele and increased fat mass, and leptin levels compared with subjects who carried the R223 allele in Mexican adolescents. The presence of the R223 allele had determined as a risk marker for developing obesity in Caucasian males (196). Another study analyzed the preliminary results of nine studies, with a total of 3263 subjects from different ethnic groups. It had observed that LEPR gene variants did not have any effect on BMI (197).

Dieguez-Campa et al. (198) compared Q223R allele frequency in non-obese and overweight/obese Mexican young adults. Allele distribution between the non-obese (Q=0.56 and R=0.44) and overweight/obese group (Q=0.62 and R=0.38) was not significantly different. In the Pacific Island population, a significant linkage had reported between the Q223R variant and BMI by Furasawa et al. (199). Results were evaluated after adjusting gender, age, and population differences. Although, no association had found between K109R polymorphism and BMI or high body weight in the study.

Novais and colleagues (200) investigated 12 gene variants, including LEPR rs1137101, in 350 obese women following Roux-en-Y gastric bypass (RYGB) surgery. The study aimed to determine gene polymorphisms that could be potential predictors of the postoperative percentage of excess weight loss (%EWL). However, they didn't find any correlation between SNP rs1137101 and %EWL among obese women. Another research group studied the possible role of LEPR K656N polymorphism in outcomes in 41 obese subjects (BMI

>40 kg/m2) 1 year after biliopancreatic diversion (BPD). Results have shown that K656N heterozygotes and N656N homozygotes lose more weight compared to K656K homozygotes (201, 202). Kops et al. (202) studied the distribution of LEPR Q223R genotypes in Brazilian subjects during the postoperative period to reveal the possible association with weight loss. There was no difference first six months. After 12 months, AA homozygous group have higher weight loss than the GG groups, with 76.5% EWL versus 52.0%, respectively. After 24 months follow up this difference remained. However, no association was observed between K656N polymorphism and %EWL. Another study tried to determine the possible role of 11 obesity-related genes (including, LEPR) in weight loss or regain in Swedish obese patients. None of the obesity-risk SNPs of these genes have found related to weight loss or regain over six years of follow-up after bariatric surgery (203).

Sahin et al. (204) reported a difference that was not statistically discernible (p=0.05) in the distribution of Q223R gene variant between obese and control groups in Turkish population. They observed that the distribution of GG genotype was slightly high in obese patients (14.3%) than in control groups (8.5%) (p=0.134). Also, the frequency of the G allele had determined at 37% in obese and 30.5% in the control group (p=0.089). Kanat et al. studied K656N, Q223R, and K109R gene variants of the LEPR gene in childhood obesity in the Turkish population. They involved 150 obese and 140 control subjects in the study. Although the AG genotype of the K109R variant has been found less frequently in the obese group, the prevalence of AA genotypes was higher. Homozygous A allele carriers of Q223R polymorphism were significantly higher in the control group than obese subjects. No difference had determined in K656N genotype prevalence and allele frequency. In our study, the distribution of the K109R genotypes didn't show a substantial difference between the two groups (p=0.940). Obese individuals had an allele frequency of 18.3%, whereas control individuals had an allele frequency of 19.5%. Also, we didn’t observe any relationship between the K656N gene variant and obesity (p=0.804). The GG genotypes of the Q223R polymorphism were 8% in patients and 13.4% in the comparison group (13.4%). s

Numerous studies have revealed the role of the dopaminergic system in rewards and behaviors associated with obesity. Especially DRD2 gene TaqI A1 polymorphism has received more attention for its role in the neuromodulation of appetite.

Hardman et al. (206) exposed that DRD2 TaqI A1 and OPRM1 A118G polymorphisms were not associated with adiposity. Another study carried out by Jenkins et al. (207) determined that the DRD2 rs1800497 polymorphism is not responsible for BMI in Pima Indians. The study had confined to full-heritage Pima Indians due to the effect of the admixture population on the allele frequency. Duran-Gonzales et al. (208) determined that the A1 allele of TaqI A1 polymorphisms related to central obesity in the young Mexican-American population. They also emphasized the genetic admixture of the Mexican population should take into account because the association between rs1800497 and obesity is not universal and did not observe in Pima Indians, Australians, and the Nauruan population.

Thompson et al. (209) reported that the D2 receptor density was lower (30-40%) in the carriers with one A1 allele compared with the homozygous (A2/A2) subjects. Additionally, Pohjalainen et al. (210) determined that the A1 allele carriers among healthy volunteers showed decreased D2 receptor availability. They also emphasized the role of aging in D2 receptor density/availability together with the A1 allele.

Also, Nisoli et al. (211) established that the A1 allele is associated with pathological eating disorders like bulimia nervosa and anorexia.

Winkler et al. (212) investigated the influence of the TaqI A1 polymorphism on weight loss success. Although young participants (21-40 years old) of a 1-year weight loss program had weight regain in the weight maintenance phase, this effect didn't observe in older adults (41-60 years old). In a study conducted by Nonino et al. (213) patients who regained weight following bariatric surgery were found to have the TaqI A1 gene variant associated with binge eating disease (BED). CT and TT variants have been detected most frequently in patients with BED, so the T allele was evaluated as a risk for developing BED.

Obregon et al. (214) reported that the TaqI A1 allele could be a risk factor that predisposed Chilean children to overeat and obesity. Additionally, Yeh et al. (215) detected that higher consumption of fast food and carbohydrates is related to the TaqI A1 allele in Asian Americans. However, when comparing BMI between A1 and A2 allele carriers difference was not observed. According to the studies, the prevalence of the DRD2 A1 allele show differences among ethnicities. The A1 allele prevalence was found approximately %70 in the Asian population, especially in women. So, this data supported that the distribution of the A1 allele depends on both ethnicity and gender.

Roth et al. (216) carried out a longitudinal study to determine the effect of DRD2 rs1800497 A1 allele on overweight/obesity and weight loss after a 1-year lifestyle intervention. They reported that there was no association between rs1800497 polymorphism and weight status. However, homozygous (A1/A1) subjects may have a risk for a weak response in lifestyle interventions.

Steele et al. (217) investigated DRD2 receptor density after laparoscopic Roux-en Y gastric bypass (LGBP) in five female subjects. DRD2 availability was measured with [11C] raclopride binding and observed an increase in receptor density in patients who lost weight after surgery. But lower DRD2 density has been evaluated as a consequence, not a cause of obesity in this study. The results were consistent that the overstimulation of dopamine receptors in obesity causes secondary downregulation. Karlsson et al. (164) emphasized the apparent relation between opioid-dopamine interaction with obesity in their study. It has shown that the dysfunctional opioid-dopamine interaction leads to overeating and obesity. Therewithal, six months after bariatric surgery, recovery was observed between opioid and dopamine interaction due to the weight loss.

Taysun et al. (218) investigated TaqI A1 gene variant in the Turkish population to reveal the relationship with obesity. This study compared 140 obese patients with 120 control subjects, and obese patients had a higher frequency of the A1 allele. However, Ergun et al. (219) compared the distribution of the A1 allele between 46 obese and 50 control patients and didn’t observe any association between BMI and TaqI A1 polymorphism. In our study, the A1 allele frequency wasn't different in obese subjects than in the comparison group ($p=0.427$).

The critical role of mu-opioid receptor (MOR) in hedonic eating has interpreted in human studies. It has been shown that MOR agonists increase food consumption, whereas antagonists have the opposite effects. Thus, variations in MOR may cause changes in feeding behavior and be responsible for eating disorders such as obesity, bulimia nervosa, and anorexia. The A118G gene variant has been linked to obesity in some studies despite controversial results.

Researchers examined the gene variant OPRM1 A118G in obese individuals with binge eating disorders (BEDs) and those without BEDs (163). They determined 18.5% of obese

subjects were carriers of the G allele, as opposed to 9.6 % of non-BED. Succurro et al. (220) demonstrated that obese patients with BED exhibited altered eating behaviors.

Xu et al. (139) investigated the prevalence of rs1799971 G allele prevalence to determine the possible association with BMI in Uyghur population. The study has revealed that the G allele carriers exhibited 25% reduced risk for the developing obesity compared with A allele carriers. Another study has reported the presence of the A118G G allele is related to reduced expression of μ-opioid receptor mRNA and protein yield. So, Xu et al. (139) suggested that anti- obesogenic effect of G allele is related to the less or inactive μ-opioid receptor. Davis et al. (163) determined the role of the G allele in fatty and sweet food preference in 300 hundred healthy adult subjects. They observed that GG homozygous subjects showed a higher preference for fatty and sweet foods compared with AA and AG groups. This study emphasized the association between the consumption of sweet-fat foods and obesity.

Unfortunately, few studies in the literature investigated the distribution of A118G gene polymorphism in the Turkish population. Also, these studies have focused more on the association between A118G and opioid dependency. Turkan et al. (221) tried to determine the possible realtionship between A118G polymorhism and addiction in Turkish population. They determined G allele frequency was higher in subjects who have opioid or other substance addiction. However, Gurel et al.(222) didn't observe the difference in genotype distribution among alcohol dependency (AD) and non-alcoholic subjects. The presence of A118G gene polymorphism in obese subjects in the Turkish population has not been investigated before. At this point, we tried to find out the effect of the OPRM1 A118G gene variant in bariatric surgery outcomes. We observed that G allele frequency was higher in the obese group (14.9%) compared with the control group (5.3%). According to the results, OPRM1 G allele carriers showed a 3-fold increased risk for obesity ($p=0.001$). These findings supported that A118G gene variant is associated with BMI ($p=0.05$).

Carpenter et al. (50) investigated DRD2 (TaqI A1), LEPR (K109R), and OPRM1 (A118G) gene variants in severely obese patients who underwent weight-loss treatment. It had observed that carrying the DRD2 TaqI A1 allele is a risk factor for increasing BMI ($p=0.04$). However, OPRM1 A118 G and LEPR K109R variants didn’t show any association with BMI. A noteworthy interaction had observed between DRD2 TaqI A1 and LEPR K109R polymorphisms. In the study, subjects who were homozygous for the LEPR K109R and

carrying one or more copies of the TaqI A1 allele (p=0.04) had higher BMI. Also, there was a marginally significant interaction between DRD2 and OPRM1 gene variants (p=0.06). In contrast, carriers of the A1 allele of theTaqI A1 and the G allele of the K109R variant had lower BMI in our study. However, substantial significance didn't observe according to the results (p=0.1 for DDR2, p=0.08 for LEPR (K109R).

We aimed to gain a better understanding of how polymorphisms in DRD2, LEPR, and OPRM1 contribute to weight regain after bariatric surgery. We failed to identify that genetic polymorphisms DRD2, LEPR K656N, K109R, and Q223R might affect weight regain after bariatric surgery. The exception is the OPRM1 A118G gene variant, where the 118G allele might increase the risk of weight and fat regain after surgery. Further clinical investigations focusing on the role of G allele in patients undergoing bariatric surgery might influence post-surgical complications. The results might help develop new medical treatment strategies for weight maintenance after surgery.

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