INVESTIGATION OF MAO-A, COMT, VMAT2, DAT1 GENE POLYMORPHISMS AMONG OVERWEIGHT AND OBESE ADULTS IN TURKISH POPULATION

by

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Dedicated to my mother,

Aynur AVŞAR who died...

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ABSTRACT

INVESTIGATION OF MAO-A, COMT, VMAT2 AND DAT1 GENE POLYMORPHISMS AMONG OVERWEIGHT AND OBESE ADULTS IN TURKISH POPULATION

Obesity is a health problem which is increasingly becoming prevalent in the worldwide and risky for several diseases. Due to the multifactorial, the prevention and treatment of obesity is so difficult. The idea that obesity is a neurobiological disease rather than a metabolic disorder, is the basis of our hypothesis. Changes in dopamine neurotransmission affect the brain reward system in a direct way. Furthermore, changes in the reward system influence the eating behavior in human. DAT1 and VMAT2 transporter proteins terminate DA function by moving into presynaptic neurons and vesicles, and MAOA and COMT enzymes terminate the function by metabolizing DA. In our study, the control group which includes 214 individuals and obese group that involve 234 subjects were investigated for *MAOA-u* VNTR, 3' UTR *DAT1* VNTR, *COMT* (rs4680), *DAT1* (rs27072), *VMAT2* (rs363399), *VMAT2* (rs4752045) polymorphisms. There is no study which clarifies the association between *DAT1* (rs27072), *VMAT2* (rs363399), *VMAT2 (*rs4752045) polymorphisms and obesity in the literature. In our study, statistical analysis has showed that in control group Val/Met COMT genotype is significantly higher according to obese group (p=0.04). When obese and control groups are compared, it has been observed that *DAT1* A/A genotype is dramatically higher in patient group (p=0.018). When the groups were compared in terms of eating behavior, the number of the subjects who ate for reward was significantly higher in obese group (p=0.03).

Our findings demonstrate that eating behavior may affect the development of obesity and the dopaminergic gene polymorphisms could be a risk factor for the pathogenesis of obesity in adults in Turkish population.

ÖZET

TÜRK POPÜLASYONUNDA AŞIRI KİLOLU VE OBEZ ERİŞKİNLERDE MAO-A, COMT, VMAT2 VE DAT1 GEN POLİMORFİZMLERİNİN ARAŞTIRILMASI

Obezite dünya genelinde gittikçe yaygınlaşan ve birçok hastalık için risk oluşturan ciddi bir sağlık sorunudur. Multifaktöriyel bir hastalık olmasından ötürü önlenmesi ve tedavisi oldukça zordur. Obezitenin metabolik bir hastalıktan ziyade nörobiyolojik bir hastalık olması düşüncesi bizim de hipotezimizin temelini oluşturmaktadır. Dopamine nörotransmisyonundaki değişiklikler beyindeki ödül sistemini doğrudan etkilemektedir. Ödül sistemindeki değişiklikler de bireyin yeme davranışını etkilemektedir. DAT1 ve VMAT2 taşıyıcı proteinleri dopamini presinaptik nöron içine ve vesikül içine taşıyarak, MAOA ve COMT enzimleri de dopamini metabolize ederek DA fonksiyonunu sonlandırmaktadır. Bizim çalışmamızda 214 kişiden oluşan kontrol grubu ile 234 kişiden oluşan hasta grubu *MAOA-u* VNTR, 3' UTR *DAT1* VNTR, *COMT* (rs4680), *DAT1* (rs27072), *VMAT2* (rs363399), *VMAT2* (rs4752045) polimorfizmleri açısından araştırıldı. Literatürde, *DAT1* (rs27072), *VMAT2* (rs363399), ve *VMAT2 (*rs4752045) polimorfizmleri ile obezite arasındaki ilişkiyi açıklayan herhangi bir çalışma bulunmamaktadır. Bizim çalışmamızda, istatistik analizi sonrası kontrol grubunda Val/Met *COMT* genotipinin obez gruba göre anlamlı şekilde yüksek olduğu gösterilmiştir (p=0.04). Obez grubu ile kontrol grubu karşılaştırıldığında, *DAT1* A/A genotipinin obez grupta istatistiksel açıdan anlamlı şekilde yüksek olduğu gözlemlenmiştir (p=0.018). Gruplar yeme davranışı açısından kıyaslandığında, obez grupta yemek yemeyi ödül olarak görenlerin sayısı anlamlı olarak yüksek çıkmıştır (p=0.03).

Bulgularımız Türk popülasyonundaki erişkin bireylerde, yeme davranışının obezite gelişimini etkileyebileceği ve dopaminerjik gen polimorfizmlerinin obezite için risk faktörü olabileceğini göstermektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

ADC	Aromatic amino acid decarboxylase
AC	Adenylate cyclase
ADRB3	Adrenergic receptor beta 3
ADSCs	Adipose-derived stem cells
AgRP	Agouti-related peptide
Ala	Alanine amino acid
AMPH	Amphetamine
a- MSH	alpha melanocyte stimulating hormone
AN	Anorexia nervosa
Arg	Arginine amino acid
BAT	Brown adipose tissue
BBS	Bardet-Biedl Syndrome
BBS1	Bardet-Biedl Syndrome 1 protein
BBS10	Bardet-Biedl Syndrome 10 protein
BED	Binge eating disorder
BMI	Body Mass Index
BMSCs	Bone marow-derived stem cells
BN	Bulimia nervosa
BRRS	Bannayan-Riley-Ruvalcaba Syndrome

BSx	Bariatric surgery
cAMP	Cyclic adenosine monophosphate
CART	Cocaine- and amphetamine-related transcript
CCK	Cholecystokinin
C/EBP α	CCAAT/enhancer-binding protein alpha
C/EBP β	CCAAT/enhancer-binding protein beta
C/EBP δ	CCAAT/enhancer-binding protein delta
CREB	cAMP response element-binding protein
CRF	Corticotropin-releasing factor
COMT	Catechol-O-methyltransferase
CTAB	Hexadecyltrimethylammoniumbromide
DA	Dopamine
DAT	Dopamine transporter
DBS	Deep Brain Stimulation
DDC	Dopa decarboxylase
DNMT1	DNA (cytosine-5)-methyltransferase 1
DNMT3A	DNA (cytosine-5)-methyltransferase 3A
DNMT3B	DNA (cytosine-5)-methyltransferase 3B
DOPAC	3,4-dihydroxyphenylacetic acid
DSM-5	The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (5th edition)
DTAB	Dodecyltrimethylammoniumbromide

EDTA	Ethylenediaminetetraaceticacid
EFSA	European Food Safety Authority
EGR2	Early growth response 2
FTO	Fat mass and obesity-associated
GHRL	Ghrelin
GPCR	G-protein coupled receptor
HVA	Homovanillic acid
IGF-1	Insulin-like growth factor-1
LEP	Leptin
MAO	Monoamine oxidase
MC4R	Melanocortin 4 receptor
MCE	Mitotic clonal expansion
MCH	Melanin-concentrating hormone
METH	Methamphetamine
mRNA	Messenger Ribonucleic acid
NAc	Nucleus accumbens
NPY	Neuropeptide Y
PAGE	Polyacrylamide Gel Electrophoresis
PCR-RFLP	Polymerase Chain Reaction Restriction Fragment Length Polymorphism
PFC	Prefrontal cortex
PKA	Protein kinase A

POMC	Pro-opiomelanocortin
PPAR γ	Peroxisome proliferator-activated receptor gamma
Pro	Proline amino acid
PWS	Prader-Willi Syndrome
RDS	Reward Deficiency Syndrome
SLC6A3	Solute Carrier Family 6 Member A3
SLC18A2	Solute Carrier Family 18 Member A2
SNP	Single Nucleotide Polymorphism
SNpc	Substantia nigra pars compacta
TAE	Tris-acetate-EDTA
TGs	Triglycerids
TH	Tyrosine hydroxylase
Trp	Tryptophan amino acid
UCP1	Uncoupling protein 1
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3
VMAT2	Vesicular monoamine transporter-2
VNTR	Variable Number Tandem Repeat
VTA	Ventral tegmental area
YFAS	The Yale Food Addiciton Scale
WAT	White adipose tissue
WHO	World Health Organization

1. INTRODUCTION

1.1. GENERAL DEFINITION, DIAGNOSIS, AND PREVALENCE OF OBESITY

The vocables ‘obese’ and ‘obesity’ are originally used in the Latin and transfer to English via French. The meaning of ‘obedere’ is ‘over eat’ and the meaning of ‘obesitas’ is being fatty. [1]. Obesity is increasingly spreading worldwide and a health problem which causes various diseases. Obesity is a chronic disorder defined as an abnormal proliferation of adipose tissue in vivo [2]. Obesity arises from the instability between food intake and energy consumption. Human bodies require energy to carry out essential daily function such as breathing, digesting the food eaten, and physical activity. Weight gain happens when the calorie intake is more than the expenditures. The World Health Organization (WHO) described obesity as “abnormal or excessive fat accumulation that may impair health” in 2006 [3]. Obesity which shows non-infectious complex etiology, is thought that both environmental and genetic diversities might be substantial for the pathogenesis [4]. Body Mass Index (BMI) which is scaled as weight (kg)/height squared (m^2) is the universal indicator for detecting overweight and obesity. It is used to define the comparative weight for height, and is markedly related to total body fat. The BMI could be a helpful indicator for obesity since there is no difference about sex and age in adults [5].

Table 1.1. BMI scale for the description of weight situation [6].

Weight status	**BMI scale (kg/m2)**
Underweight	Under 18.50
Normal	18.50 to less than 25
Overweight	25 to less than 30
Obese	30 to less than 40
Morbidly obese	40 and over

In addition to BMI, other methods to determine the percentage and distribution of body fat are listed in the below.

Waist circumference: It is associated with BMI to be able to detect obesity with low risk ≤ 102 cm for males, ≤ 89 cm for females, great risk >102 cm for males, >89 cm for females.

Waist-to-hip ratio: Another simple and significant tool to evaluate overweight and obesity and risk of health problems. The optimal ratio is 0.9 to 0.95 for males and 0.7 to 0.8 for females.

Skinfold thickness: It is a simple and noninvasive tool used for the anthropometric determination of subcutaneous body fat.

Bioelectrical Impedance: It is a simple, noninvasive and cost-effective tool to determine the percentage of body fat according to the electrical current through tissues in the body [7].

Most recently, the World Health Organization (WHO) reports that over 1 billion individuals round the globe are overweight and 600 million people are obese. It is anticipated that by the end of 2020 approximately half of total American adults will experience the WHO guidelines for obesity and then by the end of 2030 approximately 90% will show a BMI >25.0 [8]. The percentage of overweight and obese children and

adults have been increasing since 1990 in Turkey. Overweight is more prevalent among men while obesity is more common among women in Turkey. On the other hand, there is no difference for genders in children. The accelerating rate of incidence of overweight and obesity is higher in Turkey than the European countries, but it is similar to that of USA [9].

Obesity might be classified in various ways according to the origin of fat, distribution of fat, and age. Fat accumulation has been varied as mild (excess fat of 20 per cent to 40 per cent), moderate (41 per cent to 100 per cent), severe (over 100 per cent).

When the enhancement of body weight occurs because of the number of fat cells, it is named as hyperplastic obesity. When it occurs according to the expanding of fat cells with little or no alteration in number of these cells, it is named as hypertrophic obesity. Hyperplastic obesity is also named as child obesity, otherwise hypertrophic obesity is also named as adult obesity. Obesity might be classified as central or abdominal and peripheral or gluteal due to the distribution of fat in the body. Abdominal obesity occurs due to the increasing of fat excessively around stomach and abdomen. Abdominal obesity is significantly associated with hearth diseases, metabolic syndrome, diabetes mellitus type 2 and mortality. On the other hand, the peripheral type, accumulation of fat around hip and thigh, is less dangerous than abdominal obesity. Contrarily, scientists assume that peripheral fat accumulation might have protective effects on diseases [10].

Lastly, obesity might be classified according to the age of onset as childhood obesity, early childhood and middle age obesity [11]. Childhood obesity is significantly increasing in various countries worldwide. It is a very important issue to be solved by reason of the risks of heart diseases, type 2 diabetes and cancers during childhood or adulthood. Additionally, childhood obesity is crucially related with the health of later life.

Table 1.2. Waist circumference values (cm) of the populations for the diagnosis of obesity [173].

Population	Female	Male
US	≥88	≥102
Europe	≥80	≥94
Turkey	≥90	≥100
South Asia and China	≥80	≥90
Japan	≥90	≥85

1.2. GENETICS OF OBESITY

Several genes, environmental factors such as lack of physical activity, highly palatable, energy dense foods, and food-seeking behavior, and interactions between them cause obesity in human beings. Thus, the prevention and the treatment of obesity is especially difficult because of the multifactorial pathogenesis [12].

Obesity is mostly inherited and does not display normal Mendelian transmission. Correlations of mutations, single nucleotide polymorphisms, insertions, and deletions are all being involved in the development of obesity. For instance, the SNP Pro12Ala of (*PPARG*) gene is implicated in increasing of body mass index [13]. Loss of *ADRB3* gene function could cause obesity via its effect on energy consumption and there is a significant relationship between the SNP Trp64Arg of the *ADRB3* gene and weight status [14]. The uncoupling proteins, UCP-1, UCP-2, UCP-3, cause the heat production by the generation of adenosine triphosphate via phosphorylation of adenosine diphosphate. The BMI is affected by the 3′ Indel (Insertion/Deletion) variation in the *UCP-2* gene and also, in the adipose tissue of obese people, *UCP-2* messenger RNA amounts are decreased [15].

Scientists assume that 40-77 per cent of the variety of body weight is affected by genetic elements in humans [16]. Some kinds of obesity (e.g., monogenic obesity) in humans are really uncommon and very intense, usually begin in childhood [17]. On the other hand, polygenic obesity which is affected by environmental conditions is more prevalent [18]. Genes such as leptin (LEP), uncoupling protein1 (UCP1), ghrelin (GHRL), cholecystokinin (CCK) which influence homeostasis of energy, leptin-insulin signaling, adipogenesis, and thermogenesis take roles in the development of obesity [19]. CCK which is most significant among these peptides regulates satiety by decreasing food intake [20].

1.2.1. Monogenic Obesity

It is a type of obesity which is influenced by only one gene mutation. In these kind of situations, single gene variations can cause the development of obesity in the presence of food abundancy. This type of obesity generally starts in childhood and is associated with severe characteristics such as hyperphagia and hypogonadism [21]. The genetic background of monogenic obesity is significantly correlated with the leptin-melanocortin signalling pathway and this path is crucial for food intake and energy metabolism. Loss of function mutations give rise to deficiencies of regulator hormones of appetite such as POMC, MC4R, leptin receptor, and leptin. MC4R is a 7-trans-membrane protein and its expression is so high in hypothalamus. MC4R is activated by the endogenous agonist, (α-MSH) alpha-melanocyte stimulating hormone and its antagonist is (AgRP) agouti-related peptide. Leptin promotes the synthesis of POMC which is the precursor of alpha-MSH, and interrupts the production of AgRP, providing anorexigenic signal [22]. The most prevalent cause of monogenic obesity is MC4R gene mutations, but it just accounts for about 6 per cent of the cases in children [23]. The homeostatis of energy includes the combination of signals of insulin, meal-associated signals from gut, and leptin. And all the signals integrate in the brain and provide the modulation of food intake, consumption of energy, and neuroendocrine status [24].

Table 1.3. Orexic molecules which enhance food intake [25].

Central nervous system	**Peripheral**
Neuropeptide Y	Ghrelin
Melanin-concentrating hormone (MCH)	
Orexins/Hypocretins	
Agouti-related peptide (AgRP)	
Galanin	
Endogenous opioids	
Endocannabinoids	

Table 1.4. Anorexic peptides which decrase food intake [25].

Central nervous system	**Peripheral**
Cocaine- and Amphetamine-related transcript (CART)	Leptin
Melanocortins (POMC)	Peptide YY
Insulin	CCK
Corticotropin-releasing factor (CRF)	Insulin
Serotonin	Amylin
Glucagon-like peptides	Glucagon-like peptides
Neurotensin	Bombesin

The Greek word *leptos* which means ‘thin’ refers to leptin since 1994. It is located on chromosome 7 and its product is a peptide with 167 amino acid and its molecular weight is 16kD. Adipose tissue secretes the leptin hormone according to the body fat content and leptin has the ability for crossing the blood-brain barrier. Additionally, leptin induces some types of hypothalamic neurons to synthesize peptides that decrease food intake and increase energy consumption (the pathway of leptin-melanocortin). Furthermore, leptin blocks the neurons in the hypothalamus which synthesize peptides stimulating food intake and reducing energy consumption [26]. On the other hand, leptin modulates endocrinological processes such as lipogenesis, bone mineralization, kidney function and puberty. In several tissues, leptin receptors are available for the action of leptin on these tissues [27].

Congenital leptin deficiency: It was reported that two cousins from Pakistan were severely obese in 1977. In spite of the severe obesity, their serum leptin levels were significantly high and a gene mutation in the leptin gene was detected. Hyperphagia and elevated food intake is significantly correlated with the deficiency of leptin. Additionally, other clinical characteristics such as hypogonadism, aberrant T-cells, increased plasma insulin and elevated bone age might be seen in this situation [28].

Later, the leptin function in monogenic obesity was confirmed by the leptin treatment in severely obese child with congenital leptin deficiency. In 9 year old child with congenital leptin deficiency, recombinant human leptin was injected subcutaneously for one year and he recovered at the end of the year [29].

Mutation of leptin receptor gene: After discovering of the leptin deficiency, approximately comparable feature, with increased plasma leptin levels was described. It occured because of the homozygous mutation of leptin receptor gene. And another study proposed that nearly three per cent of severe morbid obesity might be clarified by the leptin receptor gene mutations [30].

Melanocortin-4 receptor deficiency (MC4R): MC4R deficiency is the most prevalent type of monogenic obesity. Obese people with MC4R deficiency display hyperphagia, but it is not so serious like in the leptin deficiency. Increasing of bone mineral density, lean-mass and fat-mass are seen in the obese people with MC4R deficiency. Furthermore, mutations

(both homozygous and heterozygous) in the MC4R gene are associated with linear growth and current therapy for MC4R deficiency is not available [31].

Pro-opiomelanocortin (POMC) deficiency: The percentage of the POMC mutations in several populations is not high. Otherwise, this deficiency causes hyperphagia and early-onset obesity because of the melanocortin signaling loss at MC4R [32].

1.2.2. Syndromic Obesity

Syndromic obesity involves some rare genetic disorders with Mendelian inheritance, both autosomal and X-linked. These syndromes such as Prader-Willi, Fragile X, Cohen, Borjeson-Forssman-Lehmann, Bannayan-Riley-Ruvalcaba, Bardet-Biedl, Weaver, Simpson-Golabi-Behmel syndromes are characterized by obesity. Most of the diseases are related with mental retardation, developmental abnormalities, and dysmorphic properties [33].

Prader-Willi syndrome: PWS is a rare genetic disorder (1/15 000 – 1/30 000). PWS is the first detected human disease correlated with genomic imprinting and the first disorder caused by uniparental disomy. This disorder shows autosomal dominant inheritance. Principal properties involve obesity, mental retardation, hyperphagia, muscular hypotonia, short stature, and hypogonadotrophic hypogonadism. It was proposed that the increased biosynthesis of ghrelin peptide which is secreted by the stomach could elevate appetite by the interactions of POMC/CART and NPY hypothalamic neurons [34].

Bannayan-Riley-Ruvalcaba syndrome: BRRS is a rare autosomal dominant genetic disorder. The Phosphatase and Tensin Homolog (PTEN) gene is a tumor suppressor gene and mutations of this gene have a relationship with nearly 55-60 per cent of all cases. The characteristics of BRRS are; macrocephaly, mental retardation, developmental and cognitive delay, anomalies of vascular system, penile lentigines, thyroid diseases involving thyroid cancer, lipomas and hamartomas, hypotonia, and postnatal overgrowth [35,36].

Bardet-Biedl syndrome: BBS is a rare autosomal recessive genetic disorder. Mutations in different genes such as BBS1,BBS10, NPHP1, IFT1 are associated with BBS. The

characteristics of BBS are; central obesity, polydactyly, menstrual irregularities in female, learning disabilities, retinitis pigmentosa, renal anomalies, and hypogonadism in males [37,38].

1.2.3. Polygenic Obesity

Polygenic obesity is the most prevalent form of obesity and refers to the conjuctions between environmental, and behavioral, and genetic factors to affect the reactions of human to diet and physical activity. When compared with the syndromes which are characterized by obesity or monogenic obesity, environmental factors have significant influence on body weight in common obese people [39].

In 2007, FTO (fat mass and obesity associated) which is the first gene significantly related to polygenic obesity was identified. FTO gene is located on chromosome 16 and the protein involves 505 amino acids. The expression of FTO gene occurs in different parts of the body, specifically in the adrenal glands, hypothalamus, muscles, and pituitary [40]. In humans, FTO gene is fundamental for cardiovascular and central nervous system development. In mice, loss of FTO gene causes some clinical features such as critical attenuation of lean and adipose tissue mass and retardation of postnatal growth. The risk allele of FTO gene is significantly correlated with the reduction of the satiety and elevated food intake in human [41].

1.3. EPIGENETICS OF OBESITY

Epigenetics means the alterations for gene regulations without changing the DNA sequence. Epigenetic alterations are reversible and they are important for the drug discovery and improved disease management [42]. Epigenetic events are tissue-specific and involves DNA methylation and histon modifications which regulate biological events such as imprinting. Genomic imprinting defines the allele expressions according to the maternal or parental origins. Imprinted genes take a place in growth, viability, differentiation, metabolic procedures, and development.

Inefficacy in imprinting may cause obesity by alteration of the expression of cellular differentiation and growth factors might be occurred by several genetic events such as inversion, translocation, hyper and hypo-methylation, duplication, and paternal disomy. For instance, PWS is caused by uniparental disomy at 15q11–q13 or paternal deletion [43].

Methylation is so common for the genome and occurs by a methyl group (CH3) insertion to a cytosine localized near guanine nucleotide (CpGs), generally in areas with a great amount of CpG dinucleotides (>60 per cent). DNA methyltransferases including DNMT1, DNMT3A, DNMT3B modulate the methylation. If the methylation arises in the promoter of the gene, it causes silencing of the gene by alteration of chromatin structure. Histone modifications are the post-translational modifications and the lysine residues can be modulated by acetylation, methylation, phosphorylation, biotinylation, ubiquitination, and sumoylation. Histone modifications and methylation processes are tissue-specific and might be various according to the stages of development and age. On the other part, untranslated noncoding RNA such as long noncoding RNA (lncRNA), small nucleolar RNA (snoRNA), micro-RNA (miRNA), and piwi-interacting RNA (piRNA) are involved in the epigenetic processes [44].

Epigenetic regulatory processes are known to participate in obesity-associated mechanisims such as food intake, adiposity, and energy expenditure. Some nutrients are able to affect the epigenetic machinery directly or indirectly. Retinoid acid which is the metabolite of vitamin A activate retinoid X receptors (RXRs) and these receptors modulate the transcription of fat cells with binding to the promoter regions of histone modifier genes in a direct way [45]. Conversely, niacin and methyl donors such as choline and folate are thought to affect DNA and histone methylation reactions [46].

Pro-opiomelanocortin is a precursor protein and participates in several kinds of biological activities such as appetite, energy homeostasis, sexual behaviors and reward system. While its expression is high in the pituitary, it is also found in different regions of the body such as in the skin, hypothalamus. Reduced hypothalamic expression of pro-opiomelanocortin is asscociated with weight gain and obesity [47]. Additionally, it is proposed that the levels of DNA methylation of the pro-opiomelanocortin promoters in cord blood might be an important indicator of metabolic syndromes in later life [48]. In a study conducted rats, gestational and post-weaning high-folate diet caused the hypomethylation of pro-

opiomelanocortin promoter regions in the offspring' hypothalamus, reduced food consumption and weight gain [49]. In animal models, high-sugar and fat diet stimulated hypermethylation and hypomethylation of leptin promoter regions in adipocytes. In obesogenic environment, adipocyte leptin promoter is hypermethylated and results in the inhibitition of the transcription of the gene [50].

1.4. ADIPOCYTE DIFFERENTIATION

Preadipocytes, adipocytes, endothelial cells, multipotent stem cells and fibroblasts are the main components of adipose tissue or other name, fat tissue. Mature adipocytes constitute nearly one-third of adipose tissue. Other part of the adipose tissue is composed of mesenchymal stem cells (MSCs), T regulatory cells, macrophages, endothelial precursors, and preadipocytes. In mammals, two different adipose tissue types are available: 1. White adipose tissue (WAT) and 2. Brown Adipose Tissue (BAT). WAT is essential for the uptake and biosynthesis of lipids and storage of triglycerids (TG). In the absence of energy such as fasting the TGs which are stored in WAT are moved and fatty acids and glycerol are occurred by the break down of TGs for energy necessity of the body [51]. Additionally, if the functional adipocytes are not available, TGs are vulnerable to accumulate in liver and muscle and then cause to disturbances of metabolism and insulin resistance [52]. Moreover, WAT secretes different kinds of adipokines such as adiponectin in usual conditions, and pro-inflammatory cytokines such ast umor necrosis factor-α (TNF-α), monocyte chemotactic protein 1 (MCP- 1), IL-6, and IL-10 during the obesogenic situation. Overall, WAT is involved in the different kinds of biological activities such as metabolism, immune response, and inflammation [53, 54].

The other adipose tissue type, BAT regulates the body temperature by the usage of stored energy as heat. BAT includes several mitochondria and vessels and strongly related to the central nervous system [55]. It is supposed that the size of adipose tissue cell influence insulin sensitivity, synthesis and release of adipokines. Furthermore, the equilibrium between adipocyte differentiation, proliferation, expanding, and lipolysis is important for adipose tissue mass. Increasing of adipogenesis (adipocyte differentiation), expanding of adipocyte (lipogenesis), diminishing of lipolysis cause accumulation of fat in WAT, and

then obesity and related diseases occur. For these reasons, determining the mechanisms of adipogenesis is so crucial for obesity and other correlated disorders [56].

Adipogenesis is a complicated and systematical biological process and the differentiation occurs through multipotent stem cells or preadipocyte precursor cells. Additionally, environmental and genetic factors influence the adipogenesis. Mainly, four steps, consisting of initial growth arrest, mitotic clonal expansion (MCE), early differentiation, and terminal differentiation are available for the development of mature adipocytes [57]. MSCs which are multipotent stem cells are involved in the differentiation of adipocytes. MSCs might be extracted from bone marrow (bone marrow-derived stems cells, BMSCs) and adipose tissue (adipose-derived stem cells, ADSCs) and then maturation of adipocytes are occurred in vitro [58].

Throughout the adipocyte differentiation process, several transcription factors regulate various genes for the generation of the mature adipocyte phenotype. Peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) family members are the principal modulators of the adipocyte differentiation. According to the adipogenic stimulus, C/EBPβ and C/EBPδ are activated and trigger MCE of preadipocytes. PPARδ regulates adipocyte differentiation negatively by elevating lipolysis, and inducing the catabolism of fatty acids and energy consumption in the adipose tissue [59, 60]. Additionally, early growth response 2 (EGR2), Kruppel-like factor 4 (KLF4), and cyclic adenylyl monophosphate (cAMP) response element-binding protein (CREB), are the elements for transcription and modulate the C/EBPβ expression in the period of adipogenesis. C/EBPα is essential for adipocyte differentiation and function of adipocyte in the presence of PPARγ. On the other hand, in the absence of C/EBPα, PPARγ can stimulate adipogenesis. In adipose tissue, PPARγ activation induces expansion of adipose tissue by adipocyte hyperplasia, and then causes the concentration of TG in adipose tissue [61].

cAMP is a seconder messenger and discovered in the 1960s. Activation of cAMP-dependent protein kinase A (PKA) is necessary for the function of cAMP. Furthermore, PKA is the main target of cAMP and includes two catalytic and two regulatory subunits. After adipogenic hormone stimulation, cAMP attaches to the regulatory parts of PKA, then

catalytic parts of PKA phosphorylate CREB, resulting in the binding to the promoter region of C/EBPβ [62].

IGF-1 (Insulin-like growth factor-1) and insulin are proadipogenic agonists. Activation of insulin through binding to its receptor, promotes the phosphorylation of the insulin receptor substrate proteins (IRSs) and IRSs signaling transduction is crucial for adipogenesis [63].

1.5. NEUROBIOLOGICAL ASPECT OF OBESITY

Obesity might be defined as neurological disorder rather than metabolic disease and an increasing risk factor for neurological and psychiatric disorders such as dementia, headache, multiple sclerosis, stroke, and sleep disorders. Additionally, some neurobiological diseases and drugs may trigger the devolopement of obesity [64].

An increasing general agreement is that obesity and addiction may share the same neurobiological aspect. The opinion is that obesity concludes from food addiction which significantly similar to drug addiction, in behavioral and nerve-related process. Several types of foods may be abused by people like addictive drugs such as alcohol, opioids, cocaine, and nicotine (table 1.5). These kind of drugs are related to regular usage featured by physiological dependence (tolerance) and negative consequences (abuse). And it has been supposed that several food substances such as fat, salt, sugar, sweeteners may show similar addictive properties [65]. Abused drugs and palatable food have several similar characteristics such as strong reinforcing features which are modulated by dopamine rise in the reward system of the brain.

Table 1.5. Abused substances.

Drugs	**Food components**
Nicotine	Sugars (glucose, fructose, sucrose)?
Cocaine	Sweeteners (e.g., stevia, sucralose, aspartame)?
Alcohol	Fats, fatty acids?
Opiates (e.g., heroin)	Salt (NaCl)?
Methamphetamine	Others?

The nonhomeostatic mechanisms of weight gain and obesity is significantly correlated with the reward system of the brain. And the neurotransmitter dopamine has nonhomeostatic effect on food intake. Despite the similar properties of drugs and food substances, they separate in quantitative and qualitative ways. Abused drugs such as methamphetamine, nicotine, and heroin affect the dopamine circuitry in the brain in a direct way. On the other hand, food substances affect the dopamine circuits in two ways indirectly. The first way is through neuronal inputs from the taste receptors to the dopaminergic neurons, and the second one is via signals and hormones which are produced by the absorption and digestion of foods [66].

Reward refers a biological process which modulates behaviors and gives pressure, then may cause addicton. The 5th edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM-5) declares the criteria for addiction, and for the diagnosis, it needs two or more of the followings: tolerance, usage of large doses of drugs for a long time, usage of the substance for a long time, repeated attempts to quit, withdrawal, going on using although serious adverse effects (table 1.6) [67]. In other respects, Reward Deficiency Syndrome (RDS, explained in 1995) refers to Brain Reward Cascade dysfunction that release of dopamine is negatively affected by several genes and polymorphisms such as serotonergic-2A receptor (5-HTT2a), DAD2 receptor (DRD2).

This alteration causes hypo-dopaminergic function and then aberrant craving behavior which contains drugs, sex, excessive food, gambling, and other behaviors [68].

Table 1.6. DSM-5 criteria for abused substances. Diagnosis is clarified as mild (2 or 3 of them), moderate (4 or 5 of them), or severe (6 or more of them).

Criteria
Withdrawal
Tolerance
Taken more than intented
Taken longer than intented
Wish for attempts to quit
Ineffective attempts to quit
Use although cognition of problems related to the usage
Desist from doing important things because of use
Recurrent use causing to dangerous behavior such as driving
Recurrent use although the important social problems related to usage
Craving

The reward system of the brain is composed of a network of different brain areas. The mesocorticolimbic pathway is the principal component of the system. The dopaminergic neurons inhabit in the midbrain nuclei (Ventral tegmental area, VTA and substantia nigra pars compacta,SNpc) that project to striatal (dorsal sitriatum and nucleus accumbens, NAc), limbic (hypothalamus and amygdala) and cortical regions (temporal pole, cingulate gyrus,

prefrontal cortex). The projections of the dopaminergic neurons from the VTA target the limbic forebrain, especially the NAc and the prefrontal cortex. The prefrental cortex supplies decreasing projections to the NAc and the VTA [69].

For the anticipated role of the mesocorticolimbic pathway, in rats, exposure to food, and sex have enhanced the amounts of dopamine in the NAc. Additionally, administration of drugs such as cocaine, morphine, and ethanol causes dopamine increases in the NAc in rats. The amounts of dopamine also are directly affected by the amounts of sweet and drug in rats [70, 71].

Abused drugs and palatable foods activate the brain reward circuitry in a similar way in both animals and human. Animal experiments remarks that continuous usage of abused drugs lead to diminishing of amounts of DAD2 receptors and dopamine, sensivity of reward areas to drug use, and electrical stimulation. In addition to this, overfeeding in animals, leads to decreasing of D2 receptors, DA levels, sensivity of reward areas to food intake, and electrical stimulation [72]. Consistent with these findings, obese people have less D2 receptors and have decreased reward response to palatable food according to lean people [73].

Neurotransmitters such as dopamine and serotonin are involved in both drug-seeking behaviors and food intake. On the other hand, the peptides (e.g., leptin and ghrelin) and hormones (e.g., insulin) which modulate food intake, also affect the drug's reinforcing features [74].

Unlike abused drugs, food is essential for survival. On the other side, highly palatable food (e.g., refined carbohydrate, fatty, and salty food) may significantly affect the mood, for this reason, some people may consume these kind of foods in large amounts to be able to get rid of psychiatric disorders such as anxiety, depression. Same as the abused drugs, stress and negative moods can stimulate the obligatory food intake. In obese people, good smelling, looking, and tasting foods have reinforcing features like abused drugs. Additionally, overconsumption of foods might be affected by drugs such as naloxone, naltrexone, baclofen, topiramate, Rhodiola rosea and Hypericum perforatum extracts [75].

The term 'food addiction' has been proposed by Randolph in 1956. The publications about the term 'food addiction' has been increasing since 2009 [76]. Food addiction model refers

that food is taken for pleasure and hedonistic food intake might be associated with drug addiction and eating disorders. Food addiction is a result of craving of spesific food substances which leads to euphoria. Despite that food addiciton is not clinically described disease, it has been proposed that overconsumption of highly palatable, refined, and energy-dense foods in an addictive-like way and then, it may cause weight gain in susceptible individuals. Food addiction may be identified by the usage of The Yale Food Addiction Scale (YFAS). In 2009, YFAS has been devised to be able to confirm 'food addiction' and to find out its prevelance. YFAS is a method for detecting of the addictive-like eating patterns and crucial for the determination of food addiction [77].

Specific foods (full of fat, salt and sugar) are similar to addictive substances and processed foods are addictive because they are full of sugar or fat. On the other hand, natural foods do not have the content of modified foods. Food addiction might be seen in some groups of obese people and appears like drug addiction [78].

Obesity can not be seen only as a metabolic disorder anymore, it is also a result of eating behavior disturbances (e.g., excessive dieting, bulimia). Binge eating disorder, bulimia nervosa, and anorexia nervosa are the main eating disorders. Additionally, eating disorders are strongly heritable and the genes associated with mood, anxiety, appetite, body weight, and estrogen activity are involved in the development of EDs [79].

A food-addiction property is mainly appeared in people with BED that is characterized by uncontrolled, excessive, fast consumption of great amount of food, despite not being hunger. But there are no compensative behaviors such as self-induced vomitting, fasting or excessive exercise. Even though BED is relevant to obesity, several individuals who are obese do not show BED pattern and some people who display binge-eating behavior are not obese [80]. Binge-eating is the most common among the eating disorders and the percentage of the heritability of BED is from 41 per cent to 57 per cent. Particularly, individuals with BED show elevated impulsivity, compulsivity, and changed reward sensitivity. Various neurotransmitter systems (dopaminergic, glutamatergic, GABAergic, serotonergic, opioidergic, noradrenergic, and cholinergic systems) are included in the development of BED. For instance, decreased D2-like receptor levels may cause to BED [81]. It has been reported that obese people with BED commonly have a gain-of-function allele (A118G) of the (*OPRM1*) which has been concerned with elevated vulnerability to

reward, further desire for fatty and sweet foods and substance addiction. In fact, vulnerability to reward is a characteristic which has been relevant to drug addiction and obesity [82].

Bulimia Nervosa (BN) is a heavy psychiatric disorder and its prevelance in the general population is one per cent. It is less prevalent in males than females and according to the DSM-5 criteria, the prevelance of BN in females is predicted to be as high as 2.6 per cent. The binge eating features of BN are; eating foods in large portions and in a short time and loss of control while eating. And then, the binge eating episodes are compensated to be able to control body weight. Furthermore, the compensative behaviors for BN are self-induced vomitting, laxatives abused, abuse of enemas, abuse of diuretics, fasting, and excessive exercise. The individuals with BN are always worried about their body weight [83].

The last type of eating disorders, anorexia nervosa, is a severe and lethal mental disorder and usually starts during puberty in females. AN patients are below-normal weight and generally are seriously underweight. Anorexia nervosa is characterized by permanent food intake restriction, resulting in so low body weight, afraid of weight gain, and disturbances in sense of body weight and shape. The self-starvation causes excessive weight lose in AN patients. Additionally, the individuals with AN might display other clinical features such as hyperactivity, disturbances in mood. Although the etiology of AN is not clear, it is a complex disease affected by prenatal, perinatal and genetic factors, hormonal alterations during puberty, and stressful lifestyle. One suggestion is that reduced neurotransmission of serotonin as a result of malnutrition is included in the hyperactivity and mood disturbances in the individuals with AN. Furthermore, it has been supposed that abnormal reward processes related to dopaminergic system take a role in the development of AN. Unfortunately, whether these anomalies are the reason or the consequences of chronic defects in eating behaviors is not clear. On the other hand, AN patients display lower seeking and appear to be less hedonic than other eating disorders. Some scientists suppose that AN is related to food anhedonia and hyporesponsive striatal dopamine system [84, 85].

Specific foods are addictive and activate brain reward systems like drugs do. Additionally, specific people (with obesity) show a characteristic of overconsumption of foods that resembles drug addiction/dependence. Reduced response in reward-related neural areas has

been connected with obesity in the course of consumption of food. Substance use disorders and obesity have been related to further activation in the striatum, anterior cingulate cortex, and medial orbitofrontal cortex in reaction to drugs and foods. Therefore, obesity should be conceptualized and treated as a brain disease [86].

Reward processing for overconsumption of foods and addiction activate the mesencephalic dopaminergic system. Repetitive triggering of this area subsequently stimulates activity in other transmitter systems, causing cumulative compulsory behavior and uncontrolled over-consumption of food and substance. Repetitive exposure to sweet and fatty foods brings about obligatory food intake, and uncontrolling of food consumption [87].

Several neuronal pathways in the hypothalamus, brainstem, and higher cortical centers modulate appetite and food intake. The cerebral 'appetite center,' the hypothalamus, is influenced by external endocrinological and neurological feedback signals, and regulates appetite, food intake, and energy homeostasis [88].

Food intake activates the reward circuits and the expenditure of highly palatable and high calorie foods elevates the amount of dopamine in the brain and activates reward-related pathways. In animals, food has the ability to stimulate the release of dopamine in the NAc and the ability of food to elevate dopamine is thought by some to be considerable for its rewarding effects. Alteration in the dopaminergic reward system of the mesolimbic area could bring about overconsumption of food [89]. Food-related stimuli activates brain centers which take roles for the synthesis, release or projections of dopamine. Additionally, highly palatable foods enhance a further dopamine release, stimulating more pleasant emotions [90]. Nonetheless, long-term high-fat diets (HFD) cause to the reduction of DA availability and release and then results in diet-induced obesity. It has been supposed that elevated DA turnover, diminished DA biosynthesis and/or DA inactivation might make contribution to less DA availability and release in diet-induced obesity [91].

1.5.1. Dopamine

The catecholamine neurotransmitter dopamine (C8H11NO2; 3-hydroxytyramine; DA) is significant for hedonism, mood, cognition, motivation, food intake, overeating, attention, stress, and the development of addiction of the substances and it is precursor of

norepinephrine (NE). In 1957, the neurotransmitter dopamine was found out in the brain, and in 2000 the Swedish pharmacologist Arvid Carlsson and Paul Greengard and Eric Kandel shared the Nobel Prize in Physiology and Medicine. DA is present as organic cations in the body fluids and brain tissues and so it is electrochemically active and has a net positive charge. In mammalian brain, four main dopaminergic pathways have been described; the nigrostriatal, mesocortical, mesolimbic, and tuberoinfundibular systems which consist of the A9 (nigrostriatal), A10 (mesocortical and mesolimbic, generally entitled the mesocorticolimbic pathway), and A12 and A14 (tuberoinfundibular) clusters of dopamine-including cells. These systems play significant roles in cognition, locomotion, reward processing, neuroendocrine function. Additionally, abnormalities of the dopaminergic pathways are involved in diseases such as Huntington's disease, bipolar disorder, schizophrenia, drug addiction and Parkinson's disease [92]. Neurons from the substantia nigra project to the striatum via nigrostriatal way and this way is particularly effective in the regulation of motor behavior. Degeneration of these projections of the nigrostriatal pathway has been shown to be relevant to feeding behavior and Parkinson's Disease. Neurons from the ventral tegmental area project to the areas of limbic (mesolimbic pathway) and cortical (meso-cortical pathway) and these two pathways are important for the modulation of cognition, motivation, and rewarding. Dopamine neurons of the arcuate project to the pituitary gland via the tuberoinfundibular path and this pathway regulates the release of prolactin hormone [93]. VTA, SNpc and NAc are places for the food intake modulation. Additionally, dopamine release in the VTA is particularly important for appetite. So that, it is not surprising that the dysfunction of DA neurotransmission has been correlated with eating disorders and obesity [94].

Dopamine or dopaminergic neurons utilize dopamine to communicate with each other and these kind of neurons are old with regard to phylogeny and approximately 400,000 dopaminergic neurons are available in the human brain [95]. Dopaminergic neurons have the incomparable feature of synthesis, translocation, metabolism and utilization of DA, and lastly direct attention to synaptic transmission. Dopamine is found in neurotranmitter vesicles, extravesicular cytoplasmic regions, and extracellular spaces and is a reason of neuronal damage because of the free radicals if the translocation or vesicular storage is interrupted [96]. Dopamine neurotransmission may be affected by various factors such as

the levels of DA produced and released, the amount of DA receptors at the synapse, the duration of DA expends in the synaptic region [97].

The presence of the tyrosine amino acid is the key for the synthesis of dopamine. The enzyme tyrosine hydroxylase (TH) converts the tyrosine into dihydroxyphenylalanine (dopa) (hydration reaction). Afterwards, dopa is converted into dopamine by the enzyme dopa decarboxylase (DDC) in a rapid manner. DDC is also termed as aromatic l-amino acid decarboxylase, because at the same time it affects other aromatic amino acids. Other enzymatic reactions do not occur in neurons which utilize dopamine as a neurotransmitter. Furthermore, dopamine-β-hydroxylase (DBH) catalyze the generation of norepinephrine from dopamine in some neurons, and later phenylethanolamine-N-methyltransferase (PNMT) can convert norepinephrine into epinephrine (Figure 1.1) (adapted from [98])

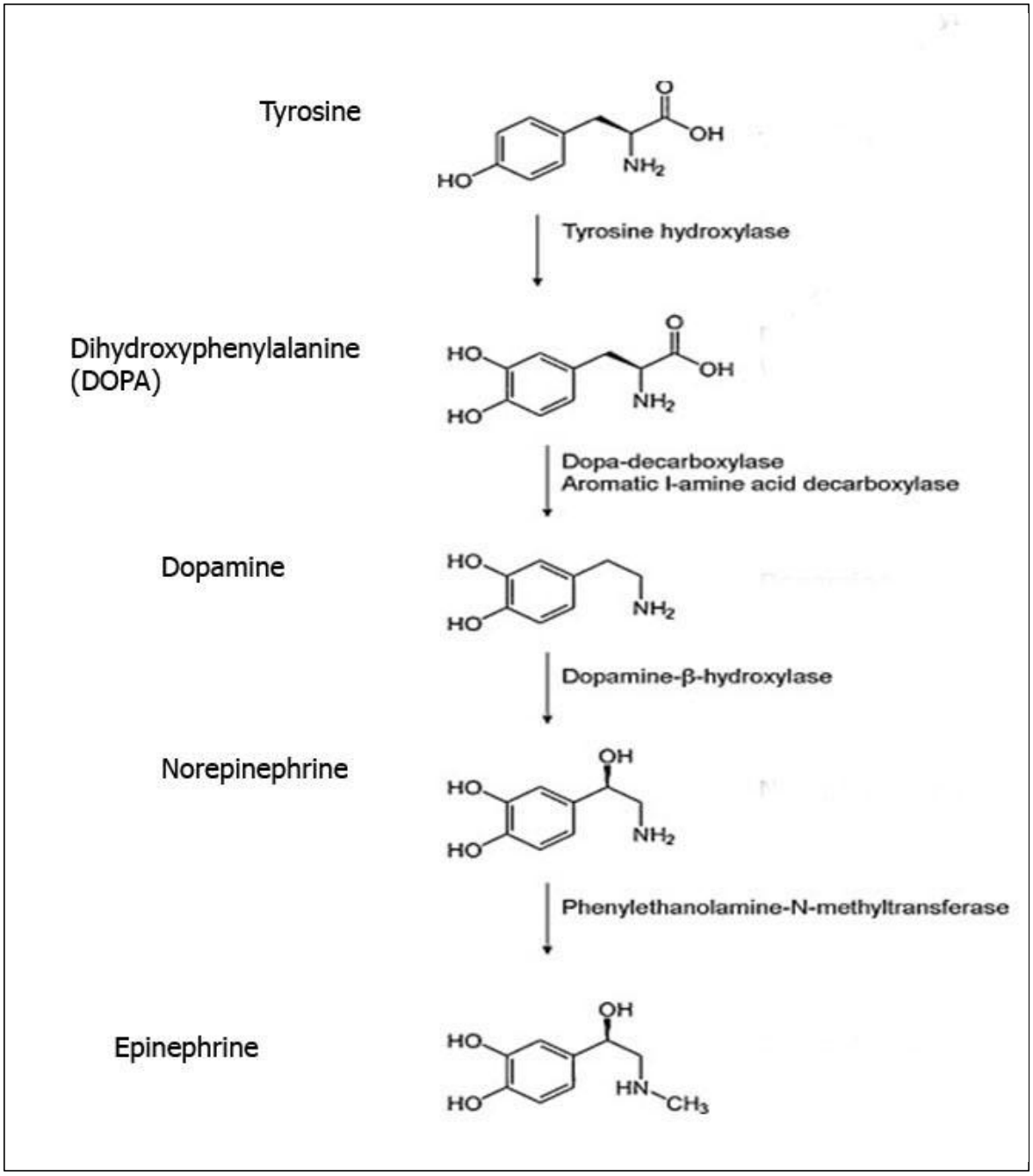


Figure 1.1. Biosynthesis of catecholamine neurotransmitters (Dopamine, norepinephrine, and epinephrine).

After releasing of dopamine from the terminal of the neuron, it interacts with and activates G-protein-coupled receptors on synaptic and extrasynaptic membranes and so that shows the biological functions of it. The dopamine receptors might be categorized by two classes,

the dopamine D1-like receptors (receptors D1 and D5) and the dopamine D2-like receptors (receptors D2, D3 and D4). Dopamine transporters (DAT) ensure the re-uptake of DA for the termination of receptor activation. Another way for termination of receptor stimulation is the degradation of dopamine by enzymes. Two main enzymes, catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO), may metabolize dopamine. They degrade unpackaged dopamine in synaptic regions and physiologically inactive molecules are produced. COMT converts dopamine into 3-methoxytyramine and dopamine is converted into 3,4-dihydroxyphenylacetic acid (DOPAC) by MAO. Lastly, degradation of dopamine by COMT and MAO forms homovanillic acid (HVA) which is the main metabolite.

Disrupted dopamine release from presynaptic neurons, disrupted catabolism or impaired translocation could cause disturbances in dopamine neurotransmission [99].

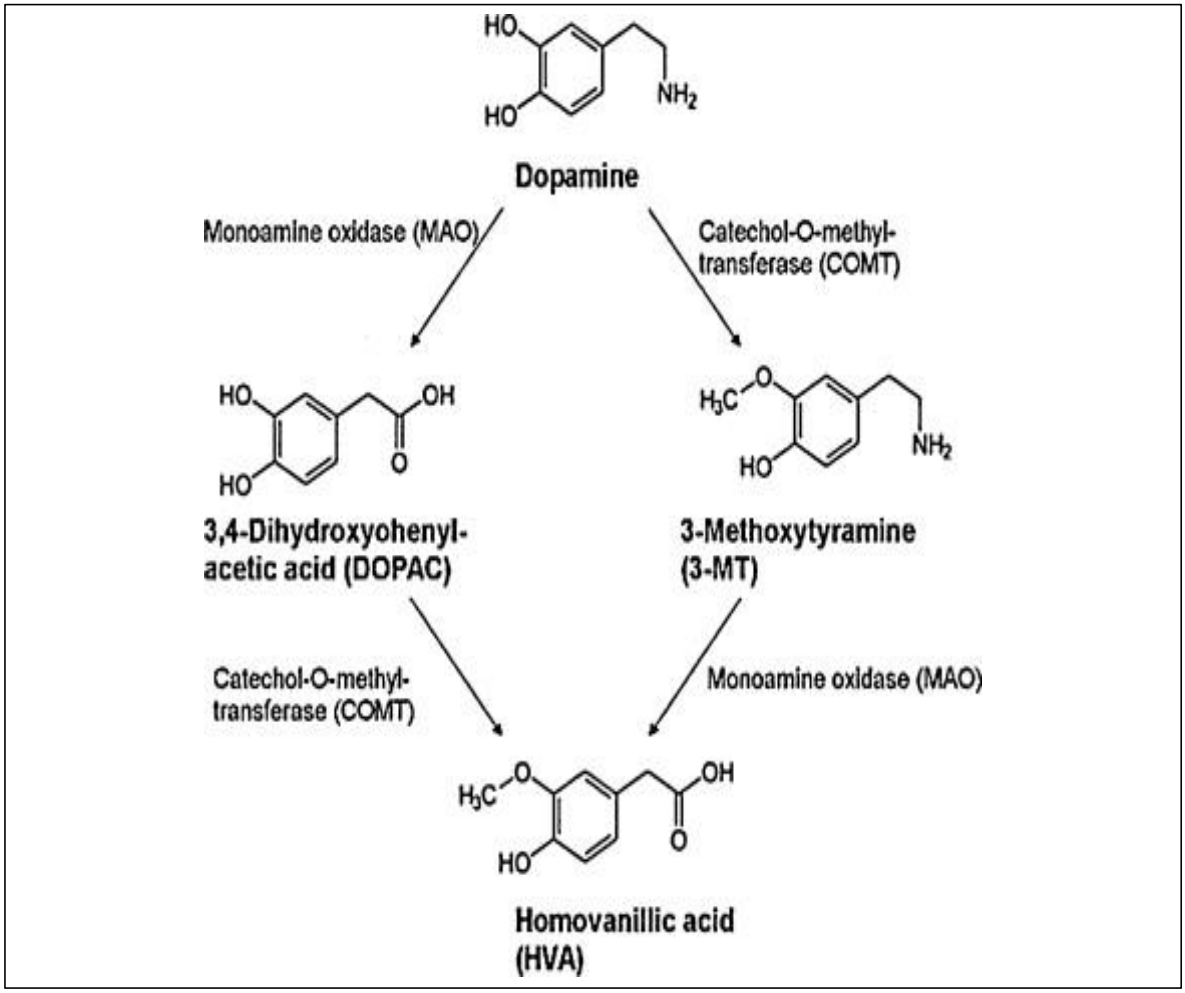


Figure 1.2. Catabolism of dopamine (adapted from [100]).

Some scientists suppose that dopamine is included in the alterations in insulin sensitivity and adipose tissue and regulates the circadian rhythm metabolic alterations related to winter sleep. In insulin resistant animals, the hypothalamic dopamine levels are shown to be reduced. Additionally, animal studies have showed that administration of dopamine stimulates the insulin sensitive condition. FDA approved Bromocriptine-QR, a dopamine D2 receptor agonist, to treat type 2 diabetes. Bromocriptine-QR modulates the glycemic parameters, weight, cholesterol and decreases the cardiovascular risk [101].

1.5.2. Tyrosine Hydroxylase

Neurons of the dopaminergic system include tyrosine hydroxylase (TH) which is dopamine synthesis's rate limiting enzyme and converts L-tyrosine to L-dihydroxyphenylalanine (L-DOPA)[102]. Furthermore, TH is a member of aromatic amino acid hydroxylases [103]. Phosphorylation of tyrosine hydroxylase enhances its activity and stimulates catecholamine synthesis and this modification adjusts the enzyme stability [104]. In mammals, dopaminergic mechanisms and TH have taken place in the modulation of food intake and locomotion [105].

TH gene is located on the short arm of chromosome 11 in band 15.5, and that region is abundance of genes in humans [106]. It has been demonstrated that alpha-synuclein is crucial for the modulation of DA synthesis, providing the decrease of TH activity. The α-synuclein aggregation generates 'Lewy Bodies' in neurons, and then causes the inhibition of neurotransmission throughout the CNS. The α-synuclein overexpression in the dopamine neurons decreases the activity of TH. Eventually, the decrease of DA biosynthesis leads to the degeneration of the neurons in the dopaminergic system and then Parkinson's disease [107].
It has been suggested that diet-induced obesity cause to the diminishing of the TH expression in NAc and VTA in mice [108].

1.5.3. Dopa Decarboxylase (DDC)

Dopa decarboxylase (DDC) was described in kidney tissue which belongs to a mammalia in 1938 and it was announced that it has an important role for the biosynthesis of epinephrine. After years, it is understood that it converts decarboxylate L-Dopa to dopamine, L-5-hydroxytryptophan to serotonin, p-tyrosine, tryptophan and phenylalanine to the concerned amines. Therefore, DDC is also termed as aromatic amino acid decarboxylase (AADC). DDC is a member of aminotransferases and is not thought to be rate-limiting enzyme in catecholamines synthesis in the body, but it becomes rate-limiting enzyme in many disesases such as Parkinson disease (PD). In mammalian neurons, the DDC have a function for the biosynthesis of neurotransmitter, and in other tissues, such as

kidney, lungs, liver, pancreas, and gastrointestinal tract and its function have not been detected [109]. The DDC gene is located on the short arm of the chromosome seven in band 12.1-12.3, and includes 15 exons, covering more than 85 kb of genomic DNA [110].

1.5.4. Dopamine Receptors

Dopamine activities are intervened by five receptors termed as D1, D2, D3, D4, D5. These receptors are divided into two subgroups (according to their pharmacological, functional properties, sequence homology, and structure): 1) The D1-like receptors (D1 and D5), that trigger the cAMP generation and the protein kinase A (PKA) activity and 2) The D2-like receptors (D2, D3 and D4), that adjust the production of cAMP in a negative manner, consequently the activity of PKA is reduced [111]. All of these dopamine receptors belong to G-protein coupled receptor (GPCR) family [112]. The biological function of dopamine is done by the interaction between dopamine and receptors [113]. In some diseases such as depression, Parkinson's disease, and schizophrenia amount and sensitivty of DA receptors are changed in some brain centers [114].

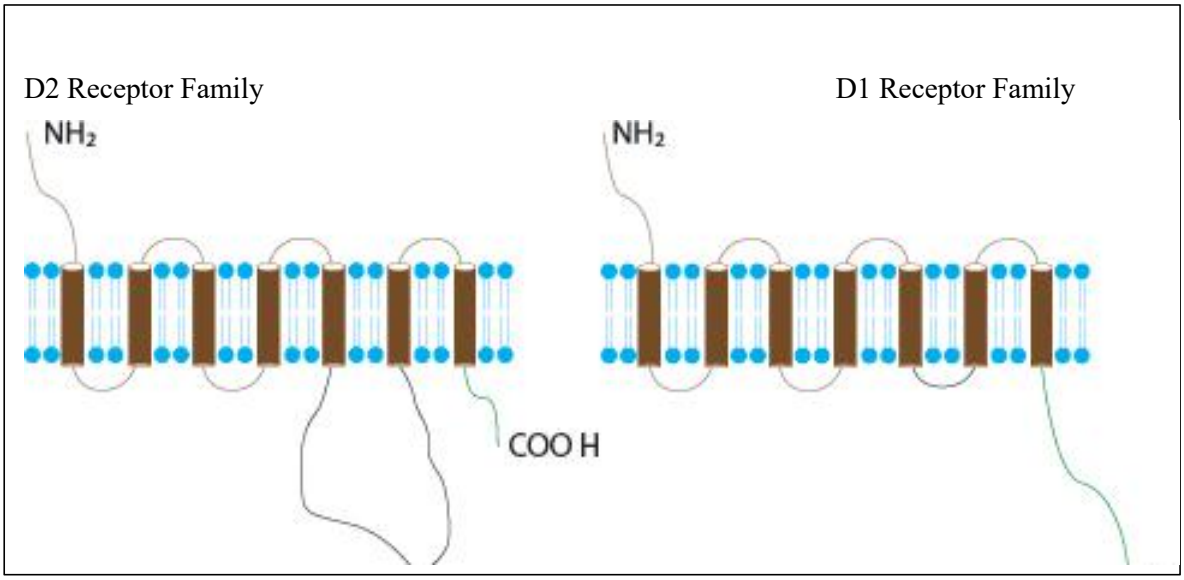


Figure 1.3. The structure of D2 and D1 Receptor Families (adapted from [115]).

1.5.4.1. Dopamine D1-like Receptors

Dopamine D1-like receptors are the class A of rhodopsine-like seven-transmembrane G-protein coupled receptors and induces Gs which is positively matched to adenylate cyclase and gives rise to increased cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) activation. D1-like receptors, by inducing cAMP generation, are excitatory [116].

The synthesis of the D1-like receptors occur in the cell cytoplasm and then consequently these receptors are moved into the plasma membrane of cell bodies and dendrites. The distribution of D1 and D5 receptors in the cell is distinguished and the D1 receptors are localized mainly in axon terminals and dendrites. Non-dopamine neurons include the D1-like receptors to induce neuronal signaling and elevate the amounts of cAMP. Among the dopamine receptors, the dopamine D1 receptor is synthesized in a highest level, in other respects, the affinity of dopamine D1 receptor for dopamine is 10-fold lower than dopamine D5 receptor. The dopamine D1 receptor gene is located on chromosome 5q35.1. The dopamine D5 receptor gene is located on chromosome 4p16.1-15.3. Additionally, the D1-like receptors do not involve introns. In humans, the D1-like

dopamine receptors are 79 per cent identical for significant transmembrane domains. Conversely, the D1-like and the D2-like receptors have only 40–45 per cent similarities [117].

1.5.4.2. Dopamine D2-like Receptors

Dopamine D2-like receptors are the class A of rhodopsine-like seven-transmembrane G-protein coupled receptors. In the nucleus accumbens, olfactory tubercle, and striatum D2-like receptors are available in great amounts, and in the hypothalamus, amygdala, hippocampus, and cortical regions in lower levels. Autoreceptors which localize on dopamine neurons are composed of the D2-like receptors. D2-like receptors activate Gi proteins which supress the generation of cAMP by blocking AC. Furthermore, D2-like receptors directly modulate ion channels which are important for modulation and formation of calcium influx via release of the Gβγ subunit upon receptor activation. Activation of D2-like receptors are inhibitory and after these receptors are activated, the DA release and the excitability of the neurons are decreased. For this reason, autoreceptors are so important for the regulation of dopamine neurotransmission. Pre-synaptic autoreceptors on the terminals of dopaminergic neurons adjust neurotransmission by blocking the possibility of dopamine release from vesicules, reducing the synthesis of dopamine and changing the dopamine uptake [118].

There are two isoforms of the D2R, a short form exists presynaptically, and a long form exists postsynaptically. Particularly, D2R is the major presynaptic autoreceptor of the dopaminergic neurotransmission. Dopamine D2 receptor (DRD2) gene is located on the long arm of chromosome 11 in band 22. The SNP Taq1A (rs1800497), a C (A2-allele) to T (A1-allele) substitution is the most studied polymorphism of DRD2 gene [119].

The D3Rs exist postsynaptically and found in large amounts in limbic regions such as nucleus accumbens. Dopamine D3 receptor (DRD3) gene is located on the long arm of chromosome 3 in band 13.3 and covers approximately 50.3 kb; mutations in this gene may change the receptor structure or expression. 9Ser/Gly SNP is the most common

polymorphism in this gene and homozygotes for the polymorphic allele show higher affinity for DA [120].

Dopamine D4 receptor (DRD4) gene is located on the short arm of chromosome 11 in band 15.5. A 48-bp variable number tandem repeat (VNTR) in exon 3 of DRD4 gene is the most common polymorphism in this gene. Until now, ten repeat alleles (2 to 11 repeat alleles) have been described and the 2-, 4- and 7-repeat alleles are the most prevalent throughout the world, but because of ethnicity differences, the frequency can be various [121].

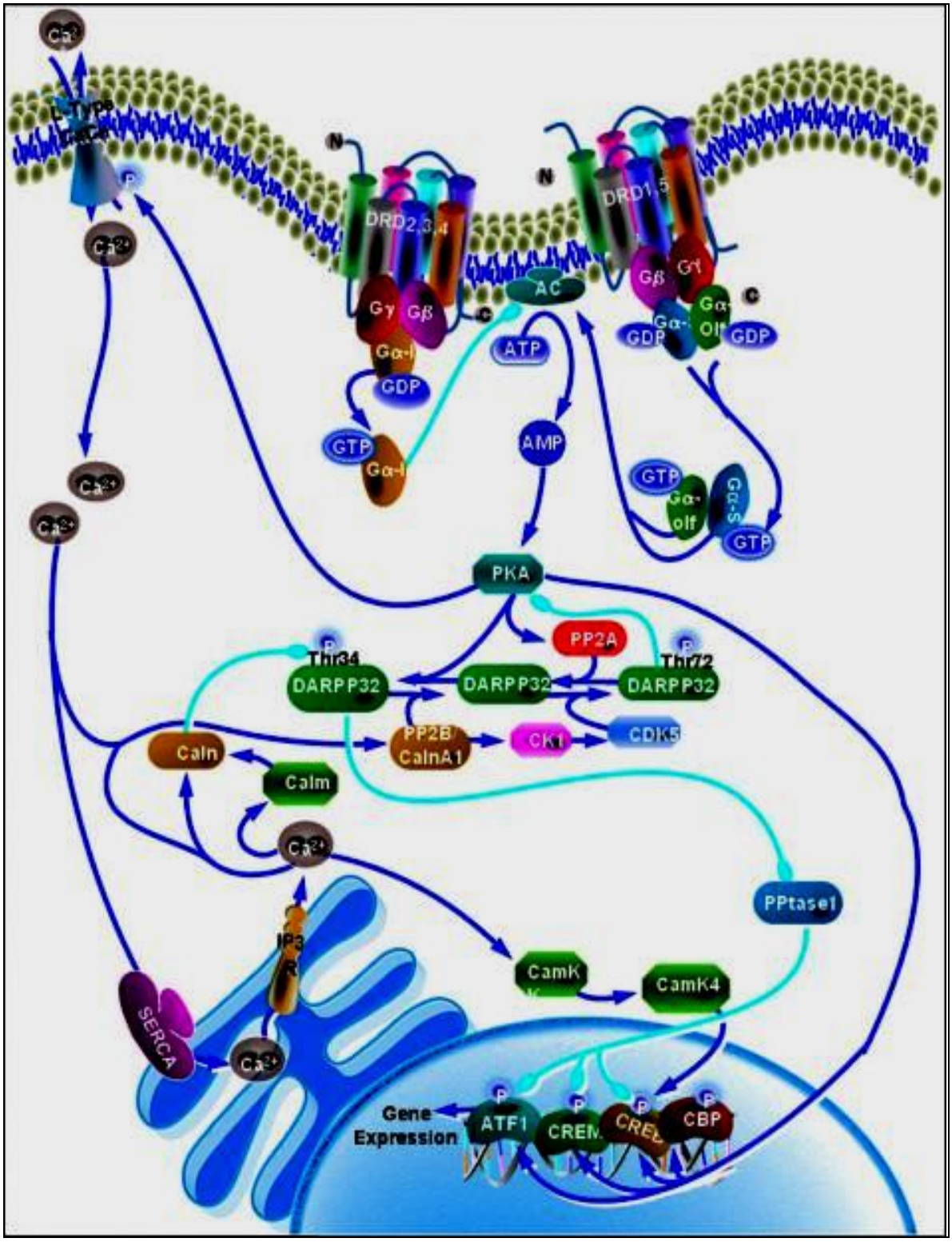


Figure 1.4. The signalling pathway of Dopamine D1-like receptors and D2-like receptors (adapted from [122]).

1.5.5. Dopamine Transporter (DAT)

Dopamine transporter (DAT) is a member of the Na^+/Cl^--dependent neurotransmitter transporter family (SLC6). DAT is a transmembrane protein which provides the reuptake of DA from the synaptic cleft and terminates the neurotransmission of dopamine. DAT decreases the presence and amount of DA at receptor sites. DAT is produced in the striatum in high levels in humans and besides, it significantly adjusts synaptic dopamine in the striatum [123]. Abused drugs such as amphetamine (AMPH), methamphetamine (METH) and cocaine target the DAT. DAT is influenced by these drugs in two ways: drugs, such as cocaine, bind to the transporter and block its activity, drugs, such as AMPH and METH, are transported into the cell and induce the transport of DA back into the synaptic cleft [124]. Additionally, acute and chronic administration of cocaine and methylphenidate reduces the availability and expression of DAT [125].

The DAT gene, the solute carrier family 6, member 3 gene (SLC6A3), is located on the short arm of the chromosome 5 in band 15.3. Generally, the gene is termed as DAT1 to make its function more clear. Mutations in the SLC6A3 gene might influence the expression of DAT, change and impair transmission of DA [126]. A 40-bp VNTR polymorphism in the 3' UTR in exon 15 is the most known one and it has been reported that DAT1 has seven alleles (3 to 11 tandem repeats), and the most common ones are 9 and 10 repeats [127]. It has been reported that DAT VNTR 10-repeat homozygotes have higher expression of DAT1 in striatum than 9/10-repeat carriers. 10-repeat homozygotes have DAT proteins in high levels, therefore clearance of synaptic DA may be enhanced [128].

1.5.6. Vesicular Monoamine Transporter-2 (VMAT2)

VMAT2 is located in the synaptic membrane and transports DA into synaptic vesicles from the extravesicular cytoplasmic region. Dopamine is a strong source for formation of free radicals and moving of dopamine into synaptic vesicles by VMAT2 inhibits autoxidation and consequently damage of dopaminergic neurons [129].

Amphetamine-like drugs inhibit DA transport into synaptic vesicles by VMAT2, enhancing the amounts of extravesicular DA in the cytoplasm and DA release through mechanisms which involve the reverse transport mediated by DAT. The increase of dopamine levels in the cell can be toxic to dopaminergic neurons because of amphetamine derivatives [130].

To package dopamine adequately is significant for the function and survival of the dopaminergic neurons. More than 90 per cent of intracellular DA is moved into vesicules, inhibiting the accumulation of DA in cytoplasm and production of reactive oxygen species which are toxic to neurons [131].

VMAT2 is produced in the CNS and peripheral tisuses such as adrenal cells, endocrine cells of the stomach, enteric nervous system, and platelets [132]. VMAT2 (SLC18A2) gene is located on the long arm of chromosome 10 in band 25.3 and in humans, it involves 16 exons and 15 introns. Polymorphisms in the VMAT2 gene which affect the expression or protein function may be genetic risk factors for Parkinson's Disease [133]. Furthermore, VMAT-2-deficient mice show elevated oxidative stress, degeneration of dopamine neurons and reduced motor activity [134].

1.5.7. Catechol-O-methyltransferase (COMT)

COMT is a degradading enzyme and metabolizes DA to inactive compounds. In the prefrontal cortex (PFC), COMT adjusts dopamine neurotransmission and provides approximately 60 per cent of the degradation of DA and it is mostly found in the rough ER in glial cells and postsynaptic neurons. COMT has two isoforms and same gene encodes these isoforms. The longer, membrane-bound COMT protein that is expressed mainly in the brain has 50 more amino acids than the shorter form, soluble COMT. The COMT gene is located on the long arm of chromosome 22 in band 11.2. and the most common polymorphism in this gene is the val158met SNP (guanine-to-adenine transition). When the Met allele and Val alleles are compared, the Val allele has four times more enzymatic activity. Generally, L is used for the Met allele for the low enzyme activity, and H is used for the Val allele for the high enzyme activity. Therefore, people homozygous for the Met

allele have a 40 per cent lower enzymatic activity in dorsolateral PFC, leading to higher synaptic dopamine levels [135]. On the other hand, the enzymatic activity of heterozygous individuals is between the enzymatic activity of homozygous individuals, because the alleles are codominant [136].

In PD patients, after the L-DOPA treatment, the plasma levels of homocysteine (Hcy) are increased because of the methylation of L-DOPA by COMT enzyme. Furthemore, homocysteine is a neurotoxic agent and causes the death of dopaminergic neurons [137].

Otherwise, in mice it has been indicated that diet-induced obesity gives rise to the decreased *COMT* expression in NAc and VTA and it supports the idea of the elevated DA turnover monitored in the NAc [138].

1.5.8. Monoamine Oxidase (MAO)

Monoamine oxidases are enzymes and localized in the outer mitochondrial membrane. They take roles in the oxidative deamination of neurotransmitters and dietary amines. Monoamine oxidases are produced in several tissues in the body, particularly in liver. Monoamine oxidases have two isoforms, MAO-A and MAO-B,encoded by two different genes which located on the short arm of X-chromosome in band 11.23. MAO-A protein is similar to MAO-B protein in terms of structure and molecular weight (70 per cent amino acid sequence identity). On the other hand, these proteins are distinct in their affinity for substrates and inhibitors [139].

The outer mitochondrial membrane of glia and monoamine releasing neurons, particularly norepinephrine releasing neurons, are places for the localization of MAO-A and it metabolizes monoamines such as dopamine, serotonin, and norepinephrine. Therefore, in the brain, MAO-A is so important for the amounts of serotonin, norepinephrine, and dopamine [140].

A 30 bp VNTR polymorphism of MAO-A gene in the promoter region is the most studied one and the most prevalent alleles involve 3 or 4 repeats, and the least common alleles include 2-, 3.5-, and 5-repeats. In subjects which carry 3.5 or 4 repeats, expression of the

enzyme is comparatively high (MAO-A High) and for carriers of 2, 3 or 5 repeats, the expression is lower (MAO-A Low) [141].

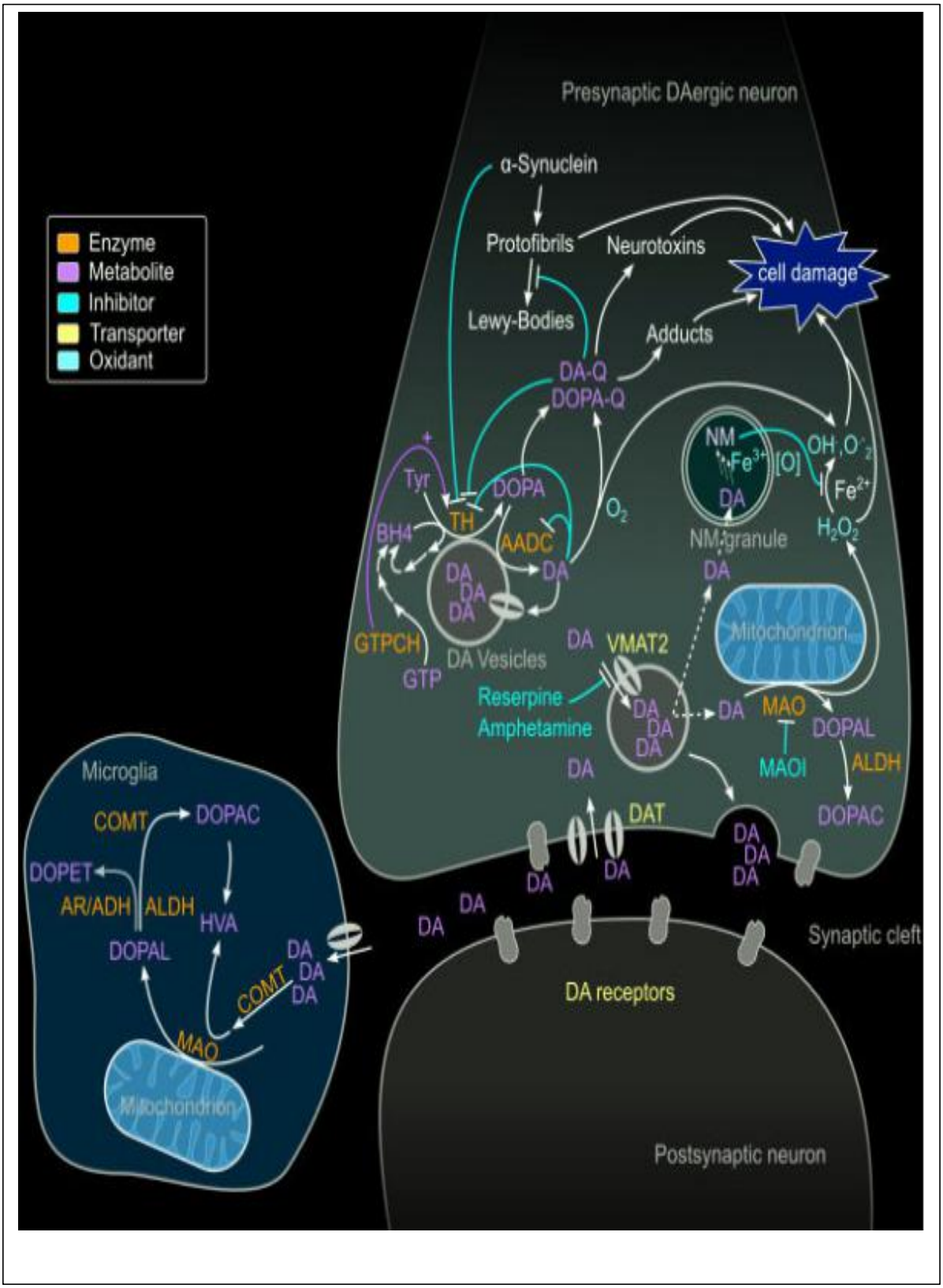


Figure 1.5. The summary of dopamine metabolism (adapted from [142]).

1.6. MANAGEMENT OF OBESITY

1.6.1. Modification of Lifestyle

The excellent combination of physical activity and diet is the main part of the management of weight. The suitable diet must involve less energy than is required for the day-care. A decrease of energy by 500 kcal/day under energy necessaries or planning a proper diet program that has 1500–1800 kcal/day for males and 1200–1500 kcal/day for females will complete the first aim successfully [143]. Furthermore, elevated physical activity is a requirement for the management of obesity. Accelerating physical activity very definitely may generate energy deficiency by ascending energy consumption. Consequently, these lifestyle changes might prevent the occurrence of obesity, and or provide the modulation of several variables such as HDL, blood pressure, tryglycerides, the risks of cardiovascular diseases and type 2 diabetes, and give rise to weight loss in a healthy way [144].

1.6.2. Bariatric Surgery

Bariatric surgery (BSx) might be a feasible treatment for morbidly obese patients (BMI ≥35 kg/m2 with obesity-associated disturbances such as diabetes mellitus, sleep apnoea, or polycystic ovarian syndrome or BMI ≥40 kg/m2) who can not succeed to lose weight by the help of lifestyle modifications with or without drug therapies. Bariatric surgery which is an invasive method, may involve risks in a short or long periods and patients require to be observed for many years after operation. Furthermore, bariatric surgery may contribute to lose weight in a regular way [145].

There are 3 main types of bariatric surgery methods: Roux-en Y gastric bypass, sleeve gastrectomy, and laparoscopic adjustable gastric banding. Laparoscopic adjustable gastric banding is a restrictive process including the implanting of a silicone band around the upper stomach. Sleeve gastrectomy is a surgical process in which the large portion of the stomach is detached. Roux-en Y gastric bypass directly connects the majority of the stomach to the intestine [146]. On the other hand, after bariatric surgery, the patients are more vulnerable to the substance use problems [147].

Currently, a medical equipment (the Maestro Rechargeable System) that may be placed surgically in the abdomen and this device has been confirmed in order to treat obesity. It has been supposed that this device is associated with neural pathway between the stomach and the brain [148].

1.6.3. Pharmacotherapy

The pharmaceutical agents that manage weight approved by the U.S. Food and Drug Administration (FDA) are generally used for overweight people (BMI ≥27 kg/m2) with obesity-associated disturbances such as diabetes mellitus, hypertension or for obese individuals (BMI ≥30 kg/m2).

Phentermine: FDA approved phentermine for the use of short period (12 weeks) in order to treat obesity. It is the most prevalent pharmaceutical agent for the treatment of obesity in US. Phentermine suppresses the appetite by inhibiting the reuptake of epinephrine.

Benzphetamine, Phendimetrazine, and Diethylpropion: FDA also approved these pharmaceutical agents for the use of short period in order to treat obesity by suppressing the appetite.

Orlistat (Xenical®, Alli®): Orlistat is approved by the FDA for the long-term treatment of obesity. Orlistat is a pancreatic lipase inhibitor which inhibits the hydrolysis and absorption of triglycerids in the intestine, contributing to excretion of approximately 30 per cent of ingested fat, and decreasing the fat absorption in the intestine. Moreover, orlistat might elevate the secretion of glucagon-like peptide 1.

Lorcaserin (Belviq®): Lorcaserin is a selective serotonin 2C (5-HT2C) receptor agonist and it modulates energy balance and satiety in the hypothalamus.

Phentermine/Topiramate (Qsymia®): FDA approved phentermine/topiramate extended release (ER) for the use of long period in order to treat obesity. Phentermine is an appetite suppressant amine which enhances the amounts of noradrenalin in the CNS. Topiramate is a γ-aminobutyric acid receptor regulator and the mechanism of its action is still unclear.

Naltrexone/Bupropion (Mysimba®) SR (Sustained Release): The combination of bupropion and naltrexone is approved for the use of long period in order to treat obesity. Naltrexone is an opioid receptor antagonist blocking all three opioid receptors and bupropion is a reuptake inhibitor of dopamine and serotonin.

Liraglutide (Saxenda®): The L-cells in the small intestine secrete the GLP-1 hormone and it triggers the insulin release. Liraglutide is a glucagon-like peptide 1 (GLP-1) receptor agonist and it is used in order to treat type 2 diabetes and weight loss. The agonistic effect of the gut-derived hormone GLP-1 forwards signals to the brain and then leads to the suppressing appetite and decreasing food intake [149].

Sodium-glucose co-transporter type 2 (SGLT2) inhibitors: SGLT2 inhibitors (empagliflozin, dapagliflozin, canagliflozin) are new anti-diabetic drugs which promote weight loss in small quantities. These agents elevate the glucose excretion and decrease hyperglycemia.

Glucomannan (Allevo®, Kilo Trim®, XLS Medical Appetite Reducer® etc.): Glucomannan is a soluble dietary fiber supplement and it has been reported to suppress appetite and stimulate weight loss. When glucomannan is hydrated in the stomach, it shapes a sticky and gel-like mass and then stimulates satiety. The glucomannan is approved by EFSA (The European Food Safety Authority) in 2010 [150].

Nevertheless, these antiobesity agents were approved by FDA, have some adverse effects. These side-effects are; elevated defecation, dizziness, vomiting, insomnia, fecal urgency, paresthesia, nausea, constipation, dry mouth, fecal incontinence, fatigue, headache, diarrhea [151].

1.6.4. Deep Brain Stimulation

Obesity is an avoidable disorder by the alterations of physical activity and food intake patterns. Lifestyle modifications are so critical but has some limitations in order to get rid of excess weight. On the other side, pharmacotherapy shows some adverse effects such as gastrointestinal intolerance by the administration of orlistat. Bariatric surgery causes the

reduction of the volume of the stomach. These surgical processes lead to protein, vitamin, and microelement deficits and are so risky after operation for several years [152].

Most recently, deep brain stimulation (DBS) is a seminal procedure in order to treat neuropsychiatric and movement diseases by neuromodulation and has been present for 25 years. DBS is an invasive surgical method including the placement of chronic electrodes (pacemaker), which project electrical impulses to the particular regions in the brain and may be used to increase memory functions [153]. It has been showed that patients with obesity, alcoholism, smoking, and drug addiction decreased cravings after NAc DBS. In one study, it has been shown that chronic NAc DBS promoted the decrease of food intake and enhanced the expressions of D2R and DA genes in diet-induced obese rats, but there were no differences for chow-fed rats. NAc is the main regulator of the brain reward system and hypomania is the most prevalent side effect of NAc DBS. It has been proposed that DBS increased the projections from the VTA to the NAc, so that resulted in the release of DA [154]. According to the animal and clinical studies, DBS may be applied for the treatment of anorexia nervosa, but the mechanism is unclear. Therefore, further studies are needed [155]. On the other hand, it has been shown that in PD patients, the bilateral DBS of the subthalamic nucleus (STN) stimulates weight gain in the first months after surgery. Additionally, some parkinsonian patients develop eating disorder approximately in 3 months after the STN DBS surgery. For these reasons, it has been suggested that after STN DBS surgery, the patients should modify their food intake and physical activity not to gain excessive weight [156].

1.7. AIM OF THE STUDY

Dopamine is the main modulator of the brain reward system and significantly regulates food intake and the mechanisms were discussed above in detail. The most studied genes are dopamine receptors in several populations around the world, for this reason we excluded these genes in this study. Our main goal was to investigate the relationship between the gene variants of the metabolism and transport of DA and obesity. Therefore, we were interested in the enzymes MAO-A and COMT, and the transporters VMAT2 and DAT1 to be able to figure out the mechanism of obesity via DA neurotransmission.

Galvao et al., have shown that 30 bp VNTR polymorphism of the MAO-A gene was effective on the eating behavior of children and more palatable food intake was observed in the children who carry the high activity allele [157].

Annerbrink et al., have shown that the COMT Val158Met polymorphism was correlated with body fat distribution in middle-aged Swedish men; there was a significant relationship between this polymorphism and waist-hip ratio, but the BMI did not change [158]. Yeh et al., have demonstrated that COMT Val15Met polymorphism alters the enzyme activity and in the case of Met/Met homozygotes, higher BMI and diastolic blood pressure were measured. As a result of the study, the COMT polymorphism was reported as a risk factor for hypertension and obesity [159].

VMAT2 gene has been reported to be related with alcohol, nicotine dependence and tardive dyskinesia [160]. In humans, it has been described that VMAT2 gene polymorphisms may be negatively correlated with BMI [161]. Therefore, we suggest that these polymorphisms which alter the expression of VMAT2 gene or the function may be effective in the pathogenesis of obesity.

It is known that the rs27072 (2319G> A) polymorphism of DAT1 gene is correlated with alcoholism and smoking [162]. Agurs-Collins et al., have shown that women with *SLC6A3* 10/10 genotype increased the intake of more high-calorie sweet food. Changes in the activity of *SLC6A3*, makes a difference in the dopamine function and in the binding affinity. In this study, it was shown that there is a direct relationship between SLC6A3 gene and high-calorie sweet food intake. Moreover, it has been thought that genes regulate dopamine affect the quantity and kind of food consumed [163]. On the other hand, Uzun et al, have shown that there was no relationship between obesity and *DAT1* VNTR polymorphism [164].

There have been no studies about the relationship between *MAO-A*, *COMT*, *VMAT2* polymorphisms and obesity in Turkish population so far. While we were writing the project of this study, there was no study about the association between *DAT1* polymophisms and obesity in Turkish population, but in 2015 Uzun et al, published an article about this polymorphism. Additionally, there is no research about the *VMAT2* SNPs around the world. We chose *VMAT2* (rs363399, C/T) and *VMAT2* (rs4752045, C/G) SNPs

because, they have been shown to be associated with depression. On the other hand, the publications about the relationship between *DAT1*, *COMT*, *MAO-A* polymorphisms and obesity are so little in the world.

In light of these evidences, we analysed the effects of dopamine neurotransmission in obesity through the polymorphisms of MAO-A, COMT, VMAT2 and DAT-1 genes in Turkish population. We expect that our findings might be helpful for the determination of the role of DA in the pathogenesis of obesity and then these results might alter the treatment of obesity according to the DA neurotransmission.

2. SUBJECTS AND METHODS

2.1. SUBJECTS

In this study, we have investigated the polymorphisms of MAO-A, COMT, VMAT2 and DAT1 genes in 2 groups. Group 1 (n=234) whose mean age was 31,4 ± 7,8 years contained overweight and obese individuals and the mean BMI was 33,8 ± 8,7 kg/m^2 (see Table 2.1). The control group selected from healthy individuals (n=214) who were compatible with age and sex and BMI between 18.50 and 24.99. The mean age of the healthy group was 27,6 ± 5,8 years and the mean BMI was 21,7 ± 1,9 kg/m^2 (see Table 2.2). Inclusion criteria were: 1.giving informed consent, 2. age between 20 and 48 years, 3. 25≤BMI<30 for overweight and ≥ 30 for obese group; BMI: 18.50-24.99 for control group. Exclusion criteria were: 1. previous use substance of abuse, 2. having a neurological or psychiatric disorder, 3. being pregnant or nursing, 4. being chronic alcoholic, 5. smoking, 6. using antihypertensive beta-blocker, 7. being menopausal, 8. having thyroid or diabetes problem 9. oral contraseptive usage. Permission for research was granted by the Bioethics Committee of Yeditepe University, Turkey. Both men and women of Turkish origin, all volunteers, were collected at the Department of Endocrinology and Metabolism Disorders at Fatih Sultan Mehmet Education and Research Hospital in Istanbul, Turkey. All volunteers were measured and weighed with standard medical device. Informed written consent was obtained from all subjects according to the Declaration of Helsinki guidelines. DNA were obtained from peripheral blood leukocytes from 448 individuals using the DTAB-CTAB DNA extraction procedure and then VNTR analysis was carried out by polymerase chain reaction and agarose gel electrophoresis and SNP analysis was carried out by PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) method.

Table 2.1. The characteristics of the overweight and obese adults.

Variables	**Female**	**Male**
Number (n)	141	93
Mean age (years)	31.1 ± 7.8	31.8 ± 7.8
Mean BMI (kg/m2)	35.1 ± 8.9	31.9 ± 8.1

Table 2.2. The characteristics of the healthy individuals.

Variables	**Female**	**Male**
Number (n)	166	48
Mean age (years)	27.3 ± 5.8	28.6 ± 5.9
Mean BMI (kg/m2)	21.3 ± 1.9	23.0 ± 1.2

2.2. MATERIALS

The list of materials and equipments used in this study were given in table 2.3 and 2.4.

Table 2.3. The list of materials used in this study, the companies they were purchased from, and product codes.

Material name	Company	Product code
DTAB	Sigma-Aldrich	D5047
CTAB	Sigma-Aldrich	H6269
Trizma base	Sigma-Aldrich	T6061
EDTA	ThermoScientific	17892
NaCl	Merck	106404
Cloroform	Sigma-Aldrich	24216
HCl	Riedel-de haen	7102
Ethanol	Sigma-Aldrich	32221
DreamTaq Green DNA Polymerase	ThermoScientific	EPO712
dNTP Mix (2 mM each)	ThermoScientific	R0241
DreamTaq PCR Master Mix (2X)	ThermoScientific	K1071
TE buffer	ThermoScientific	12090015
EtBr (10 mg/mL)	ThermoScientific	17898
TAE buffer	ThermoScientific	B49
Agarose	Sigma-Aldrich	A9539
PCR 20 bp Low Ladder	Sigma-Aldrich	P1598
50 bp DNA Ladder	ThermoScientific	SM0371
100 bp DNA Ladder	ThermoScientific	SM1143
DNA Gel Loading Dye (6X)	ThermoScientific	R0611
MspI	NEB	R0106S
N1aIII	NEB	R0125S
AciI	NEB	R0551L
Primers	BGI	

Table 2.4. The list of devices used in this study, the companies they were purchased from.

Device	**Company**
Nanophotometer	Implen
Electrophoresis	BioRad
PCR Thermal Cycler	BioRad
Ultra Centrifuge	Hettich
Water bath	Memm ert
UV Spectrophotometer	UVP
Vortex	IKA
Spin	Labnet
Autoclave	Witeg
Refrigerator (-80℃)	ThermoScientific
Micropipets	ThermoScientific
Fume Hood	Düperthal
Refrigerator (+4/-20°C)	Arçelik
Microbalances	Denver Instruments
Oven	Arçelik
Centrifuge	Labnet
Cooled block heater	BioEr

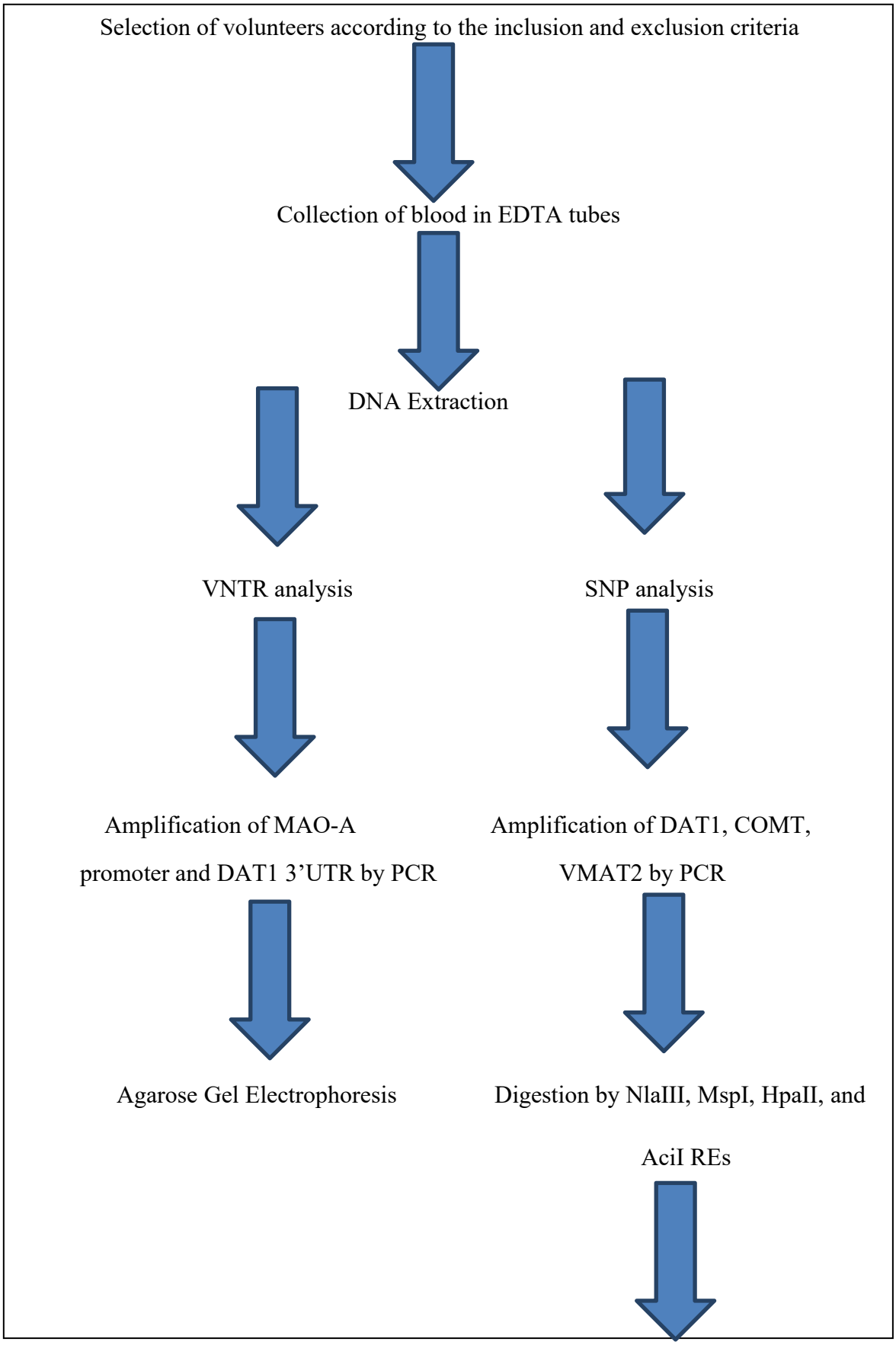


Figure 2.1. The summary of the flow of the experiments.

2.3. GENOTYPING METHODS

2.3.1. DNA Extraction

DNA extraction was performed from peripheral blood samples which were recruited in tubes including ethylenediaminetetraacetic acid (EDTA) which is an anticoagulant agent. For DNA extraction we used the DTAB-CTAB DNA extraction method. DTAB and CTAB are conventional cationic surfactants.

The protocol of the preparation of DTAB (Dodecyltrimethylammoniumbromide):

(i) 4 g of 8% DTAB
(ii) 0,605 g of 0,1 M Tris
(iii) 3,50 g of 1,2 M NaCl
(iiii) 0,930 g of 0,05 M EDTA

These chemicals were solved in 50 mL ddH_2O and the pH was adjusted at 8 by the use of HCl.

The protocol of the preparation of CTAB (Hexadecyltrimethylammonuimbromide):

(i) 1,16 g of 400 mM NaCl
(ii) 2,5 g of 5% CTAB

These chemicals were solved in 50 mL ddH_2O.

The protocol of the preparation of NaCl:
7,01 g of 1,2 M NaCl was solved in 100 mL ddH_2O.

The protocol of the preparation of %70 ethanol:
70 mL ethanol was completed to 100 mL by adding 30 mL ddH_2O.
The composition of 1X TE buffer:
10 mM Tris-HCl (pH 8.0)
0,1 mM EDTA
The protocol of DTAB-CTAB DNA extraction method:

- Blood was extracted in EDTA tubes (purple top).

- 1350 µL of DTAB buffer (8% DTAB, 100 mM Tris pH 8, 1,2 M NaCl, 50 mM EDTA) was added to 1 mL of blood and then incubated at 65°C for 5 min.
- 1700 µL of chloroform was added and quickly closed the tube and vigorously shaked the mix for at least 5 min (This was a critical step, if it was not done in a good way there would have been blood clots in the mix which would have decreased the quantity and probably the quality of DNA).
- The tubes were centrifuged at 13,000 rpm for 5 min.
- The aqueous phase was transferred to a new tube and added 350 µL CTAB buffer (5% CTAB, 400 mM NaCl) and 3 mL of ddH_2O. It was mixed gently and incubated at – 20 °C for 15 min.
- It was centrifuged at 10,000 rpm for 5 min and discarded the supernatant.
- The pellet was resuspended in 700 µL of 1,2 M NaCl and 1 mL of cold 100% ethanol. It was mixed gently (The nucleic acid precipitate was able to be seen).
- It was centrifuged at 10,000 rpm for 5 min and discarded the supernatant.
- The pellet was washed with 2300 µL of 70% ethanol.
- It was centrifuged at 10,000 rpm for 5 min.
- The EtOH was evaporated and resuspended the pellet in 100 µL TE buffer.

2.3.2. Determination of DNA Purity

After the implementation of DNA isolation, the optical density of each sample were read at 260 nm and 280 nm using a spectrophotometer. The total yield of extracted DNA were computed by the DNA concentration (ng/µL) multiplied by the final elution volume. The absorbance value of each sample was measured by using 3 µL volume of each sample. Moreover, TE buffer was used as a “Blank”. Purity were compared based on A260/A280 absorbance ratios in the range of 1.7-1.9. The absorbance value of double-stranded DNA at 260 nm is 50 µg/mL.

2.3.3. DNA Analysis

2.3.3.1. Polymerase Chain Reaction (PCR)

The 30 bp VNTR in the promoter region of *MAO-A*, Val158Met SNP of *COMT*, SNP-C/T of *VMAT2*, SNP-C/G of *VMAT2*, SNP-A/G of *DAT1*, and 40 bp VNTR in 3' the UTR of *DAT1* polymorphisms were genotyped using touch-down polymerase chain reaction (PCR) with primers given in the table 10.

Table 2.5. The sequences of the forward and reverse primers for the studied genes.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
MAOA VNTR	CCCAGGCTGCTCCAGAAAC	GGACCTGGGCAGTTGTGC	[165]
DAT1 VNTR	GCTTGGGGAAGGAAGGG	TGTGTGCGTGCATGTGG	[166]
DAT1 (G/A)	GTAGATCTGTGCAGCGAGGT	CTACTGTGAGCACGGGGATT	[167]
COMT(A/G)	TCGTGGACGCCGTGATTCAGG	AGGTCGACAACGGGTCAGGC	[168]
VMAT2 (C/T)	GCTCACGCCCAGGAAAGT	TCCGCTTGTCAAAATTCTTAGGT	[169]
VMAT2 (C/G)	CACCATGTTCCTGTTTCAGCC	TGGCAGGAGACAGTTTCTCCA	[169]

Table 2.6. The properties of the forward and reverse primers, the length of the regions of amplication, and the optimized PCR temperatures.

Primers	**%GC**	**Tm (°C)**	**The length of the amplicons (bp)**	**Opt. PCR Temp. (°C)**
MAOA-F	63	62.00	179, 209, 227, 239, 269	55-69
MAOA-R	66	60.00		
DAT1 VNTR-F	64	56.00	200, 240, 280, 320, 360, 400, 440, 480, 520, 560, 600	50-65
DAT1 VNTR-R	58	54.00		
DAT1 (G/A)-F	55	57.45	200	52-62
DAT1 (G/A)-R	55	57.45		
COMT (A/G)-F	61	61.75	217	52-62
COMT (A/G)-R	65	61.55		
VMAT2 (C/T)-F	61	58.00	92	50-60
VMAT2 (C/T)-R	39	54.86		
VMAT2 (C/G)-F	52	57.80	80	50-60
VMAT2 (C/G)-R	52	57.80		

The protocol of primer dilution:

The primers which were used in this project, had been purchased as lyophilized from the company (BGI). The primers were centrifuged at 13.000 rpm and ddH_2O was added in a volume according to the instructions of the company to each primer stock to make the concentration 100 µM. The primers were purified by the help of PAGE (Polyacrylamide Gel Electrophoresis). For the preparation of 10 µM working solution, 10 µl was taken from

the stock solution and 90 µl PCR water was added in PCR tubes. The tube was vortexed and then spinned down for 20 seconds. The primer working solution was stored in -20°C for genetic analysis.

2.3.3.2. VNTR (Variable Number Tandem Repeat) Analysis

The protocol of PCR reaction of MAO-A VNTR (30 bp sequence located nearly 1.2 kb upstream of the coding region): The PCR reaction was carried out in a total volume of 25 µL using 100 ng of genomic DNA. The PCR mix for *MAO-A* VNTR included 2,5 µL of 2 mM dNTPs , 1.0 µL each of 10 µM forward and reverse primers, 2,5 µL of 10X Dream Taq Green Buffer, 5 µL DNA template, 12,875 µL PCR water and 0.125 µL 500U Dream Taq polymerase (5U/µL). The 10X Dream Taq Green Buffer involved dye for being able to load PCR products on a gel in a direct way. Moreover, 10X Dream Taq Green Buffer involved $MgCl_2$ at a concentration of 20 mM. The dNTP Mix included mixture of dATP, dTTP, dGTP, dCTP, each at a final concentration of 2 mM.

Table 2.7. The reaction components for the amplication of 30 bp promoter region of MAOA gene.

Reaction components	**Concentration**	**Volume used (µL)**	**Final concentration**
10X Dream Taq Green Buffer	10X	2.5	1X
dNTP Mix	2 mM	2.5	0.2 mM
Forward primer	10 µM	1	0.4 µM
Reverse primer	10 µM	1	0.4 µM
Template DNA	20 ng/µL	5	100 ng/25µL
Dream Taq DNA polymerase	5 u/µL	0.125	0.625 u/µL
Water, nuclease-free	—	12.875	—
Total		25	

The PCR conditions were optimized using a thermal cycler as follows: initial denaturation at 95°C for 15 minutes, followed by 19 cycles at 95°C for 30 seconds, 69°C for 30 seconds, at 72°C for 30 seconds, followed by 24 cycles at 95°C for 30 seconds, at 55°C for 30 seconds and at 72°C for 90 seconds, with a final extension of 10 minutes at 72°C for *MAOA* gene.

The Touch-down PCR programme for *MAO-A*:

95 °C 15 min.

95 °C 30 sc.

69 °C 30 sc. ↓0.5 — 19 cycle

72 °C 30 sc.

95 °C 30 sc.

55 °C 30 sc. — 24 cycle

72 °C 1.30 min.

72 °C 10 min

4 °C ∞

The protocol of PCR reaction of DAT1 VNTR: The PCR reaction was carried out in a total volume of 25 µL using 100 ng of genomic DNA. The PCR mix for *DAT1* VNTR included 2,5 µL of 2 mM dNTPs , 1.0 µL each of 10 µM forward and reverse primers, 2,5 µL of 10X Dream Taq Green Buffer, 3 µL DNA template, 14,875 µL PCR water and 0.125 µL 500U Dream Taq polymerase (5U/µL).

Table 2.8. The reaction components for the amplication of 40 bp 3' UTR of DAT1 gene.

Reaction components	**Concentration**	**Volume used (µL)**	**Final concentration**
Dream Taq PCR Master Mix	2X	2.5	1X
dNTP Mix	2 mM	2.5	0.2 mM
Forward primer	10 µM	1	0.4 µM
Reverse primer	10 µM	1	0.4 µM
Template DNA	33.3 ng/µL	3	100 ng/25µL
Dream Taq DNA polymerase	5 u/µL	0.125	0.625 u/µL
Water, nuclease-free	—	14.875	—
Total		25	

The Touch-down PCR programme for *DAT1*:

95 °C 15 min.

95 °C 30 sc.

65 °C 30 sc. ↓0,5 } 19 cycle

72 °C 30 sc.

95 °C 30 sc.

50 °C 30 sc. } 24 cycle

72 °C 1.30 min.

72 °C 10 min 4 °C ∞

Table 2.9. The alleles and types of polymorphism of *MAOA* and *DAT1* VNTR.

Alleles	**Type of polymorphism**
MAOA-u VNTR (OMIM: 309850.0002)	Indel
ACCGGCACCGGCACCCAGTACCCGCACCAGT	
DAT1 3' UTR VNTR (rs28363170)	Indel
GGGGGCCCTGCATGCGTCCTGGGGTAGTACACGCTCCAGT	

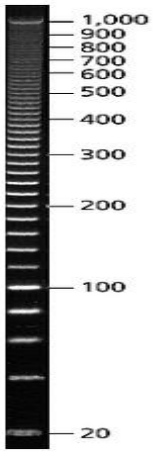
Preparation of 1X TAE Buffer:

20 µL 50X TAE Buffer was added to 980 µL ddH_2O in a glass bottle.

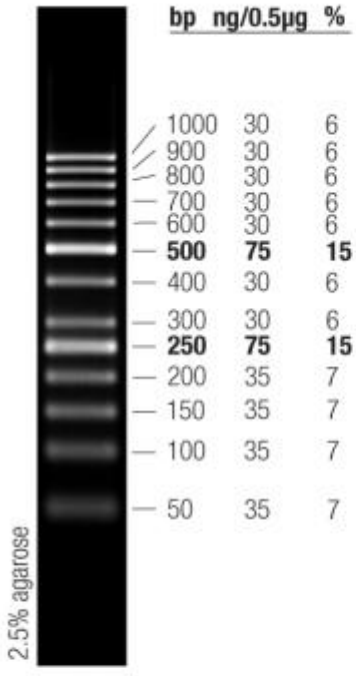
Table 2.10. The names and the components of the materials used for agarose gel electrophoresis.

Name	**Components**
1X TAE (Tris-acetate EDTA)Buffer	40 mM Tris, 20 mM acetic acid, 1 mM EDTA
Ethiduim bromide	10 mg/mL ethidium bromide, in ddH2O
DNA Gel Loading Dye (6X)	10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 60% glycerol 60 mM EDTA
Agarose	D-galactose and 3,6-anhydro-L galactose units
Gene Ruler 50 bp DNA Ladder	13 chromatography-purified individual DNA fragments
Gene Ruler 100 bp DNA Ladder	10 chromatography-purified individual DNA fragments

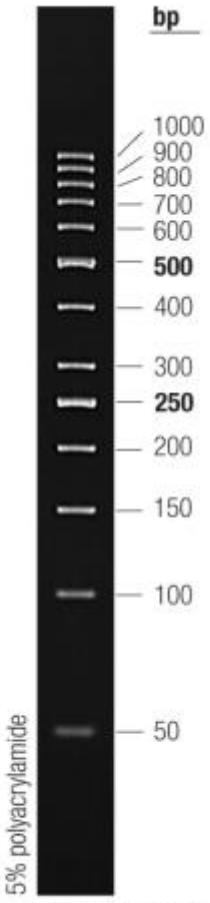
PCR 20 bp Low Ladder



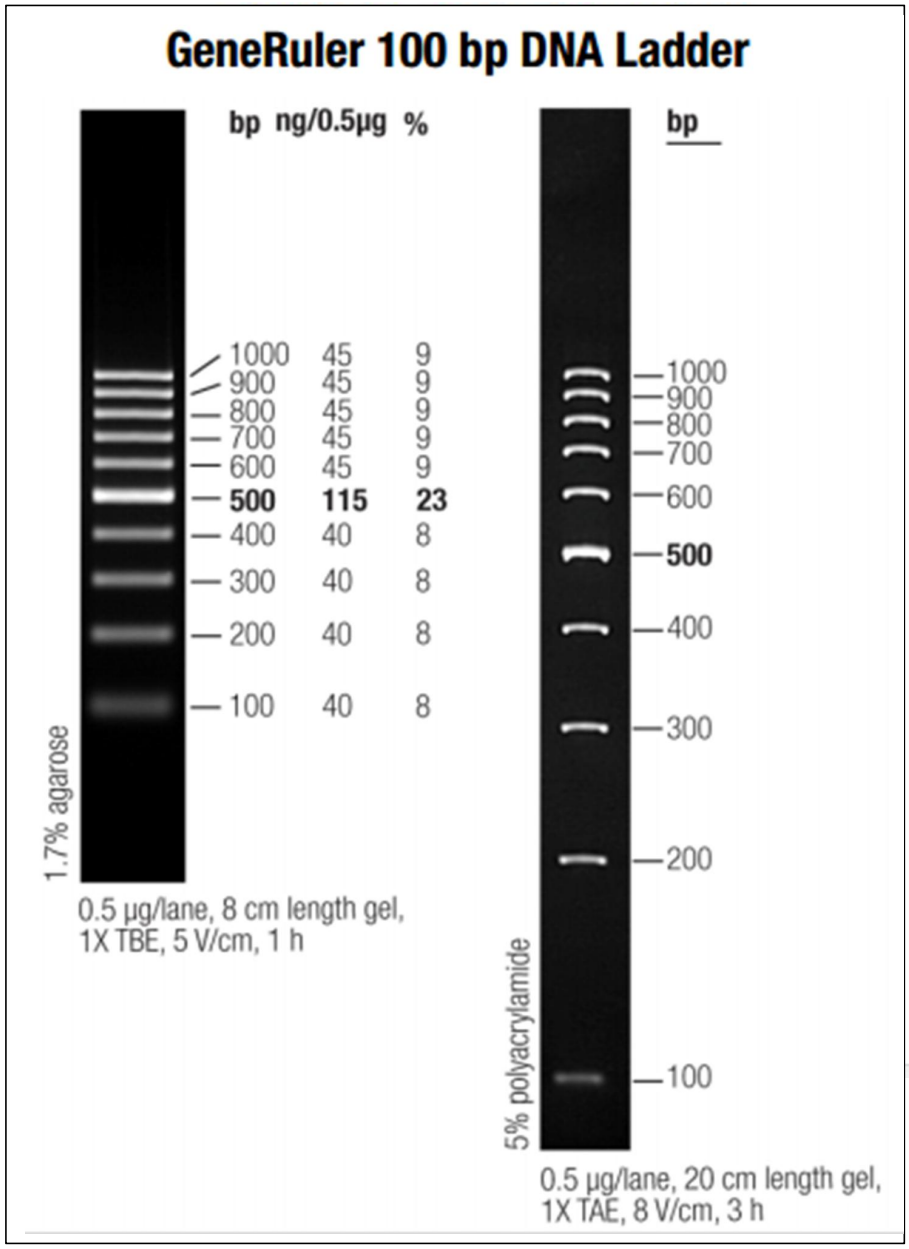
GeneRuler 50 bp DNA Ladder



0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h



0.5 µg/lane, 20 cm length gel,
1X TAE, 8 V/cm, 3 h



The protocol of preparation of agarose gel (making a 5 per cent agarose gel):

- 5 g agarose was added into 100 mL 1 per cent Tris-acetate-EDTA (TAE) buffer.
- The mixture was heated at microwave oven for 55 seconds.
- 10 µL EtBr was added in the fume hood
- The agorose was poured into a gel tray with the well comb in place.
- It was taken 30 minutes to solidify in the fume hood at room temperature.
- The agarose gel was placed into the electrophoresis unit.

- The gel box was filled with 1 per cent TAE buffer until the gel was covered.
- A molecular weight ladder (100 bp) was loaded into the first lane of the gel.
- Then original samples (5 µL,each sample) were loaded into the other lanes respectively.

The PCR products of *MAO-A* and *DAT-1* VNTR were determined by electrophoresis using a 5 per cent agarose gel which was stained with EtBr. The gel electrophoresis was run for 50 minutes at 100 volts and then visualized and analyzed under ultraviolet light.

2.3.3.3. SNP (Single Nucleotide Polymorphism) Analysis

2.3.3.3.1. PCR of rs4680 (COMT), rs27072 (DAT1), rs363399 (VMAT2), rs4752045 (VMAT2):

The PCR reaction was carried out in a total volume of 25 µL using 100 ng of genomic DNA. The PCR mix included 12,5 µL DreamTaq PCR Master Mix (2X), 1.0 µL each of 10 µM forward and reverse primers, 3 µL DNA template, 7,5 µL nuclease-free water. DreamTaq PCR Master Mix involved all the essential components for the PCR reaction except for primers and template DNA. The Master Mix was composed of dATP, dTTP, dCTP, dGTP, 0,4 mM each, DreamTaq DNA Polymerase, 4 mM $MgCl_2$, and 2X DreamTaq buffer.

Table 2.11. The reaction components for the amplication of rs4680, rs27072, rs363399, rs4752045.

Reaction components	**Concentration**	**Volume used (µL)**	**Final concentration**
Dream Taq PCR Master Mix	2X	12.5	1X
Forward primer	10 µM	1	0.4 µM
Reverse primer	10 µM	1	0.4 µM
Template DNA	33.3 ng/µL	3	100 ng/25µL
Water, nuclease-free	—	7.5	—
Total		25	

The Touch-down PCR programme for rs4680 (*COMT*) and rs27072 (*DAT1*):

95 °C 15 min.

95 °C 30 sc.

62 °C 30 sc. ↓0,5 — 19 cycle

72 °C 30 sc.

95 °C 30 sc.

52 °C 30 sc. — 24 cycle

72 °C 1.30 min.

72 °C 10 min

4 °C ∞

The Touch-down PCR programme for rs363399 and rs4752045 (*VMAT2*):

95 °C 15 min.

95 °C 30 sc.

60 °C 30 sc. ↓ 0,5 — 19 cycle

72 °C 30 sc.

95 °C 30 sc.

50 °C 30 sc. — 24 cycle

72 °C 1.30 min.

72 °C 10 min

4 °C ∞

The protocol of preparation of agarose gel (making a 2 per cent agarose gel):

- 2 g agarose was added into100 mL 1 per cent Tris-acetate-EDTA (TAE) buffer.
- The mixture was heated at microwave oven for 35 seconds.
- 4 µL EtBr was added in the fume hood.
- The agorose was poured into a gel tray with the well comb in place.
- It was taken 30 minutes to solidify in the fume hood at room temperature.
- The agarose gel was placed into the electrophoresis unit.
- The gel box was filled with 1 per cent TAE buffer until the gel was covered.
- A molecular weight ladder (100 bp) was loaded into the first lane of the gel.
- Then original samples (5 µL, each sample, and 1 µL loading dye) were loaded into the other lanes respectively.

The PCR products of rs4680, rs27072, rs363399, and rs4752045 were determined by electrophoresis using a 2 per cent agarose gel which was stained with ethidium bromide.

The gel electrophoresis was run for 30 minutes at 100 volts and then visualized and analyzed under ultraviolet light.

2.3.3.3.2. Restriction Fragment Length Polymorphism (RFLP) of rs4680, rs27072, rs363399, and rs4752045

The exact place of the substitution of the SNPs were determined by the help of NCBI (National Center for Biotechnology Information) database.

Table 2.12. The nucleotide sequences show the substitution of the SNPs.

SNP ID	Sequence
rs4680	CCAGCGGATGGTGGATTTCGCTGGC[A/G]TGAAGGACAAGGTGTGCATGCCTGA
rs27072	AGTGCCCCTGGGGCAGCCTCAGAGC[G/A]GGGAGCAGGGAGCAGGGAGGGAGGG
rs363399	GAAGTCCGTGCCCCCTGGAATTCTC[T/C]GGTAGCTGAAAAAGACAAGCAGAAC
rs4752045	TCCTGTTTCAGCCTCTTGTGTTGCC[C/G]CGGTTGCCATTCTGGTTAATTTCTG

The map of the restriction enzymes was found by the use of NEBcutter V2.0 programme and the proper enzyme was selected according to the recognition sequence.

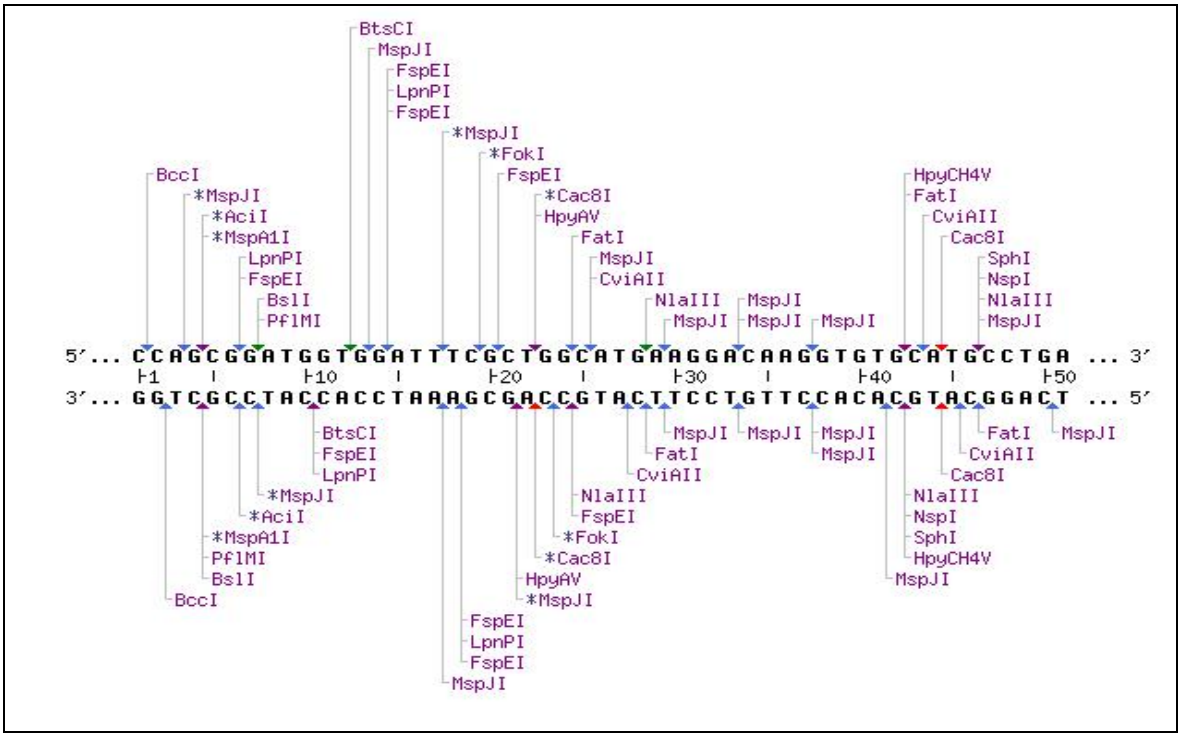


Figure 2.2. The map of restriction enzymes of rs4680 (*COMT*). (Wild type sequence). GC: 57 and AT: 43 per cent.

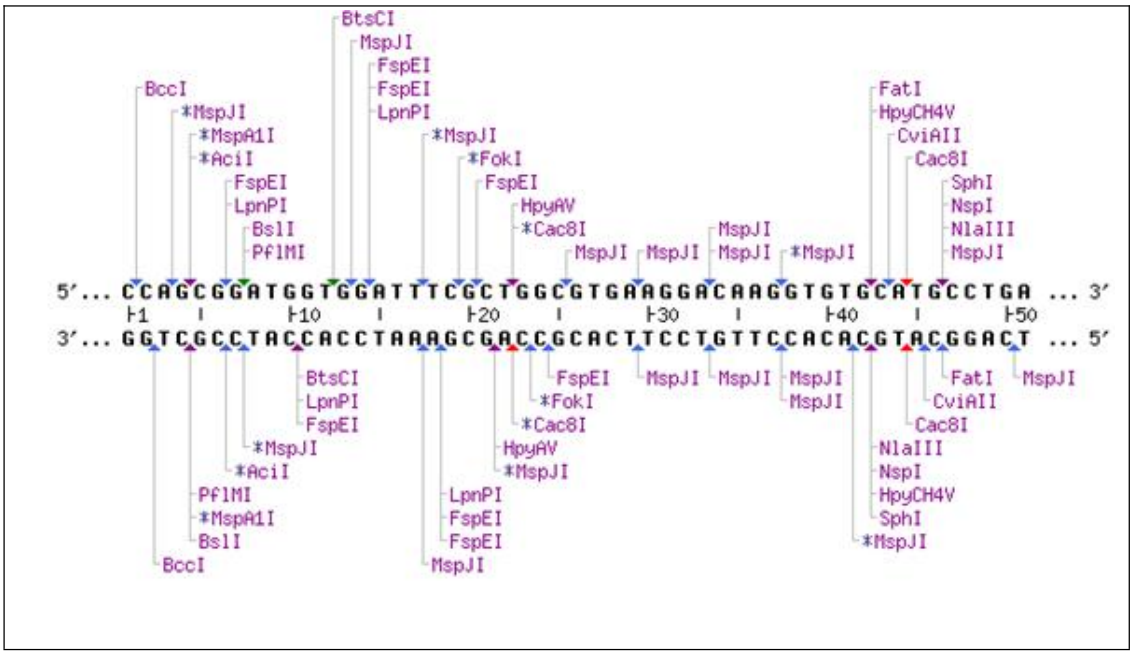


Figure 2.3. The map of restriction enzymes of rs4680 (*COMT*). (Polymorphic sequence). GC: 59 per cent and AT: 41 per cent.

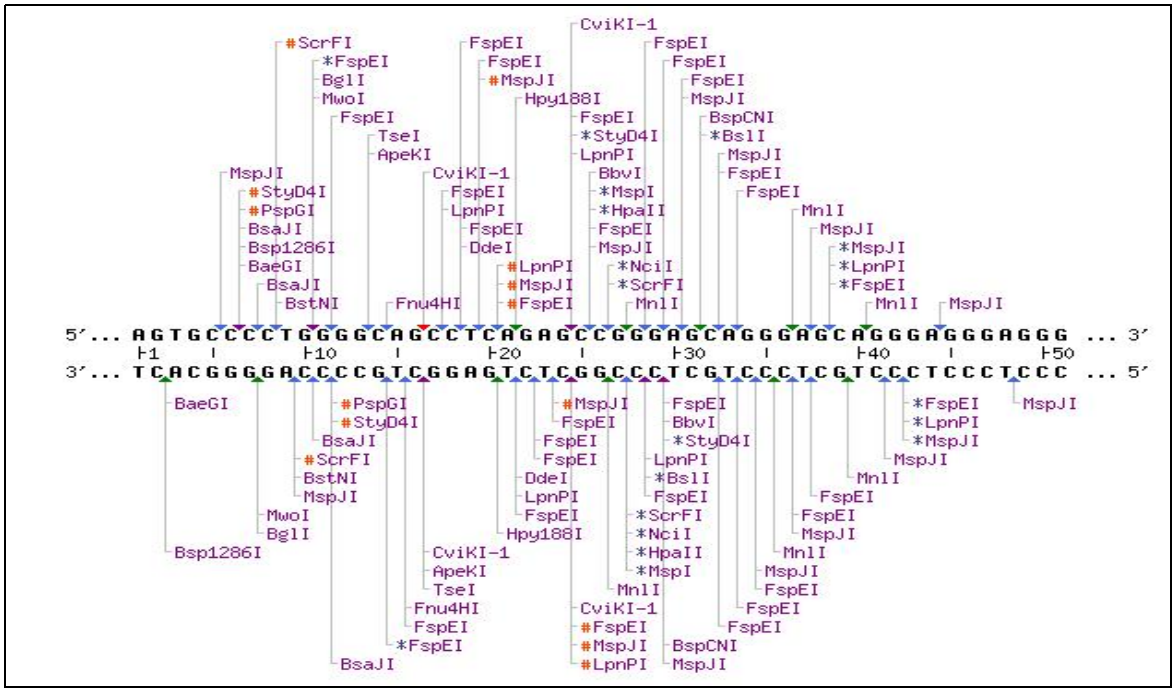


Figure 2.4. The map of restriction enzyme of rs27072 (*DAT1*). (Wild type sequence). GC: 75 per and AT: 25 per cent.

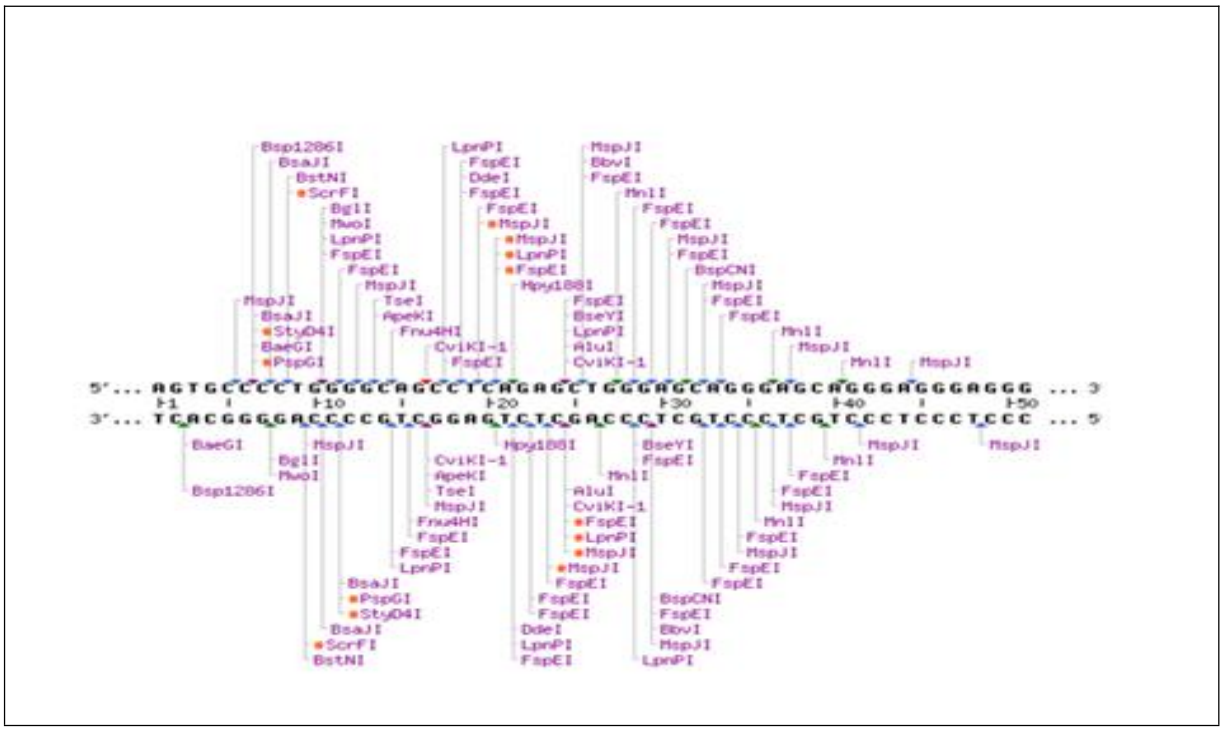


Figure 2.5. The map of restriction enzyme of rs27072 (*DAT1*). (Polymorphic sequence). GC: 73 per cent and AT: 27 per cent.

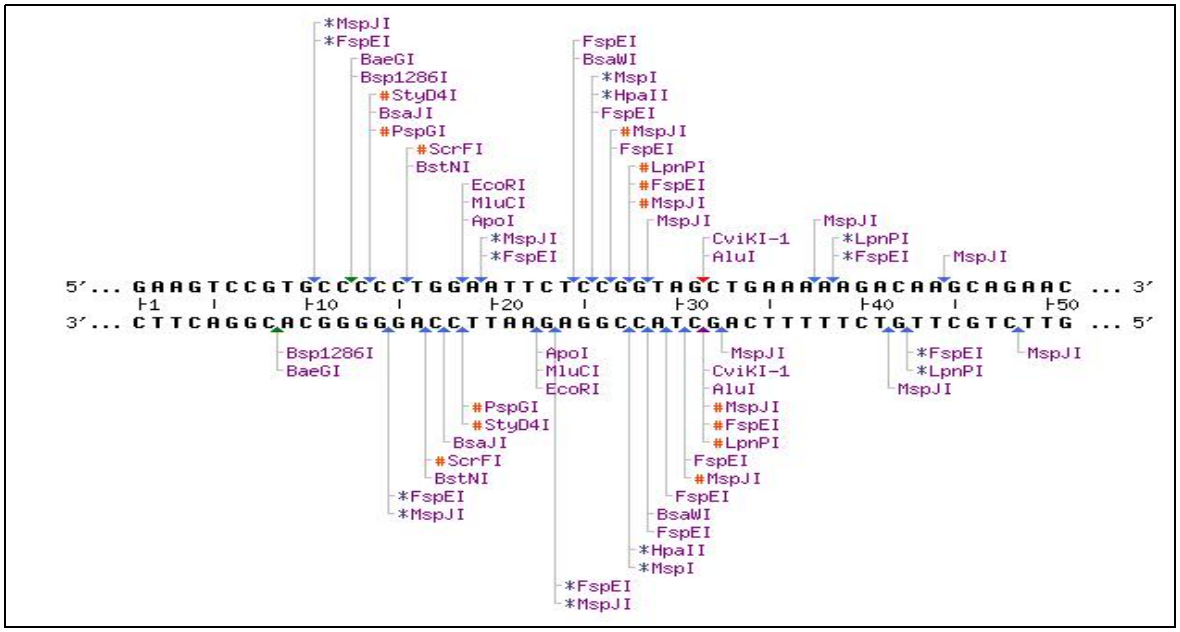


Figure 2.6. The map of restriction enzymes of rs363399 (*VMAT2*). (Wild type sequence). GC: 53 per cent and AT: 47 per cent.

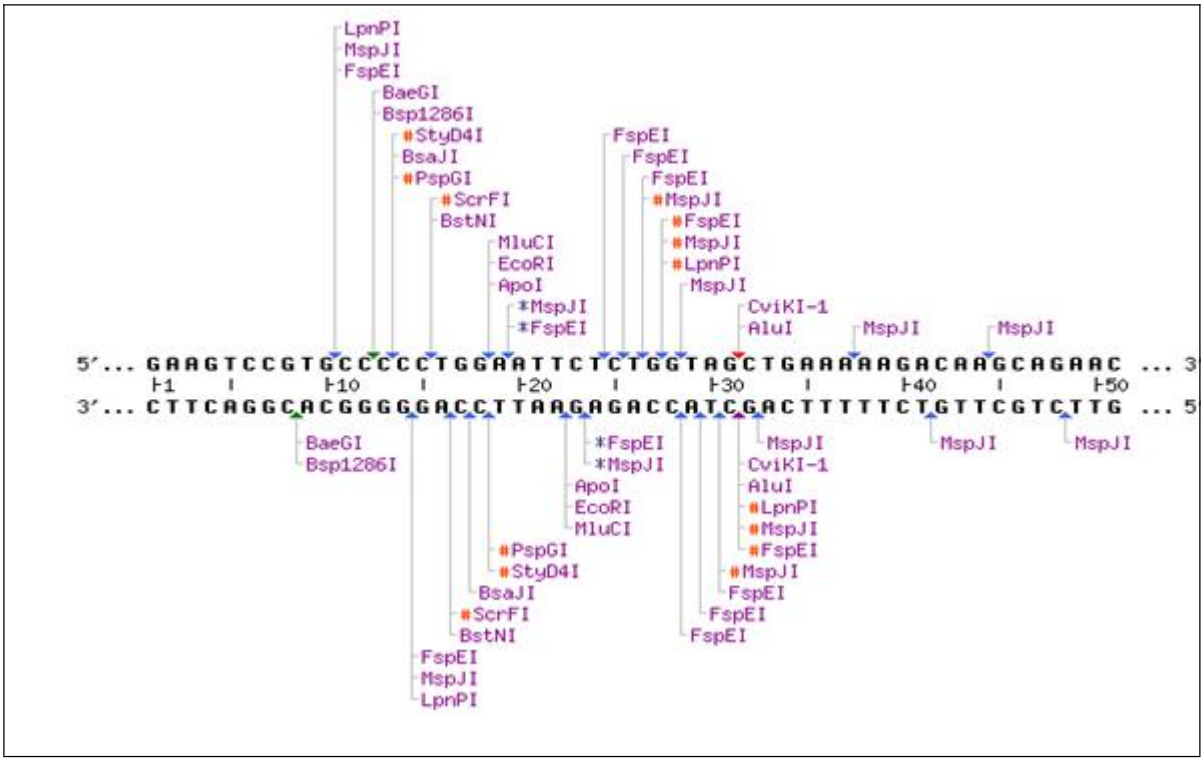


Figure 2.7. The map of restriction enzymes of rs363399 (*VMAT2*). (Polymorphic sequence). GC: 51 per cent and AT: 49 per cent.

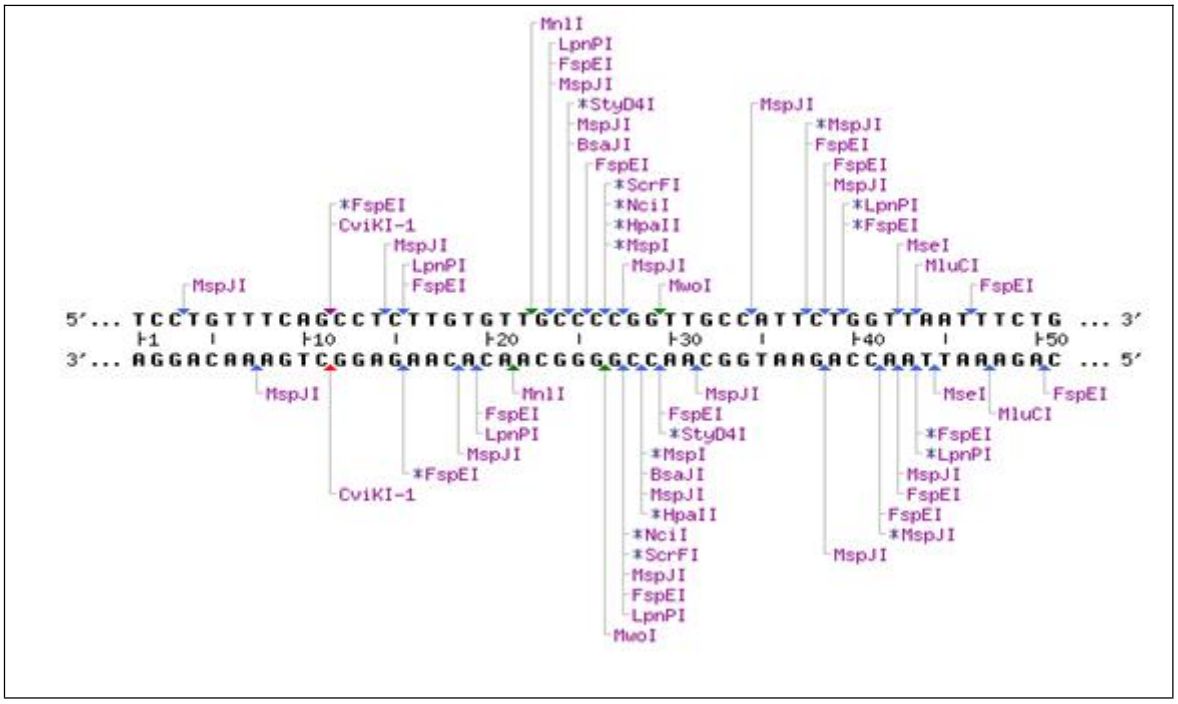


Figure 2.8. The map of restriction enzymes of rs4752045 (*VMAT2*). (Wild type sequence). GC: 49 per cent and AT: 51 per cent.

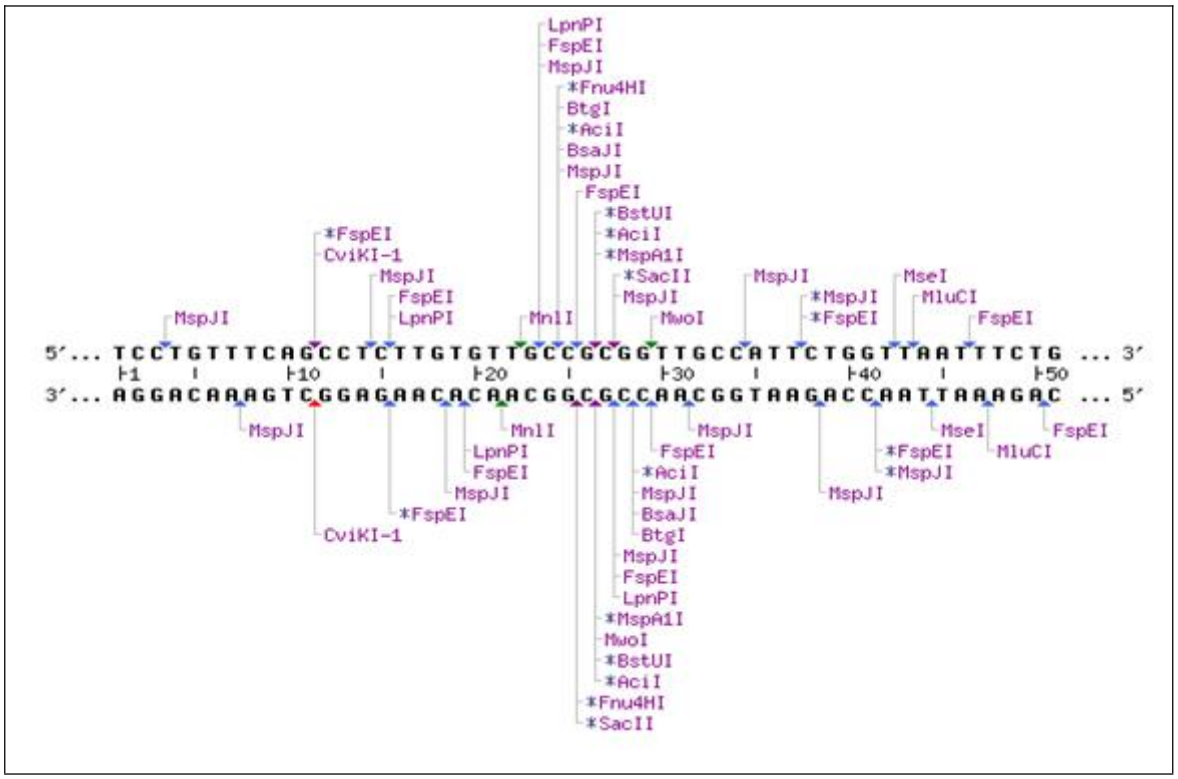


Figure 2.9. The map of restriction enzymes of rs4752045 (*VMAT2*). (Polymorphic sequence). GC: 49 per cent and AT: 51 per cent.

The PCR products of rs4680, rs27072, rs363399 and rs4752045 were cleaved by using NlaIII, MspI, MspI, and AciI restriction enzymes, respectively. The restriction reactions were carried out in a total volume of 20 µL using 7 µL PCR product, 2 µL reaction buffer, 10.9 µL nuclease-free water, and 0.1 µL restriction enzyme. The 1X NEBuffer involved 50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 100 µg/µL BSA and the pH was 7.9. The reaction was incubated at 37°C for 20 minutes and then inactivated at 65°C for 20 minutes for NlaIII and AciI restriction enzymes. On the other hand, the incubation was at 37°C for 20 minutes for MspI restriction enzyme but there was no heat inactivation.

Table 2.13. The restriction endonucleases and their sources and sensitivity of methylation.

RE	**Product source**	***dam methylation***	***dcm methylation***	***CpG methylation***
N1aIII	*Neisseria lactamica*	Not sensitive	Not sensitive	Not sensitive
MspI	*Moraxella species*	Not sensitive	Not sensitive	Not sensitive
AciI	*Arthrobacter citreus*	Not sensitive	Not sensitive	Blocked

Table 2.14. Restriction endonucleases, recognition sequences, genotypes, and restriction fragments of the SNPs which were investigated in this study.

SNP ID	RE	Recognition sequence	Genotype	Restriction fragments (bp)
rs4680	N1aIII	CATG	Val/Val	136 and 81
			Val/Met	136, 96, 81, and 41
			Met/Met	96, 81, and 41
rs27072	MspI	CCGG	A/A	107 and 93
			G/G	200
			A/G	200, 107, and 93
rs363399	MspI	CCGG	C/C	47 and 45
			T/T	92
			C/T	92, 47, and 45
rs4752045	AciI	CCGC	C/C	80
			G/G	48 and 32
			C/G	80, 48, and 32

Table 2.15. The location and function of the SNPs.

SNP ID	Location	Function
rs4680	Exon 3	Missense. Different mRNA structures and expression
rs27072	3' UTR	Regulatory. Affects mRNA expression and translation
rs363399	Intron 2	Unknown
rs4752045	Intron 7	Unknown

The protocol of agarose gel (making a 5 per cent agarose gel):

- 5 g agarose was added into 100 mL 1 per cent Tris-acetate-EDTA (TAE) buffer
- The mixture was heated at microwave oven for 55 seconds.
- 10 μL EtBr was added in the fume hood.
- The agorose was poured into a gel tray with the well comb in place.
- It was taken 30 minutes to solidify in the fume hood at room temperature.
- The agarose gel was placed into the electrophoresis unit.
- The gel box was filled with 1 per cent TAE buffer until the gel was covered.
- A molecular weight ladder was loaded into the first lane of the gel.
- Then original samples (5 μL,each sample, and 1 μL loading dye) were loaded into the other lanes respectively.

The restriction products were determined by electrophoresis using a 5 per cent agarose gel which was stained with ethidium bromide. The gel electrophoresis was run at 100 volts for 50 minutes and then visualized under ultraviolet light.

2.4. STATISTICAL ANALYSIS

The statistical assessment of our study was carried out by the use of SPSS (Statistical Package for the Social Sciences) version 24.0 (Chicago, IL, USA). The differences of genotype between control and obese groups were analyzed by Chi-square, Fisher's Exact test. Pearson x^2 test was used for the determination of the deviation from Hardy-Weinberg equilibrium (p<0.05). The effects of genotypes and alleles on eating behavior were analyzed by one-way Anova test. Allele frequencies were computed according to gene counting method. Logistic regression was used in order to calculate odds ratios (ORs) and confidence interval (CI) values. P values which were under 0.05 were considered significant. Finally, linkage disequilibrium between SNPs and haplotype analysis were evaluated by SNPStats program.

3. RESULTS

We have investigated the polymorphisms of MAO-A, COMT, VMAT2 and DAT1 genes in 2 groups. Both men and women of Turkish origin, all volunteers, were collected at the Department of Endocrinology and Metabolism Disorders at Fatih Sultan Mehmet Education and Research Hospital in İstanbul, Turkey.

In this study, *MAO-A*, *COMT*, *VMAT2* and *DAT1* genes were amplified with appropriate primers and analyzed by agarose gel electrophoresis for VNTRs and digested with proper restriction endonucleases and analyzed by agarose gel electrophoresis for SNPs by the use of DNA samples from 101 overweight, 133 obese and 214 control groups.

3.1. PCR RESULTS

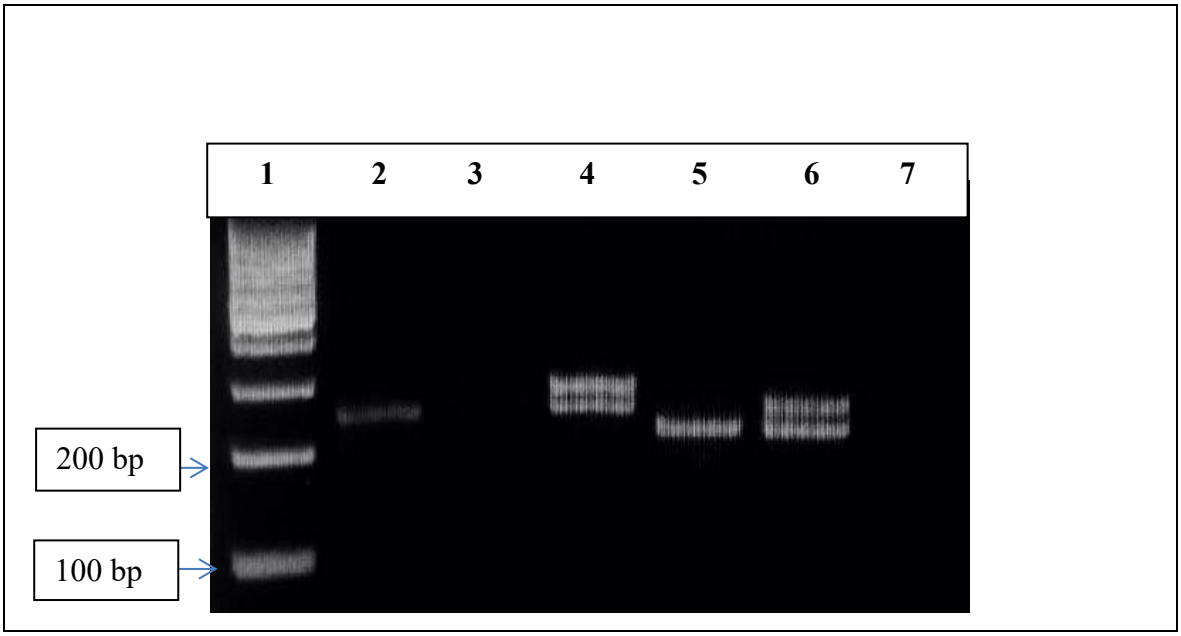


Figure 3.1. The detection of PCR products of *MAOA-u* VNTR by five per cent agarose gel electrophoresis. Lane1: GeneRuler 100 bp DNA ladder; lane 2: 4R/4R; lanes 3: No amplification, lane 4: 4R/5R; lane 5: 3R/3R; lane 6: 3R/4R; lane 7: NC. The MAOA-u VNTR alleles are: 2R (179bp), 3R (209bp), 4R (239bp), 5R (269bp).

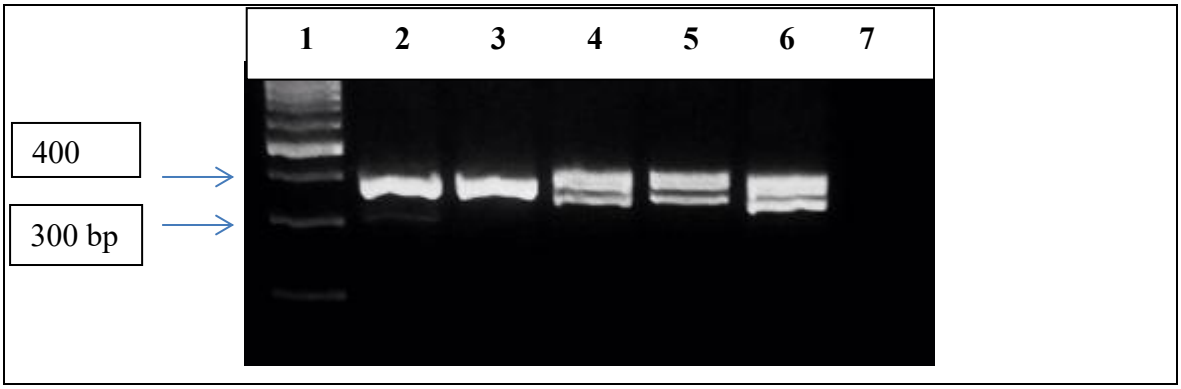


Figure 3.2. The detection of PCR products of 40 bp 3' UTR VNTR of *DAT1* by five per cent agarose gel electrophoresis. Lane 1: GeneRuler 100 bp DNA ladder; lanes 2: 6R/8R; lane 3: 8R/8R; lanes 4, 5, and 6: 7R/8R; lane 7: NC. The DAT1 3' UTR VNTR alleles are: 3R (200bp), 4R (240 bp), 5R (280 bp), 6R (320 bp), 7R (360 bp), 8R (400 bp), 9R (440 bp), 10R (480 bp), 11R (520 bp), 12R (560 bp), 13R (600 bp).

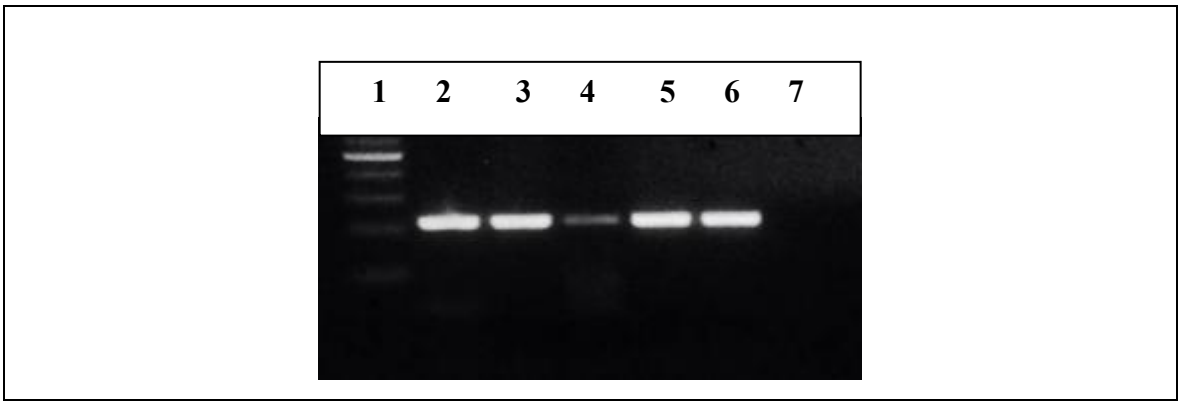


Figure 3.3. The PCR products of rs4680 (*COMT*) by two per cent agarose gel. Lane 1: GeneRuler 100 bp DNA ladder; lanes 2, 3, 4, 5, and 6: 217 bp; lane 7: NC.

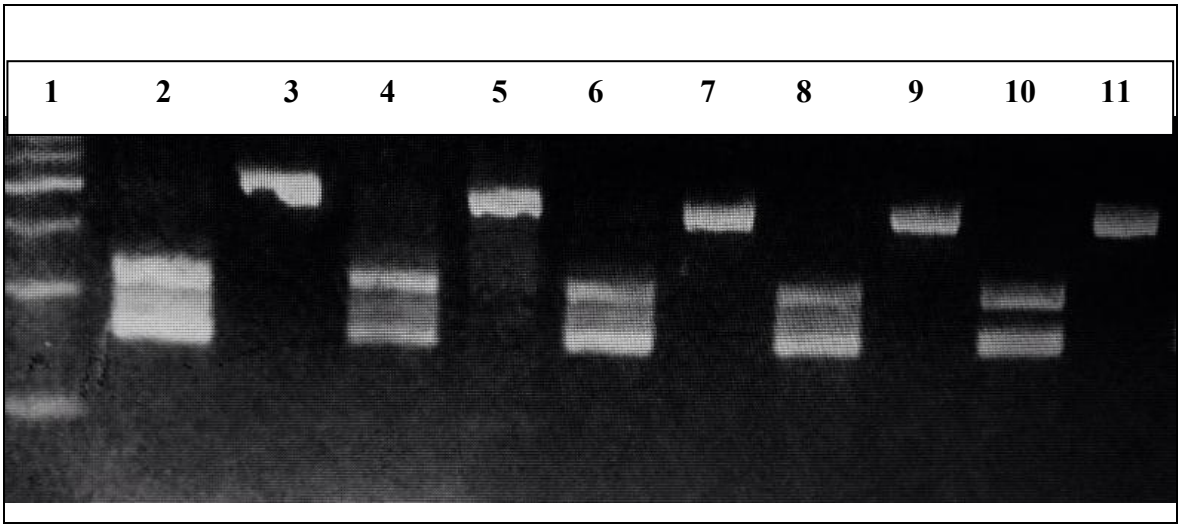


Figure 3.4. The RFLP products of rs4680 by five per cent agarose gel electrophoresis. Lane 1: GeneRuler 50 bp DNA ladder; lanes 2, 4, 6, 8 and 10: 136, 96, and 81 bp (Val/Met); lanes 3, 5, 7, 9, and 11: 217 bp (PCR product).

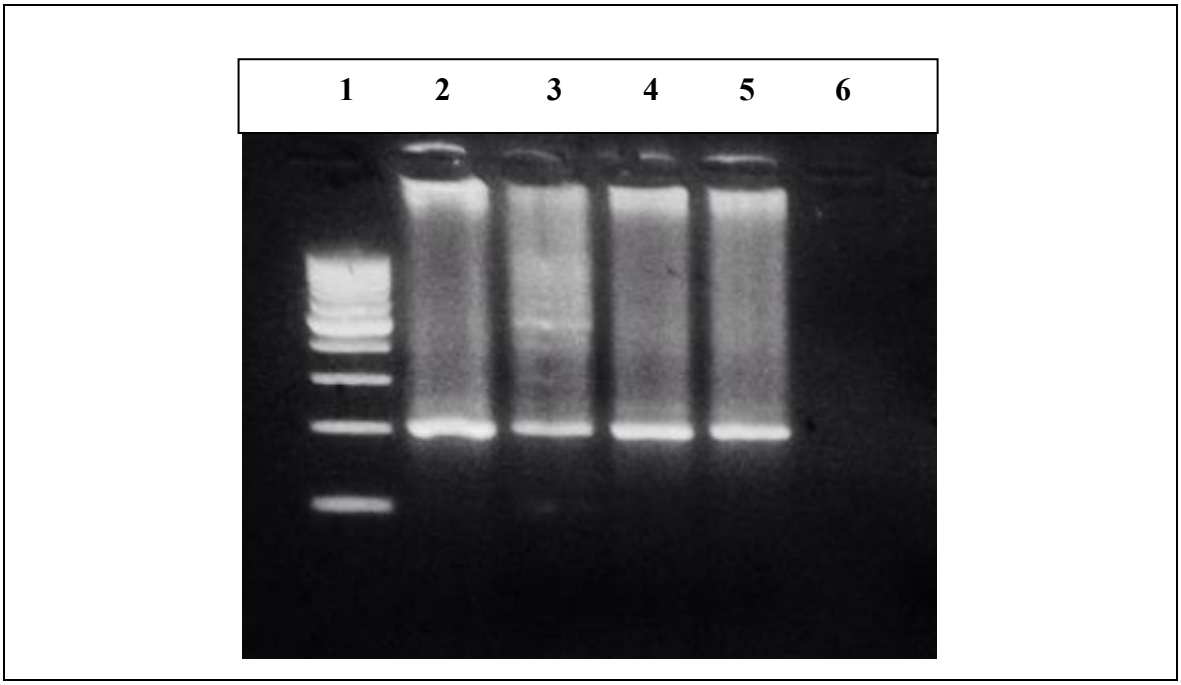


Figure 3.5. The PCR products of rs27072 (*DAT1*) by two per cent agarose gel electrophoresis. Lane 1: GeneRuler 100 bp DNA ladder; lanes 2, 3, 4, and 5: 200 bp; lane 6: NC.

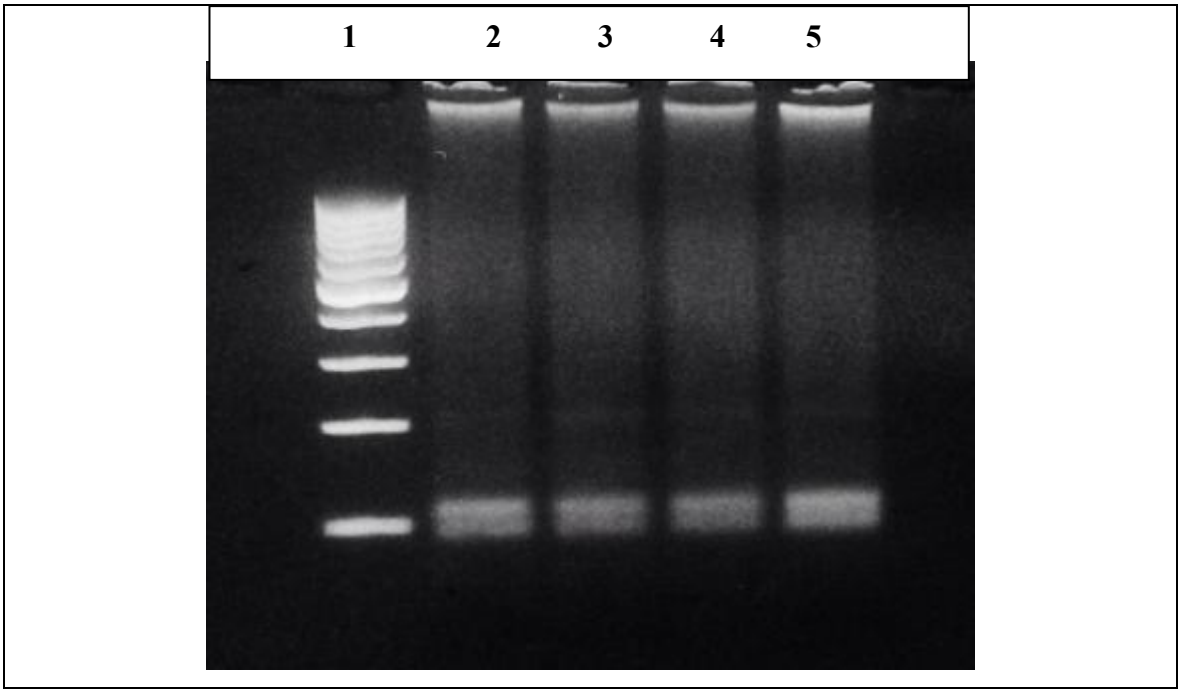


Figure 3.6. The RFLP products of rs27072 by five per cent agarose gel electrophoresis. Lane 1: GeneRuler 100 bp DNA ladder; lanes 2, 3, 4, and 5: 107 and 93 bp (C/C).

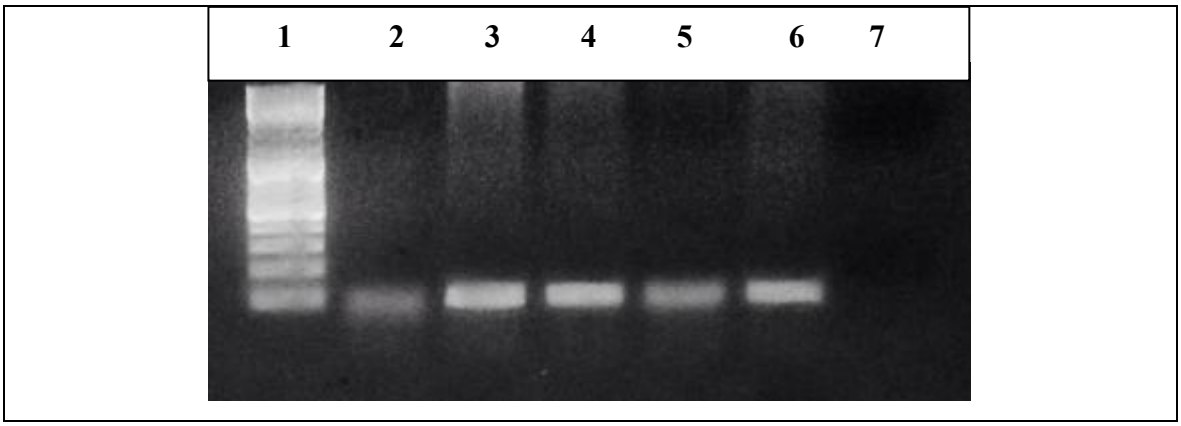


Figure 3.7. The PCR products of rs363399 (*VMAT2*) by two per cent agarose gel electrophoresis. Lane 1: GeneRuler 100 bp DNA ladder; lanes 2, 3, 4, 5, and 6: 92 bp and lane 7: NC.

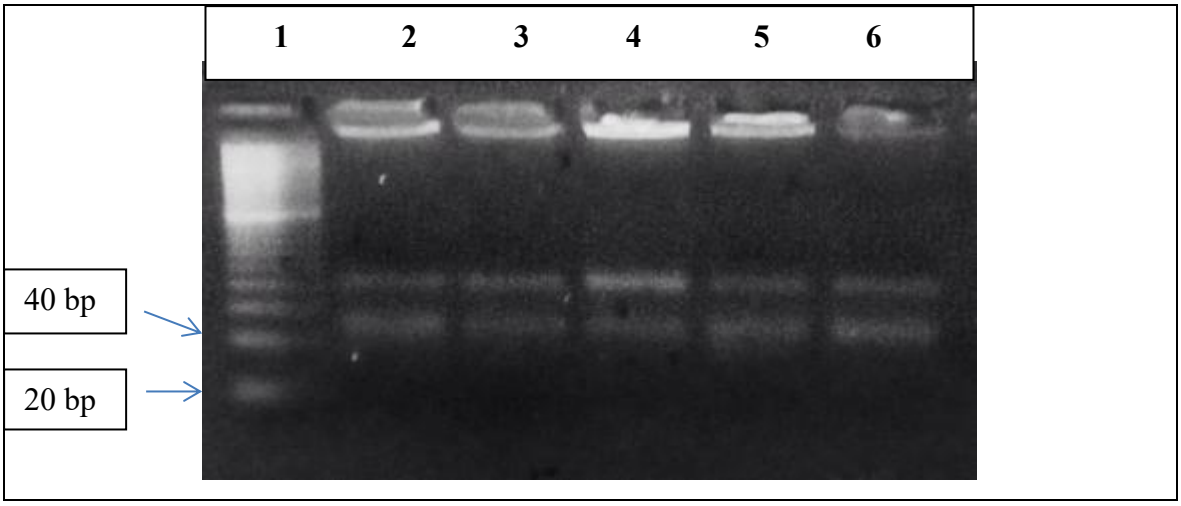


Figure 3.8. PCR-RFLP analysis of rs363399 by five per cent agarose gel electrophoresis; MspI-digested PCR fragments from the C allele (45 and 47 bp) and T allele (92 bp). Lane 1: GeneRuler 20 bp DNA ladder; lanes 2, 3, 4, 5, and 6: heterozygous variant genotype (C/T).

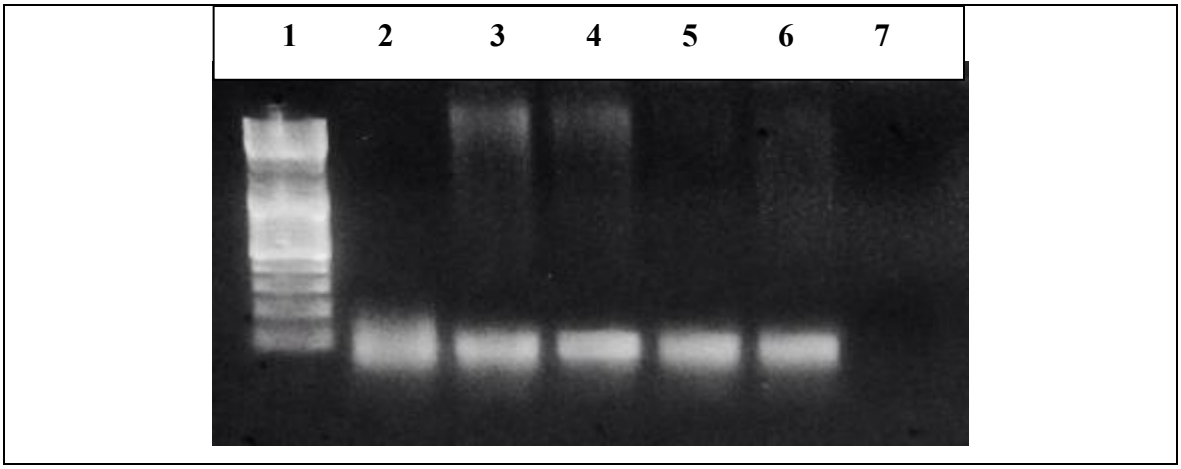


Figure 3.9. The analysis of PCR products of rs4752045 (*VMAT2*) by two per cent agarose gel electrophoresis. Lane 1: GeneRuler 100 bp DNA Ladder; lanes 2, 3, 4, 5, and 6: 80 bp; lane 7: NC.

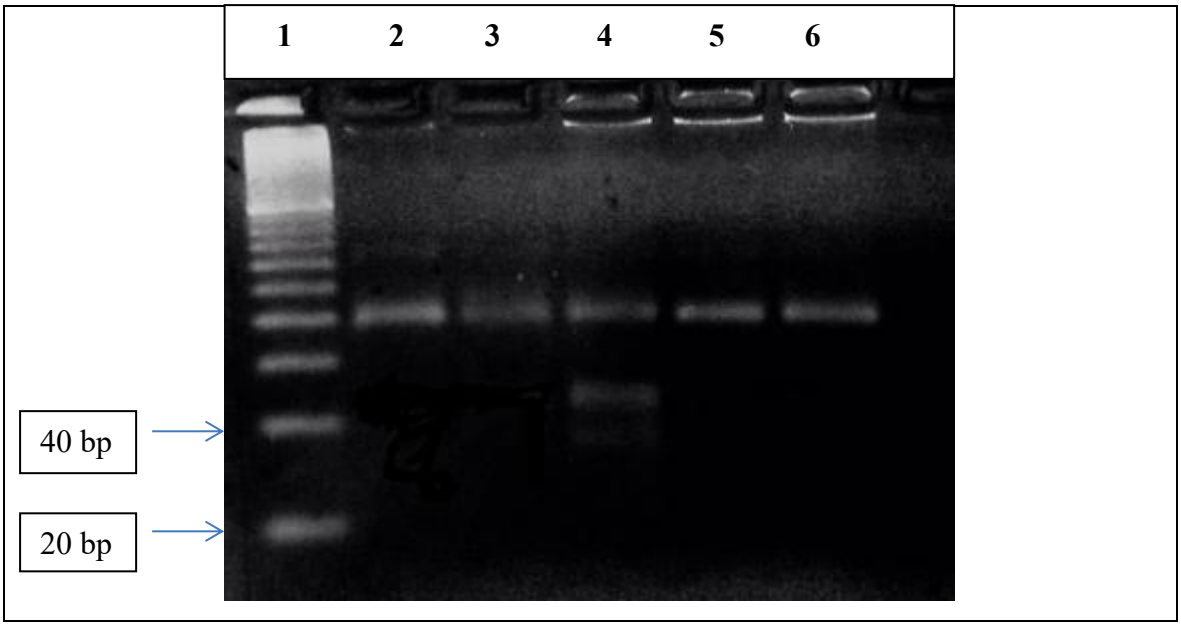


Figure 3.10. RFLP analysis of rs4752045 by five per cent agarose gel electrophoresis; AciI-digested PCR fragments from the G allele (32 and 48 bp) and C allele (80 bp). Lane 1: PCR 20 bp Low Ladder; lanes 2, 3, 5, and 6: homozygous wild-type genotype (C/C) and lane 4: heterozygous variant genotype (G/C).

3.2. THE RESULTS OF STATISTICAL ANALYSIS

Table 3.1. Demographic characteristics of the obese group.

Characteristic	Female (n=140)	Male (n=94)	p-value	OR
Age (years)	31.3 ± 8.1	31.7 ± 7.9	0.44	
BMI (kg/m2)	35.4 ± 8.9	32.7 ± 8.3	0.59	
Family history				
Yes	n=90 (62.9%)	n=53 (37.1%)		
No	n=50 (54.9%)	n=41 (45.1%)	0.22	0.718
Eating behavior				
Need	n=27 (64.2%)	n=15 (35.8%)		
Reward	n=113 (58.8%)	n=79 (41.2%)	0.51	1.258

The mean age of female subjects was 31.3 and for males it was 31.7 in obese group. The mean BMI of females was 35.4 and the mean BMI for males was 32.7. The number of female subjects who had family history of obesity was 90 (64.2 per cent) and who had not was 50 (35.7 per cent). On the other hand, the number of male subjects who had family history of obesity was 53 (56.3 per cent), and who had not was 41(43.6 per cent). The *p* value between female and male groups about family history was 0.22. The number of females who ate for need was 27 (19.2 per cent), and who ate for reward was 113 (80.7 per cent). The number of male subjects who ate for need was 15 (15.9 per cent) and who ate for reward was 79 (84 per cent). The *p* value between female and male groups about eating behavior was 0.51.

Table 3.2. Demographic characteristics of the control group.

Characteristic	**Female (n=166)**	**Male (n=48)**	**p-value**	**OR**
Age (years)	27.5 ± 5.8	29.1 ± 6.1	0.98	
BMI (kg/m2)	21.3 ± 1.9	23.1 ± 1.2	Not significant	
Family history				
Yes	n=68 (82.9%)	n=14 (17.1%)		
No	n=98 (74.2%)	n=34 (25.8%)	0.11	1.788
Eating behavior				
Need	n=42 (75%)	n=14 (25%)		
Reward	n=124 (78.4%)	n=34 (21.6%)	0.78	1.241

The mean age of female subjects was 27.5 and for males it was 29.1 in control group. The mean BMI of females was 21.3 and the mean BMI for males was 23.1. The number of female subjects who had family history about obesity was 68 (40.9 per cent), and who had not was 98 (59 per cent). On the other hand, the number of male subjects who had family history about obesity was 14 (29.1 per cent), and who had not was 34 (70.8 per cent). The *p* value between female and male groups about family history was 0.11. The number of females who ate for need was 42 (25.3 per cent) and who ate for reward was 124 (74.6 per cent). The number of male subjects who ate for need was 14 (29.1 per cent) and who ate for reward was 34 (70.8 per cent). The *p* value between female and male groups about eating behavior was 0.78.

Table 3.3. Demographic characteristics of the study population.

Characteristic	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Sex			
Female	n=166 (54.2%)	n=140 (45.8%)	**<0.001**
Male	n=48 (33.8%)	n=94 (66.2%)	
Age (years)	27.8 ± 5.9	31.4 ± 7.9	**<0.001**
BMI (kg/m2)	21.7 ± 1.9	34.4 ± 8.7	**<0.001**
Family history			
Yes	n=82 (36.6%)	n=142 (63.4%)	Not significant
No	n=132 (59%)	n=92 (41%)	
Eating behavior			
Need	n=56 (57.1%)	n=42 (42.9%)	**0.03**
Reward	n=158 (45.1%)	n=192 (54.9%)	
Regular exercise			
Yes	n=11 (78.5%)	n=3 (21.4%)	**0.02**
No	n=203 (46.7%)	n=231 (53.3%)	

There were 166 female and 48 male subjects in control group and 140 females and 94 males in obese group. The mean age of control group was 27.8 and for obese group it was 31.4. The mean BMI of healthy subjects was 21.7 and the mean BMI for patients was 34.4. The number of healthy subjects who had family history about obesity was 82 (38.3 per cent), and who had not was 132 (61.6 per cent). On the other hand, the number of patient subjects who had family history about obesity was 142 (60.6 per cent), and who had not

was 92 (39.3 per cent). The *p* value between control and obese groups about family history was not significant. The number of healthy individuals who ate for need was 56 (26.1 per cent), and who ate for reward was 158 (73.8 per cent). The number of patient subjects who ate for need was 42 (17.9 per cent) and who ate for reward was 192 (82 per cent). The *p* value between control and obese groups about eating behavior was **0.03.** The number of healthy subjects who did regular exercise was 11 (5.1 per cent), and did not was 203 (94.9 per cent). On the other hand, the number of patients who did regular exercise was 3 (1.3 per cent), and did not was 231 (98.7 per cent). The *p* value between control and obese groups for regular exercise was **0.02.**

Table 3.4. Genotype and allele frequencies of 30 bp *MAOA* VNTR in the study groups.

MAOA-u VNTR	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Genotypes			
3R/3R	n=9 (31%)	n=20 (69%)	0.38
3R/4R	n=37 (48%)	n=40 (52%)	
3R/5R	n=2 (50%)	n=2 (50%)	
4R/4R	n=146 (50.3%)	n=144 (49.7%)	
4R/5R	n=10 (47.6%)	n=11 (52.4%)	
5R/5R	n=10 (37%)	n=17 (63%)	
Alleles			
3R	n=57 (41%)	n=82 (59%)	
4R	n=339 (50%)	n=339 (50%)	
5R	n=32 (40.5%)	n=47 (50.5%)	

The number of healthy subjects who had 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R genotypes was 9 (4.2 per cent), 37 (17.2 per cent), 2 (0.9 per cent), 146 (68.2 per cent), 10 (4.6 per cent), and 10 (4.6 per cent), respectively. On the other hand, the number of patient groups who had 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R genotypes was 20 (8.5 per cent), 40 (17 per cent), 2 (0.8 per cent), 144 (61.5 per cent), 11 (4.7 per cent), and 17 (7.2 per cent), respectively. The number of control groups who had 3R, 4R, 5R alleles was 57 (13.3 per cent), 339 (79.2 per cent), and 32 (7.4 per cent), respectively. Conversely, the number of obese subjects who had 3R, 4R, 5R alleles was 82 (17.5 per cent), 339 (72.4 per cent) , and 47 (20 per cent), respectively. The *p* value between control and obese groups about genotype frequencies was 0.38.

Table 3.5. Genotype and allele frequencies of 40 bp *DAT1* 3' UTR VNTR of the study groups.

DAT1 VNTR	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Genotypes			
7R/7R	n=41 (45.6%)	n=49 (54.4%)	0.42
7R/8R	n=23 (46.9%)	n=26 (53.1%)	
8R/8R	n=76 (44.7%)	n=94 (53.3%)	
7R/9R	n=2 (40%)	n=3 (60%)	
8R/9R	n=27 (62.8%)	n=16 (37.2%)	
9R/9R	n=42 (48.8%)	n=44 (51.2%)	
8R/12R	n=1 (100%)	n=0 (0%)	
12R/12R	n=2 (66.7%)	n=1 (33.3%)	
12R/13R	n=0 (0%)	n=1 (100%)	
Alleles			
7R	n=107 (45.7%)	n=127 (54.3%)	
8R	n=51 (54.8%)	n=42 (45.2%)	
9R	n=113 (51.3%)	n=107 (48.7%)	
12R	n=5 (62.5%)	n=3 (37.5%)	
13R	n=0 (0%)	n=1 (100%)	

The number of healthy subjects who had 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes was 41 (19.1 per cent), 23 (10.7 per cent) , 76 (35.5 per cent), 2 (0.9 per cent), 27 (12.6 per cent), 42 (19.6 per cent), 1 (0.4 per cent), 2 (0.9 per cent), and 0 (0 per cent), respectively. On the other hand, the number of patient groups who had 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes was 49 (20.9 per cent), 26 (11.1 per cent), 94 (40.1 per cent), 3 (1.2 per cent), 16 (6.8 per

cent), 44 (18.8 per cent), 0 (0 per cent), 1 (0.4 per cent), and 1 (0.4 per cent), respectively. The number of control groups who had 7R, 8R, 9R, 12R, 13R alleles was 107 (38.7 per cent), 51 (18.4 per cent), 113 (40.9 per cent), 5 (1.8 per cent), and 0 (0 per cent), respectively. Conversely, the number of obese subjects who had 7R, 8R, 9R, 12R, 13R alleles was 127 (45.3 per cent), 42 (15 per cent), 107 (38.2 per cent), 3 (1 per cent), and 1 (0.3 per cent), respectively. The *p* value between control and obese groups about genotype frequencies was 0.42.

Table 3.6. Genotype and allele frequencies of *COMT* (rs4680) of the study groups.

COMT (rs4680)	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Genotypes			
Val/Val	n=104 (39%)	n=163 (61%)	**0.001**
Val/Met	n=82 (65%)	n=44 (35%)	
Met/Met	n=28 (51%)	n=27 (49%)	
Alleles			
Val	n=290 (43.9%)	n=370 (56.1%)	
Met	n=138 (58.5%)	n=98 (41.5%)	

The number of healthy individuals who had Val/Val, Val/Met, Met/Met genotypes was 104 (48.5 per cent), 82 (38.3 per cent), and 28 (13 per cent), respectively. The number of obese subjects who had Val/Val, Val/Met, Met/Met genotypes was 163 (69.6 per cent), 44 (18.8 per cent), and 27 (11.5 per cent), respectively. The number of control groups who had Val, Met allele was 290 (67.7 per cent) and 138 (32.2 per cent), respectively. The number of obese subjects who had Val, Met allele was 370 (79 per cent) and 98 (20.9 per cent), respectively. The *p* value between control and obese groups about the genotype frequencies of *COMT* was **0.001.**

Table 3.7. Genotype and allele frequencies of *DAT1* (rs27072) of the study groups.

DAT1 (rs27072)	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Genotypes			
G/G	n=147 (51.3%)	n=139 (48.7%)	**0.016**
G/A	n=48 (47.5%)	n=53 (52.5%)	
A/A	n=19 (31.1%)	n=42 (68.9%)	
Alleles			
G	n=342 (50.8%)	n=331 (49.2%)	
A	n=86 (38.5%)	n=137 (61.5%)	

The number of healthy individuals who had G/G, G/A, A/A genotypes was 147 (68.6 per cent), 48 (22.4 per cent), and 19 (8.8 per cent), respectively. The number of obese subjects who had G/G, G/A, A/A genotypes was 139 (59.4 per cent), 53 (22.6 per cent), and 42 (17.9 per cent), respectively. The number of control group who had G, A allele was 342 (79.9 per cent) and 86 (20.1 per cent), respectively. The number of obese subjects who had G, A allele was 331 (70.7 per cent) and 137 (29.2 per cent), respectively. The *p* value between control and obese groups about the genotype frequencies of *DAT1* was **0.016.**

Table 3.8. Genotype and allele frequencies of *VMAT2* (rs363399) of the study groups.

VMAT2 (rs363399)	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Genotypes			
T/T	n=150 (45.5%)	n=179 (54.5%)	0.262
T/C	n=45 (55.5%)	n=36 (44.5%)	
C/C	n=19 (50%)	n=19 (50%)	
Alleles			
T	n=345 (46.6%)	n=394 (53.4%)	
C	n=83 (52.8%)	n=74 (47.2%)	

The number of healthy individuals who had T/T, T/C, and C/C genotypes was 150 (70 per cent), 45 (21 per cent), and 19 (8.8 per cent), respectively. The number of obese subjects who had T/T, T/C, and C/C genotypes was 179 (76.4 per cent), 36 (15.3 per cent), and 19 (8.1 per cent), respectively. The number of control groups who had T, C allele was 345 (80.6 per cent) and 83 (19.3 per cent), respectively. The number of obese subjects who had T, C allele was 394 (84.1 per cent) and 74 (15.8 per cent), respectively. The *p* value between control and obese groups about the genotype frequencies of *VMAT2* (rs363399) was 0.26.

Table 3.9. Genotype and allele frequencies of *VMAT2* (rs4752045) of the study groups.

VMAT2 (rs4752045)	**Controls (n=214)**	**Patients (n=234)**	***p*-value**
Genotypes			
C/C	n=97 (44.4%)	n=121 (55.6%)	0.222
C/G	n=94 (52.8%)	n=84 (47.2%)	
G/G	n=23 (44.2%)	n=29 (55.8%)	
Alleles			
C	n=288 (47%)	n=326 (53%)	
G	n=140 (49.6%)	n=142 (50.4%)	

The number of healthy individuals who had C/C, C/G, and G/G genotypes was 97 (45.3 per cent), 94 (43.9 per cent), and 23 (10.7 per cent), respectively. The number of obese subjects who had C/C, C/G, and G/G genotypes was 121 (51.7 per cent), 84 (35.8 per cent), and 29 (12.3 per cent), respectively. The number of control groups who had C, G allele was 288 (67.2 per cent) and 140 (32.7 per cent), respectively. The number of obese subjects who had C, G allele was 326 (69.6 per cent) and 142 (30.3 per cent), respectively. The *p* value between control and obese groups about the genotype frequencies of *VMAT2* (rs4752045) was 0.22.

Table 3.10. The relationship between MAOA-u VNTR and family history of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**No**	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
3R/3R	n=6 (66.7%)	n=3 (33.3%)	**0.016**	n=7 (35%)	n=13 (64%)	0.126
3R/4R	n=31 (83.8%)	n=6 (16.2%)		n=11 (27.5%)	n=29 (72.5%)	
3R/5R	n=0 (0%)	n=2 (100%)		n=2 (100%)	n=0 (0%)	
4R/4R	n=86 (58.9%)	n=60 (41.1%)		n=63 (43.8%)	n=81 (56.3%)	
4R/5R	n=4 (40%)	n=6 (60%)		n=4 (36.4%)	n=7 (63.6%)	
5R/5R	n=5 (50%)	n=5 (50%)		n=4 (23.5%)	n=13 (76.5%)	

The number of healthy subjects who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and had not family history about obesity was 6 (4.5 per cent), 31 (23.4 per cent), 0 (0 per cent), 86 (65.1 per cent), 4 (3 per cent), and 5 (3.7 per cent), respectively. The number of healthy subjects who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and had family history about obesity was 3 (3.6 per cent), 6 (7.3 per cent), 2 (2.4 per cent), 60 (73.1 per cent), 6 (7.3 per cent), and 5 (6.1 per cent), respectively. The *p* value between having family history and not having in control group about genotype frequencies was **0.016.** The number of obese subjects who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and had not family history about obesity was 7 (7.6 per cent), 11 (12 per cent), 2 (2.1 per cent), 63 (69.2 per cent), 4 (4.3 per cent), and 4 (4.3 per cent), respectively. The number of obese individuals who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and had family history was 13 (9 per cent), 29 (20.2 per cent), 0 (0 per cent), 81, 7, and 13, respectively. The *p* value for obese group between having family history or not having about genotype frequencies was 0.12.

Table 3.11. The relationship between 40 bp 3'UTR DAT1 VNTR and family history of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**No**	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
7R/7R	n=23 (56.1%)	n=18 (43.9%)		n=24 (49%)	n=25 (51%)	
7R/8R	n=13 (56.5%)	n=10 (43.5%)		n=11 (42.3%)	n=15 (57.7%)	
8R/8R	n=45 (59.2%)	n=31 (40.8%)		n=30 (31.9%)	n=64 (68.1%)	
7R/9R	n=2 (100%)	n=0 (0%)		n=0 (0%)	n=3 (100%)	
8R/9R	n=18 (66.7%)	n=9 (33.3%)	0.68	n=2 (12.5%)	n=14 (87.5%)	**0.02**
9R/9R	n=28 (66.7%)	n=14 (33.3%)		n=23 (53.3%)	n=21 (47.7%)	
8R/12R	n=1 (100%)	n=0 (0%)		n=0 (0%)	n=0 (0%)	
12R/12R	n=2 (100%)	n=0 (0%)		n=0 (0%)	n=1 (100%)	
12R/13R	n=0 (0%)	n=0 (0%)		n=1 (100%)	n=0 (0%)	

The number of healthy subjects who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R *DAT1* genotypes and had not family history about obesity was 23 (17.4 per cent), 13 (9.8 per cent), 45 (34.1 per cent), 2 (1.5 per cent), 18 (13.6 per cent), 28 (21.2 per cent), 1 (0.75 per cent), 2 (1.5 per cent), and 0 (0 per cent), respectively. The number of healthy subjects who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes and had family history about obesity was 18 (21.9 per cent), 10 (12.1 per cent), 31 (37.8 per cent), 0 (0 per cent), 9 (10.9 per cent), 14 (17.0 per cent), 0 (0 per cent), 0 (0 per cent), and 0 (0 per cent), respectively. The *p* value between having family history and not having in control group about genotype frequencies was 0.68. The number of obese subjects who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes and had not family history about obesity was 24 (26.3 per cent), 11 (12.1 per cent), 30 (32.9 per cent), 0 (0 per cent), 2 (2.2 per cent), 23 (25.2 per cent), 0 (o per cent), 0 (o per cent), and 1 (1.1 per cent), respectively.

The number of obese individuals who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes and had family history was 25 (17.4 per cent), 15 (10.4 per cent), 64 (44.7 per cent), 3 (2.1 per cent), 14 (9.8 per cent), 21 (14.6 per cent), 0 (0 per cent), 1 (0.7 per cent), and 0 (o per cent), respectively. The *p* value for obese group between having family history or not having about genotype frequencies was **0.02.**

Table 3.12. The relationship between *COMT* (rs4680) and family history of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**No**	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
Val/Val	n=60 (57.7%)	n=44 (42.3%)	0.483	n=72 (44.4%)	n=91 (55.6%)	**0.03**
Val/Met	n=53 (64.6%)	n=29 (35.4%)		n=14 (31.8%)	n=30 (68.2%)	
Met/Met	n=19 (67.9%)	n=9 (32.1%)		n=5 (18.5%)	n=22 (81.5%)	

The number of healthy subjects who carried Val/Val, Val/Met, Met/Met COMT genotypes and had not family history about obesity was 60 (45.4 per cent), 53 (40.1 per cent), and 19 (14.3 per cent), respectively. The number of healthy subjects who carried Val/Val, Val/Met, Met/Met genotypes and had family history about obesity was 44 (53.6 per cent), 29 (35.3 per cent), and 9 (10.9 per cent), respectively. The *p* value between having family history or not having in control group about genotype frequencies was 0.483. The number of obese subjects who carried Val/Val, Val/Met, Met/Met genotypes and had not family history about obesity was 72 (79.1 per cent), 14 (15.3 per cent), and 5 (5.5 per cent), respectively. The number of obese individuals who carried Val/Val, Val/Met, Met/Met genotypes and had family history was 91 (63.6 per cent), 30 (20.9 per cent), and 22 (15.3 per cent), respectively. The *p* value for obese group between having family history or not having about genotype frequencies was **0.03.**

Table 3.13. The relationship between *DAT1* (rs27072) and family history of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**No**	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
G/G	n=89 (60.5%)	n=58 (39.5%)	0.871	n=54 (38.8%)	n=85 (61.2 %)	0.18
G/A	n=31 (64.6%)	n=17 (35.4%)		n=25 (47.2%)	n=28 (52.8%)	
A/A	n=12 (63.2%)	n=7 (36.8%)		n=12 (28.6%)	n=30 (71.4%)	

The number of healthy subjects who carried G/G, G/A, A/A *DAT1* genotypes and had not family history about obesity was 89 (67.4 per cent), 31 (23.4 per cent), and 12 (9.1 per cent), respectively. The number of healthy subjects who carried G/G, G/A, A/A genotypes and had family history about obesity was 58 (70.7 per cent), 17 (20.7 per cent), and 7 (8.5 per cent), respectively. The *p* value between having family history or not having in control group about genotype frequencies was 0.87. The number of obese subjects who carried G/G, G/A, A/A genotypes and had not family history about obesity was 54 (59.3 per cent), 25 (27.4 per cent), and 12 (13.1 per cent), respectively. The number of obese individuals who carried G/G, G/A, A/A genotypes and had family history was 85 (59.4 per cent), 28 (19.5 per cent), and 30 (20.9 per cent), respectively. The *p* value for obese group between having family history or not having about genotype frequencies was 0.18.

Table 3.14. The relationship between *VMAT2* (rs363399) and family history of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**No**	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
C/C	n=11 (57.9%)	n=8 (42.1%)	0.366	n=10 (52.6%)	n=9 (47.4%)	0.400
C/T	n=24 (53.3%)	n=21 (46.7%)		n=13 (36.1%)	n=23 (63.9%)	
T/T	n=97 (64.7%)	n=53 (35.3%)		n=68 (38%)	n=111 (62%)	

The number of healthy subjects who carried C/C, C/T, T/T *VMAT2* genotypes and had not family history about obesity was 11 (8.3 per cent), 24 (18.1 per cent), and 97 (73.4 per cent), respectively. The number of healthy subjects who carried C/C, C/T, T/T genotypes and had family history about obesity was 8 (9.7 per cent), 21 (25.6 per cent), and 53 (64.6 per cent), respectively. The *p* value between having family history or not having in control group about genotype frequencies was 0.36. The number of obese subjects who carried C/C, C/T, T/T genotypes and had not family history about obesity was 10 (10.9 per cent), 13 (14.2 per cent), and 68 (74.7 per cent), respectively. The number of obese individuals who carried C/C, C/T, T/T genotypes and had family history was 9 (6.2 per cent), 23 (16.1 per cent), and 111 (77.6 per cent), respectively. The *p* value for obese group between having family history or not having about genotype frequencies was 0.4.

Table 3.15. The relationship between *VMAT2* (rs4752045) and family history of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**No**	**Yes**	***p*-value**	**No**	**Yes**	***p*-value**
C/C	n=57 (58.8%)	n=40 (41.2%)	0.72	n=44 (36.4%)	n=77 (63.6%)	0.28
C/G	n=60 (63.8%)	n=34 (36.2%)		n=38 (45.2%)	n=46 (54.8%)	
G/G	n=25 (65.2%)	n=8 (34.8%)		n=9 (31%)	n=20 (69%)	

The number of healthy subjects who carried C/C, G/C, G/G *VMAT2* genotypes and had not family history about obesity was 57 (43.1 per cent), 60 (45.4 per cent), and 15 (11.3 per cent), respectively. The number of healthy subjects who carried C/C, G/C, G/G genotypes and had family history about obesity was 40 (48.7 per cent), 34 (41.4 per cent), and 8 (9.7 per cent), respectively. The *p* value between having family history or not having in control group about genotype frequencies was 0.72. The number of obese subjects who carried C/C, G/C, G/G genotypes and had not family history about obesity was 44 (48.3 per cent), 38 (41.7 per cent), and 9 (9.8 per cent), respectively. The number of obese individuals who carried C/C, G/C, G/G genotypes and had family history was 77 (53.8 per cent), 46 (32.1 per cent), and 20 (13.9 per cent), respectively. The *p* value for obese group between having family history or not having about genotype frequencies was 0.28.

Table 3.16. The relationship between *MAOA*-u VNTR and eating behavior of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**Need**	**Reward**	***p*-value**	**Need**	**Reward**	***p*-value**
3R/3R	n=1 (11.1%)	n=8 (88.9%)	0.211	n=3 (15%)	n=17 (85%)	0.420
3R/4R	n=10 (27%)	n=27 (73%)		n=8 (20%)	n=32 (80%)	
3R/5R	n=1 (50%)	n=1 (50%)		n=0 (0%)	n=2 (100%)	
4R/4R	n=41 (28.1%)	n=105 (71.9%)		n=31 (21.5%)	n=113 (78.5%)	
4R/5R	n=6 (60%)	n=4 (40%)		n=4 (36.4%)	n=7 (63.6%)	
5R/5R	n=4 (40%)	n=6 (60%)		n=1 (5.9%)	n=16 (94.1%)	

The number of healthy subjects who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and ate for need was 1 (1.5 per cent), 10 (15.8 per cent), 1 (1.5 per cent), 41 (65.1 per cent), 6 (9.5 per cent), and 4 (6.3 per cent), respectively. The number of healthy subjects who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and ate for reward was 8 (5.2 per cent), 27 (17.8 per cent), 1 (0.6 per cent), 105 (69.5 per cent), 4 (2.6 per cent), and 6 (3.9 per cent), respectively. The *p* value between genotype frequencies and eating for need or for reward in control group was 0.21. The number of obese subjects who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and ate for need was 3 (6.3 per cent), 8 (17 per cent), 0 (0 per cent), 31 (65.9 per cent), 4 (8.5 per cent), and 1 (2.1 per cent), respectively. The number of obese individuals who carried 3R/3R, 3R/4R, 3R/5R, 4R/4R, 4R/5R, 5R/5R MAOA genotypes and ate for reward was 17 (9.1 per cent), 32 (17.1 per cent), 2 (1.1 per cent), 113 (60.4 per cent), 7 (3.7 per cent), and 16 (8.5 per cent), respectively. The *p* value for obese group between genotype frequencies and eating for need or for reward was 0.42.

Table 3.17. The relationship between 40 bp 3' UTR *DAT1* VNTR and eating behavior of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**Need**	**Reward**	***p*-value**	**Need**	**Reward**	***p*-value**
7R/7R	n=6 (14.6%)	n=35 (85.4%)	0.15	n=14 (28.6%)	n=35 (71.4%)	0.60
7R/8R	n=11 (47.8%)	n=12 (52.2%)		n=6 (23.1%)	n=20 (76.9%)	
8R/8R	n=23 (30.3%)	n=53 (69.7%)		n=17 (18.1%)	n=77 (81.9%)	
7R/9R	n=0 (0%)	n=2 (100%)		n=1 (33.3%)	n=2 (66.7%)	
8R/9R	n=9 (33.3%)	n=18 (66.7%)		n=1 (6.3%)	n=15 (93.8%)	
9R/9R	n=14 (33.3%)	n=28 (66.7%)		n=8 (18.2%)	n=36 (81.8%)	
8R/12R	n=0 (0%)	n=1 (100%)		n=0 (0%)	n=0 (0%)	
12R/12R	n=0 (0%)	n=2 (100%)		n=0 (0%)	n=1 (100%)	
12R/13R	n=0 (0%)	n=0 (0%)		n=0 (0%)	n=1 (100%)	

The number of healthy subjects who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R *DAT1* genotypes and ate for need was 6 (9.5 per cent), 11 (17.4 per cent), 23 (36.5 per cent), 0 (0 per cent), 9 (14.2 per cent), 14 (22.2 per cent), 0 (0 per cent), 0 (0 per cent), and 0 (0 per cent), respectively. The number of healthy subjects who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes and ate for reward was 35 (23.1 per cent), 12 (7.9 per cent), 53 (35 per cent), 2 (1.3 per cent), 18 (11.9 per cent), 28 (18.5 per cent), 1 (0.6 per cent), 2 (1.2 per cent), and 0 (0 per cent), respectively. The *p* value between genotype frequencies and eating for need or for reward in control group was 0.15. The number of obese subjects who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes and ate for need was 14 (29.7 per cent), 6 (12.7 per cent), 17 (36.1 per cent), 1 (2.1 per cent), 1 (2.1 per cent), 8 (17 per cent), 0 (0 per cent), 0 (0 per cent), and 0 (0 per cent), respectively. The

number of obese individuals who carried 7R/7R, 7R/8R, 8R/8R, 7R/9R, 8R/9R, 9R/9R, 8R/12R, 12R/12R, 12R/13R genotypes and ate for reward was 35 (18.7 per cent), 20 (10.6 per cent), 77 (41.1 per cent), 2 (1.1 per cent), 15 (8.1 per cent), 36 (19.2 per cent), 0 (0 per cent), 1 (0.5 per cent), and 1 (0.5 per cent), respectively. The *p* value for obese group between genotype frequencies and eating for need or for reward was 0.60.

Table 3.18. The relationship between *COMT* (rs4680) and eating behavior of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**Need**	**Reward**	***p*-value**	**Need**	**Reward**	***p*-value**
Val/Val	n=31 (29.8%)	n=73 (70.2%)	0.66	n=28 (17.3%)	n=135 (82.7%)	0.38
Val/Met	n=22 (26.8%)	n=60 (73.2%)		n=12 (27.3%)	n=32 (72.7%)	
Met/Met	n=10 (35.7%)	n=18 (64.3%)		n=7 (25.9%)	n=20 (74.1%)	

The number of healthy subjects who carried Val/Val, Val/Met, Met/Met *COMT* genotypes and ate for need was 31 (49.2 per cent), 22 (34.9 per cent), and 10 (15.8 per cent), respectively. The number of healthy subjects who carried Val/Val, Val/Met, Met/Met genotypes and ate for reward was 73 (48.3 per cent), 60 (39.7 per cent), and 18 (11.9 per cent), respectively. The *p* value between genotype frequencies and eating for need or for reward in control group was 0.66. The number of obese subjects who carried Val/Val, Val/Met, Met/Met genotypes and ate for need was 28 (59.5 per cent), 12 (25.5 per cent), and 7 (14.8 per cent), respectively. The number of obese individuals who carried Val/Val, Val/Met, Met/Met genotypes and ate for reward was 135 (72.1 per cent), 32 (17.1 per cent), and 20 (10.6 per cent), respectively. The *p* value for obese group between genotype frequencies and eating for need or for reward was 0.38.

Table 3.19. The relationship between *DAT1* (rs27072) and eating behavior of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**Need**	**Reward**	***p*-value**	**Need**	**Reward**	***p*-value**
G/G	n=44 (29.9%)	n=103 (70.1%)	0.29	n=24 (17.3%)	n=115 (82.7%)	0.23
G/A	n=11 (22.9%)	n=37 (77.1%)		n=15 (28.3%)	n=38 (71.7%)	
A/A	n=8 (42.1%)	n=11 (57.9%)		n=8 (19%)	n=34 (81%)	

The number of healthy subjects who carried G/G, G/A, A/A *DAT1* genotypes and ate for need was 44 (69.8 per cent), 11 (17.4 per cent), and 8 (12.6 per cent), respectively. The number of healthy subjects who carried G/G, G/A, A/A genotypes and ate for reward was 103 (68.2 per cent), 37 (24.5 per cent), and 11 (7.2 per cent), respectively. The *p* value between genotype frequencies and eating for need or for reward in control group was 0.29. The number of obese subjects who carried G/G, G/A, A/A genotypes and ate for need was 24 (51 per cent), 15 (31.9 per cent), and 8 (17.1 per cent), respectively. The number of obese individuals who carried G/G, G/A, A/A genotypes and ate for reward was 115 (61.4 per cent), 38 (20.3 per cent), and 34 (18.1 per cent), respectively. The *p* value for obese group between genotype frequencies and eating for need or for reward was 0.23.

Table 3.20. The relationship between *VMAT2* (rs363399) and eating behavior of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**Need**	**Reward**	***p*-value**	**Need**	**Reward**	***p*-value**
C/C	n=7 (36.8%)	n=12 (63.2%)	0.71	n=2 (10.5%)	n=17 (89.5%)	0.54
C/T	n=12 (26.7%)	n=33 (73.3%)		n=8 (22.2%)	n=28 (77.8%)	
T/T	n=44 (29.3%)	n=106 (70.7%)		n=37 (20.7%)	n=142 (79.3%)	

The number of healthy subjects who carried C/C, C/T, T/T *VMAT2* genotypes and ate for need was 7 (11.1 per cent), 12 (19 per cent), and 44 (69.8 per cent), respectively. The number of healthy subjects who carried C/C, C/T, T/T genotypes and ate for reward was 12 (7.9 per cent), 33 (21.8 per cent), and 106 (70.1 per cent), respectively. The *p* value between genotype frequencies and eating for need or for reward in control group was 0.71. The number of obese subjects who carried C/C, C/T, T/T genotypes and ate for need was 2 (4.2 per cent), 8 (17.1 per cent), and 37 (78.7 per cent), respectively. The number of obese individuals who carried C/C, C/T, T/T genotypes and ate for reward was 17 (9.1 per cent), 28 (14.9 per cent), and 142 (75.9 per cent), respectively. The *p* value for obese group between genotype frequencies and eating for need or for reward was 0.54.

Table 3.21. The relationship between *VMAT2* (rs4752045) and eating behavior of the study population.

	Controls (n=214)			**Patients (n=234)**		
Genotypes	**Need**	**Reward**	***p*-value**	**Need**	**Reward**	***p*-value**
C/C	n=26 (26.8%)	n=71 (73.2%)	0.71	n=24 (19.8%)	n=97 (80.2%)	0.83
C/G	n=31 (33%)	n=63 (67%)		n=16 (19%)	n=68 (81%)	
G/G	n=6 (26.1%)	n=17 (73.9%)		n=7 (24.1%)	n=22 (75.9%)	

The number of healthy subjects who carried C/C, C/G, G/G *VMAT2* genotypes and ate for need was 26 (41.2 per cent), 31 (49.2 per cent), and 6 (9.5 per cent), respectively. The number of healthy subjects who carried C/C, C/G, G/G genotypes and ate for reward was 71 (47 per cent), 63 (41.7 per cent), and 17 (11.2 per cent), respectively. The *p* value between genotype frequencies and eating for need or for reward in control group was 0.71. The number of obese subjects who carried C/C, C/G, G/G genotypes and ate for need was 24 (51 per cent), 16 (34 per cent), and 7 (15 per cent), respectively. The number of obese individuals who carried C/C, C/G, G/G genotypes and ate for reward was 97 (51.8 per

cent), 68 (36.3 per cent), and 22 (11.7 per cent), respectively. The *p* value for obese group between genotype frequencies and eating for need or for reward was 0.83.

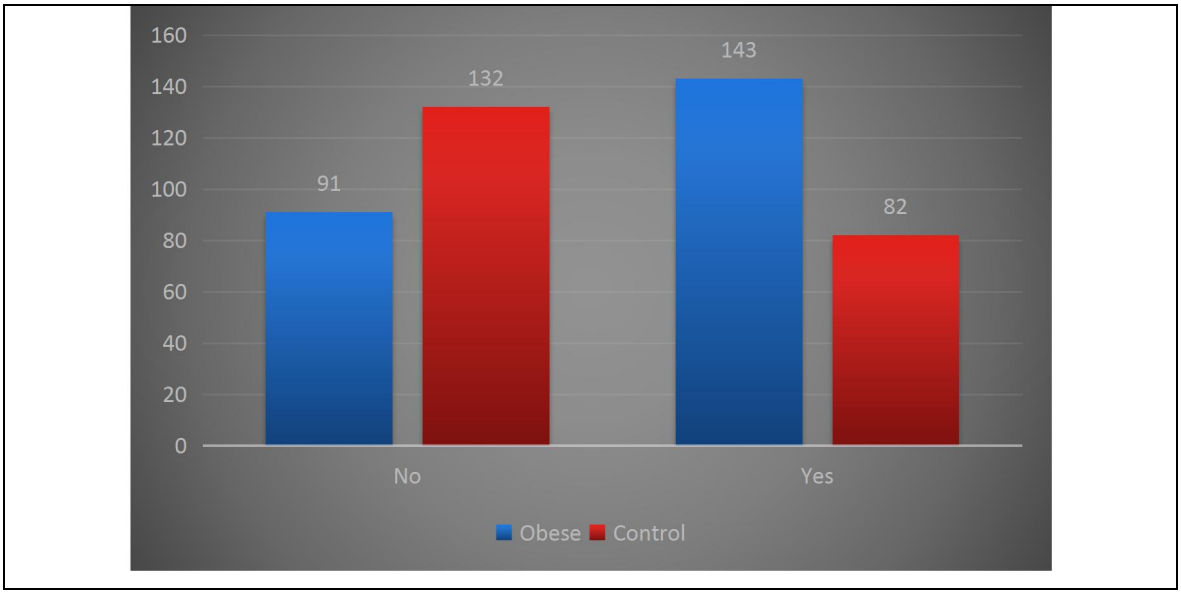


Figure 3.11. The family history distribution of the study population.

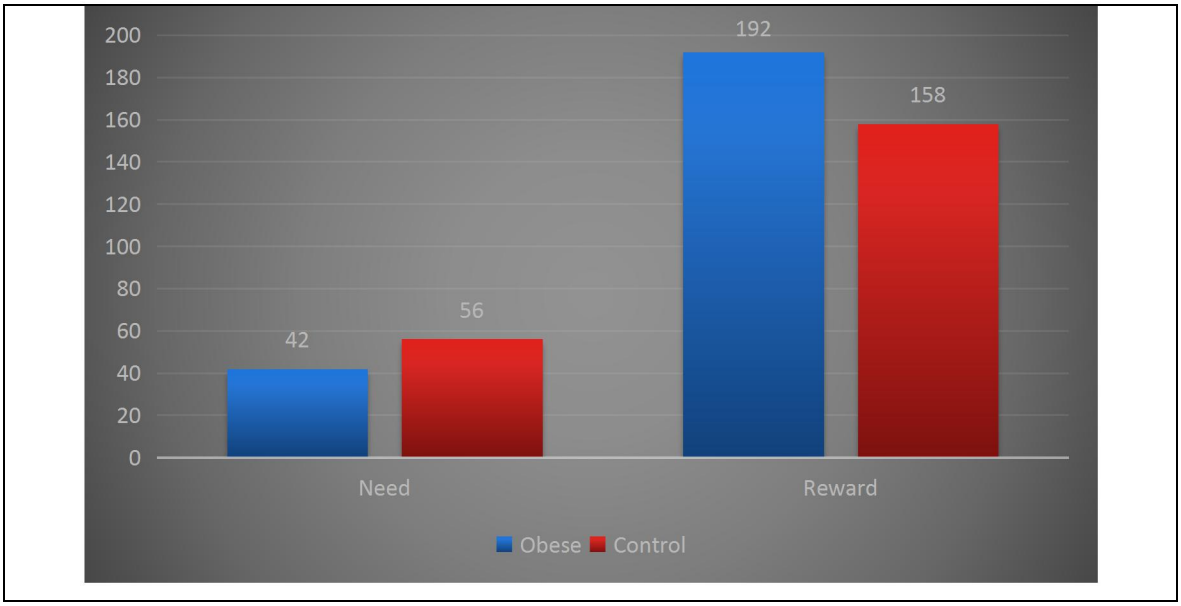


Figure 3.12. The eating behavior distribution of the study population.

Table 3.22. The relationship between genotype frequencies of 30 bp *MAOA* VNTR and eating behavior in obese group.

MAOA-u VNTR Genotypes	**Need**	**Reward**	***p*-value**	**OR (95% CI)**
3R/3R	n=3 (15%)	n=17 (85%)	0.30	3.590 (0.321-40.20)
3R/4R	n=8 (20%)	n=32 (80%)	0.16	4.679 (0.520-42.11)
3R/5R	n=0 (0%)	n=2 (100%)	-	-
4R/4R	n=31 (21.5%)	n=117 (78.5%)	0.13	5.011 (0.614-40.88)
4R/5R	n=4 (36.4%)	n=7 (63.6%)	0.30	15.69 (1.298-189.55)
5R/5R	n=1 (5.9%)	n=16 (94.1%)	-	-

Table 3.23. The relationship between genotype frequencies of 40 bp 3' UTR *DAT1* VNTR and eating behavior in obese group.

DAT1 VNTR Genotypes	**Need**	**Reward**	***p*-value**	**OR (95% CI)**
7R/7R	n=14 (28.6%)	n=35 (71.4%)	0.99	-
7R/8R	n=6 (23.1%)	n=20 (76.9%)	0.99	-
8R/8R	n=17 (18.1%)	n=77 (81.9%)	0.99	-
7R/9R	n=1 (33.3%)	n=2 (66.7%)	0.99	-
8R/9R	n=1 (6.3%)	n=15 (93.8%)	0.99	-
9R/9R	n=8 (18.2%)	n=36 (81.8%)	0.99	-

Table 3.24. The relationship between genotype frequencies of *COMT* and eating behavior in obese group.

COMT	**Need**	**Reward**	***p*-value**	**OR (95% CI)**
Genotypes				
Val/Val	n=28 (17.3%)	n=135 (82.7%)	0.28	0.579 (0.214-1.560)
Val/Met	n=12 (27.3%)	n=32 (72.7%)	0.98	1.000 (0.328-3.090)
Met/Met	n=7 (25.9%)	n=20 (74.1%)	Not significant	-

Table 3.25. The relationship between genotype frequencies of *DAT1* (rs27072) and eating behavior in obese group.

DAT1	**Need**	**Reward**	***p*-value**	**OR (95% CI)**
Genotypes				
G/G	n=24 (17.3%)	n=115 (82.7%)	**0.001**	-
G/A	n=15 (28.3%)	n=38 (71.7%)	0.09	1.923 (0.888-4.165)
A/A	n=8 (19%)	n=34 (81%)	0.49	1.389 (0.541-3.561)

The *p* value between the obese subjects who eat for need and for reward about G/G genotype was **0.001.**

Table 3.26. The relationship between genotype frequencies of *VMAT2* (rs363399) and eating behavior in obese group.

VMAT2	**Need**	**Reward**	***p*-value**	**OR (95% CI)**
Genotypes				
C/T	n=8 (22.2%)	n=28 (77.8%)	0.54	0.776 (0.339-1.774)

Table 3.27. The relationship between genotype frequencies of *VMAT2* (rs4752045) and eating behavior in obese group.

VMAT2	**Need**	**Reward**	***p*-value**	**OR (95% CI)**
Genotypes				
C/C	n=24 (19.8%)	n=97 (80.2%)	0.70	0.823 (0.301-2.250)
G/C	n=16 (19%)	n=68 (81%)	0.49	0.681 (0.226-2.051)

Table 3.28. The chi-square (x^2), degree of freedom (df), *p,* odds ratio (OR), and confidence interval (95% CI) values between control and obese groups according to eating behavior and genotypes (The reference category is control).

Variable	**x^2**	**df**	***p*-value**	**OR (95% CI)**
Eating behavior	4.60	1	**0.03**	0.600 (0.376-0.959)
MAOA-u VNTR	2.24	5	0.81	-
3R/3R	-	1	0.487	1.598 (0.490-5.214)
3R/4R	-	2	0.78	0.875 (0.335-2.285)
3R/5R	-	2	0.94	0.924 (0.095-8.986)
4R/4R	-	3	0.74	0.865 (0.365-2.047)
4R/5R	-	3	0.86	1.109 (0.322-3.817)
DAT1 VNTR	8.18	8	0.41	-
COMT (rs4680)	20.21	2	**0.001**	-
Val/Val	-	1	0.22	1.470 (0.789-2.738)
Val/Met	-	1	**0.04**	0.511 (0.259-1.011)
DAT1 (rs27072)	5.81	2	0.05	-
A/A	-	1	**0.018**	2.144 (1.137-4.041)
A/G	-	2	0.52	1.170 (0.717-1.908)
VMAT2 (rs363399)	2.34	2	0.30	-
C/T	-	1	0.66	0.837 (0.357-1.933)
T/T	-	1	0.55	1.247 (0.602-2.583)
VMAT2 (rs4752045)	3.25	2	0.19	-
C/C	-	1	0.54	0.812 (1.416-1.582)
G/C	-	1	0.13	0.591 (0.298-1.171)

The eating behavior difference between control and obese groups was significant (x^2=4.60,***p*=0.03**). The Val/Met *COMT* genotype for eating behavior was significantly different in obese and control groups (***p*=0.04**). The subjects who had A/A *DAT1* genotype were significantly higher in obese group (***p*=0.018**).

Table 3.29. The characteristics of extreme samples of the obese group.

ID	Sex	Age (yrs)	BMI (kg/m2)	Eating behavior	Explanation
1	Female	40	25.3	Reward	Morbidly obese from childhood. Lost 22 kg by acupuncture (30 yrs). Other members are obese
2	Female	28	39.6	Reward	Underwent bariatric surgery and lost 23 kg. Other members are obese
3	Female	21	45.4	Reward	Premature baby. She was told to be with mental retardation or obesity. None of family members are obese
4	Male	20	26.9	Reward	Lost 22 kg by exercise and diet within 2 years. Mother is obese
5	Male	29	38.1	Reward	Lost 37 kg by bariatric surgery in 3 months. Uses protein powder. Mother is obese

Table 3.30. The genotypes of extreme samples of the obese group.

ID	MAOA (30 bp VNTR)	DAT1 (40 bp VNTR)	COMT (rs4680)	DAT1 (rs27072)	VMAT2 (rs363399)	VMAT2 (rs4752045)
1	4R/4R	3R/3R	Val/Val	G/A	T/T	C/C
2	4R/4R	3R/3R	Val/Val	G/A	T/T	C/C
3	4R/5R	3R/3R	Val/Val	G/G	C/T	G/C
4	4R/4R	3R/3R	Met/Met	G/G	T/T	C/C
5	4R/4R	3R/3R	Val/Val	G/G	T/T	G/C

Table 3.31. The characteristics of extreme sample of the control group.

ID	Sex	Age (years)	BMI (kg/m2)	Eating behavior	Explanation
1	Female	21	19.9	Hates	Used drugs for appetite. Mother is obese

Table 3.32. The genotypes of the extreme sample of the control group.

ID	MAOA (30 bp VNTR)	DAT1 (40 bp VNTR)	COMT (rs4680)	DAT1 (rs27072)	VMAT2 (rs363399)	VMAT2 (rs4752045)
1	4R/4R	3R/3R	Met/Met	G/G	T/T	G/C

Table 3.33. Logistic regression analysis for adult obesity.

	Multivariate		
Variable	**OR**	**%95 CI**	***p*-value**
Age	0.7	1.020-3.145	**<0.001**
Sex	1.6	0.052-0.985	**<0.001**
BMI	1.3	1.321-4.010	**<0.001**
Family history	—	—	—
Eating behavior (Reward)	1.688	1.072-2.660	**0.02**
Regular exercise (Yes)	0.235	0.061-0.907	**0.03**
MAOA-u VNTR	—	—	—
DAT1 VNTR	—	—	—
COMT (rs4680)	—	—	**0.001**
DAT1 (rs27072)			
A/G	2.281	1.241-4.191	**0.008**
A/A	1.903	0.955-3.791	**0.016**
VMAT2 (rs363399)	—	—	—
VMAT2 (rs4752045)	—	—	—

OR: Odds ratio; CI: Confidence Interval; *p*-value which is significant is shown in black and boldface type.

In order to detect the factors which affect obesity, we carried out logistic regression analysis. As a result, a remarkable association was found between adult obesity and eating behavior, regular exercise, *COMT* (rs4680), and *DAT1* (rs27072) genotypes.

3.3. RESULTS OF LINKAGE DISEQUILIBRIUM AND HAPLOTYPE ANALYSIS

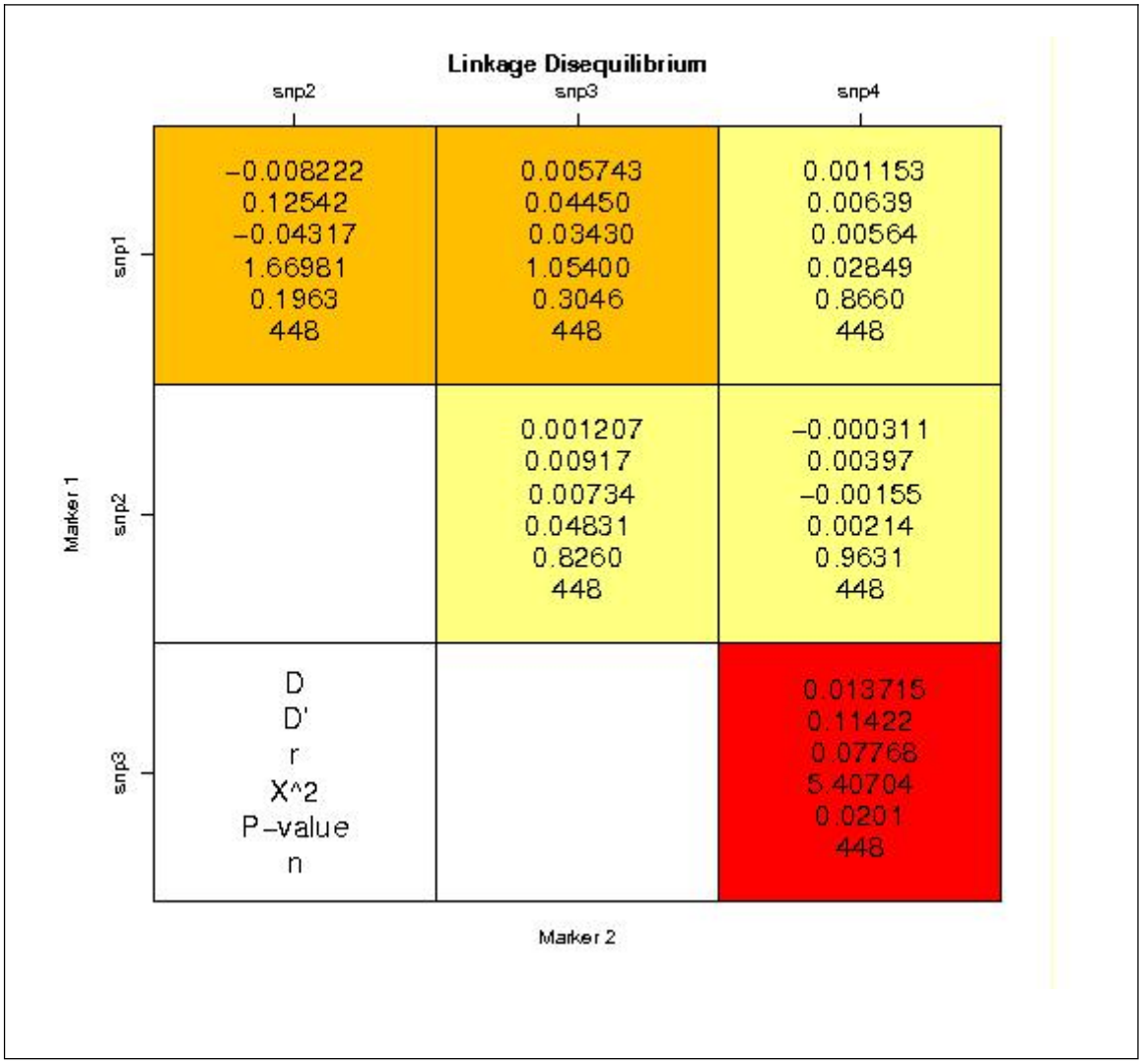


Figure 3.13. Linkage disequilibrium values between the four studied SNPs. SNP1: rs4680(COMT), SNP2: rs27072(DAT1), SNP3: rs363399(VMAT2), SNP4: rs4752045(VMAT2).

snp1	snp2	snp3	snp4	Total	Obese Group	Control Group	Cumulative frequency
A	G	T	C	0.3285	0.3563	0.2955	0.3285
A	G	T	G	0.1299	0.1237	0.1379	0.4583
G	G	T	C	0.1121	0.0829	0.1409	0.5705
A	A	T	C	0.1037	0.1192	0.0865	0.6741
A	A	T	G	0.0516	0.0646	0.0388	0.7258
A	G	C	C	0.0507	0.0516	0.0487	0.7764
G	G	T	G	0.0491	0.0388	0.0683	0.8255
A	G	C	G	0.0366	0.033	0.042	0.8621
G	A	T	C	0.0352	0.0378	0.0382	0.8973
G	G	C	C	0.0243	0.0107	0.0428	0.9216
A	A	C	C	0.0228	0.0279	0.0204	0.9445
G	G	C	G	0.02	0.0104	0.023	0.9645
G	A	T	G	0.0147	0.0186	0	0.9792
A	A	C	G	0.0128	0.0143	0.0078	0.992
G	A	C	C	0.008	0.0103	NA	1
G	A	C	G	0	0	0.0092	1

Figure 3.14. The estimation of haplotype frequencies and haplotype association with obesity.

ID	snp1	snp2	snp3	snp4	Freq	OR (95% CI)	P-value
1	A	G	T	C	0.3286	1.00	---
2	A	G	T	G	0.1302	1.40 (0.81 - 2.42)	0.23
3	G	G	T	C	0.1108	**1.82 (1.06 - 3.13)**	0.03
4	A	A	T	C	0.104	0.99 (0.59 - 1.64)	0.96
5	A	A	T	G	0.0516	0.75 (0.35 - 1.62)	0.47
6	A	G	C	C	0.0503	1.14 (0.59 - 2.20)	0.7
7	G	G	T	G	0.0498	1.73 (0.79 - 3.82)	0.17
8	G	A	T	C	0.0367	1.18 (0.53 - 2.66)	0.68
9	A	G	C	G	0.0361	1.20 (0.54 - 2.66)	0.65
10	G	G	C	C	0.0244	3.18 (0.97-10.44)	0.057
11	A	A	C	C	0.0227	0.81 (0.32 - 2.07)	0.66
12	G	G	C	G	0.0208	**4.63 (1.09 - 19.59)**	0.038
13	A	A	C	G	0.0132	1.22 (0.35 - 4.24)	0.76
14	G	A	T	G	0.013	0.33 (0.03 - 3.28)	0.34
rare	*	*	*	*	0.0077	0.72 (0.08 - 6.32)	0.76

Figure 3.15. Global haplotype association with obesity (*p*-value: **0.019**).

4. DISCUSSION AND CONCLUSION

Obesity is increasingly spreading worldwide day by day and a health problem which may cause various diseases such as cancer, diabetes, cardiovascular diseases, neuropsychiatric disorders and social problems. For these reasons, it is so significant to determine overweight and obesity in adults and children. And in 1998, the World Health Organization (WHO) has termed obesity as an important community health problem [170]. Overweight and obesity in adults is increasing in prevalence from 4.2 per cent in 1990 to 6.7 per cent in 2010. It has been anticipated that the prevalence of overweight and obesity will raise to 9.1 per cent in 2020 in adolescents [171]. The prevalence of obesity was 36.5 per cent in United States in adults during 2011-2014. Currently, nearly 70 per cent of women are overweight or obese in US [172]. The prevalence of central obesity is 64 per cent, 35 per cent, 53 per cent for females, males, and general population, respectively [173]. Furthermore, obesity is variable in prevalence between urban and rural regions of a country and also between developing and developed countries [174]. The prevalence of obesity is 41 per cent, 20.5 per cent, 30.3 per cent for females, males, and general population, respectively in Turkey[175]. The two most prevalent indicators for the diagnosis of obesity are BMI and waist circumference and the values of waist circumference are different for populations [173].

The imbalance between food intake and energy consumption may cause to the development of obesity. Obesity is a multifactorial complex disease, affected by different factors such as diet, physical activity, sleep, sedentariness, eating behavior, genetic and epigenetic factors [176]. Different kinds of molecules have been discovered to be able to understand the molecular mechanism of obesity. For instance, the novel molecule irisin might be a new target in order to understand the pathogenesis of obesity. Irisin affects white adipose tissue and triggers the expression of UCP1 and brown adipocyte development. Irisin may increase the energy consumption without any change of food intake and physical activity . Therefore, irisin will be considered as a therapeutic agent for obesity if the function of irisin is proven by other future experiments [177, 178].

On the other hand, obesity might be defined as neurobiological disease rather than metabolic disorder [179]. For several years, obese individuals were considered that they had metabolic disturbances such as low metabolic rate. In different studies, the metabolic rates were normal or higher than lean people's. Additionally, scientists supposed that obesity could be an eating disorder such as binge eating, bulimia nervosa, night eating disorder. Conversely, only a small group of patients of eating disorders were obese [180, 181].

Obesity is significantly associated with excessive food intake and choice for energy-dense palatable foods. The neurotransmitter dopamine is the main component of the neurobiological aspect of obesity. Palatable foods activate the reward system in the brain and contribute to release of DA in striatum and nucleus accumbens [182]. In obese humans, it has been demonstrated that DRD2 and DRD3 are lower and repetitive palatable food intake might be the reason [183]. Cenkinson C.P., et al [184] showed that DRD2 Ser311Cys and TaqIA polymorphisms were not responsible for the development of obesity. Ariza M., et al [185] expressed that DRD2/ANKK1-TaqA1 polymorphism was associated with obesity, on the other hand, there was no relationship between DRD4 polymorphism and food reinforcement effect. On the other side, Kaplan A.S., et al [186] demonstrated that the hypofunctional 7R allele of DRD4 leaded to the weight gain in females with bulimia nervosa. Yeh J., et al [187] showed that there was a correlation between palatable food (particularly carbohydrate) cravings and DRD2 A1 allele for Asian Americans. It has been demonstrated that the DRD2 Taq1A allele is significantly associated with obesity by the help of variable population studies [188].

In this study, pregnancy and lactation were excluded because pregnancy may cause wieght gain and obesity. Additionally, oxytocinergic system activates the brain reward circuits and this connection is about maternal caregiving behavior. Injection of oxytocin in the ventral tegmental area leads to enhancement of DA signals and these signals are inhibited by oxytocin antagonists. It has been proven that dopamine is responsible for maternal behaviors. In the period of lactation and pregnancy, both oxytocin and dopaminergic gene expressions are raised especially in substantia nigra. Addiction studies have demonstrated that abused drugs reduce the expression of oxytocin if they are administrated chronically [189]. Having thyroid or diabetes problem is excluded in this

study. First of all, the function of thyroid is significantly correlated with thermogenesis, glucose and lipid metabolism, and basal metabolism. Hypothyroidism leads to weight gain by reducing thermogenesis and basal metabolism [190]. Insulin stimulates the differantiation of preadipocytes to adipocytes and lipogenesis in mature adipocytes therefore, diabetes patients tend to gain weight [191]. The women with menopause are excluded, since this period causes to the alteration of body composition, weight gain and then obesity [192]. Hormonal and nonhormonal contraception contribute to weight gain and antihypertensive beta blockers account for weight gain as a common adverse effect. Therefore we excluded use of contraceptives and antihypertensive beta blockers [193, 194]. Moreover, in this study, smoking, chronic alcoholic condition, having a neurological or psychiatric disorder, previous use substance of abuse were excluded because these factors might affect the dopaminergic system.

The most studied genes are dopamine receptors in several populations around the world, therefore we excluded these genes in this study. We aimed to investigate the relationship between the gene variants of the metabolism and transport of DA and obesity. The metabolizing enzymes and transporter proteins terminate the action and function of DA. Therefore, we were interested in the enzymes MAO-A and COMT, and the transporters DAT1 and VMAT2 to be able to figure out the mechanism of obesity via DA neurotransmission.

In our study, the genotype frequencies were 3R/3R 9(4.2 per cent), 3R/4R 37(17.3 per cent), 3R/5R 2(0.9 per cent), 4R/4R 146(68.2 per cent), 4R/5R 10(4.7 per cent), 5R/5R 10(4.7 per cent) in control group, on the other hand, 3R/3R 20(8.5 per cent), 3R/4R 40(17.1 per cent), 3R/5R 2(0.9 per cent), 4R/4R 144(61.5 per cent), 4R/5R 11(4.7 per cent), and 5R/5R 17(7.3 per cent) in obese group. The allele frequencies of control group were 3R 57(13.3 per cent), 4R 339(79.2 per cent), and 5R 32(0.5 per cent), otherwise, 3R 82(17.5 per cent), 4R 339(72.4 per cent), and 5R 47(10.1 per cent) in obese group. Chi-square reveals that there is no difference for 30 bp promoter VNTR polymorphism of *MAOA* between control and obese groups (x^2=5.2, p=0.3). There is no relationship between MAO-A and eating behavior. Fuemmeler B.F., et al [195] has reported that in male subjects MAOA-u VNTR polymorphism is significantly associated with obesity and BMI. Goldfield G.S., et al [196] has demonstrated that pregnant women who had 4R/4R MAOA

genotype showed excessive gestational weight status. Galvao A., et al [157] reported that in 3-4 years old boys, high activity allele was associated with high-fat and high-carbohydrate food intake. Dias H., et al [197] showed that 3R/3R genotype was significantly associated with total body fat in males. Furthermore, Ducci F., et al [198] reported that low activity allele was relevant to higher BMI.

Table 4.1. The distribution of 30 bp promoter VNTR polymorphism of *MAOA* in diverse populations.

Study	Country	Year	n	Genotype	p-value
Fuemmler B.F., et al [195]	USA	2008	1584	Low activity vs High activity	0.04
Goldfield G.S., et al [196]	Canada	2013	93	High activity vs Low activity	0.03
Galvao A., et al [157]	Brazil	2012	354	High activity vs Low activity	0.03
Dias H., et al [197]	Portugal	2016	535	Low activity vs High activity	0.03
Ducci F., et al [198]	Finland	2006	505	Low activity vs High activity	0.005

MAOA 4R/4R: High activity genotype; MAOA 3R/3R, 3R/3.5R, 3R/4R: Low activity genotypes.

In our study, the genotype frequencies of 40 bp 3'UTR polymorphism of DAT1 VNTR were 7R/7R 41(19.1 per cent), 7R/8R 23(10.7 per cent), 8R/8R 76(35.5 per cent), 7R/9R 2(0.9 per cent), 8R/9R 27(12.6 per cent), 9R/9R 42(19.6 per cent), 8R/12R 1(0.4 per cent), 12R/12R 2(0.9 per cent), and 12R/13R 0(0 per cent) in control group. On the other hand, the frequencies were 7R/7R 49(20.9 per cent), 7R/8R 26(11.1 per cent), 8R/8R 94(40.1 per cent), 7R/9R 3(1.2 per cent), 8R/9R 16(6.8 per cent), 9R/9R 44(18.8 per cent), 8R/12R 0(0

per cent), 12R/12R 1(0.4 per cent), and 12R/13R 1(0.4 per cent) in obese group. The allele frequencies were 7R 107(38.7 per cent), 8R 51(18.4 per cent), 9R 113(40.9 per cent), 12R 5(1.8 per cent), and 13R 0(0 per cent) in control group. Conversely, the allele frequencies were 7R 127(45.3 per cent), 8R 42(15 per cent), 9R 107(38.2 per cent), 12R 3(1 per cent), and 13R 1(0.3 per cent) in obese group. The chi-square value clarifies that there is no relationship between DAT1 VNTR polymorphism and adult obesity (x^2=16.62, p=0.08). There is no association between DAT1 genotypes and eating behavior. In agreement with our study, Goldfield G.S., et al [196] reported that 3' UTR polymorphism of DAT1 VNTR did not affect excessive gestational weight gain and Uzun M., et al [164] found no correlation between them. On the other hand, Agurs-Collins T., et al [163] demonstrated that DAT1 10R/10R genotype was associated with higher amount of palatable food intake in females. Sikora M., et al [94] has reported that in obese females, BMI for S/S genotype was remarkably higher than L/L and L/S genotypes.

Table 4.2. The distribution of 40 bp 3' UTR polymorphism of *DAT1* VNTR in diverse populations.

Study	Country	Year	n	Genotype	p-value
Goldfield G.S., et al [196]	Canada	2013	93	9R/9R, 9R/10R vs 10R/10R	1.00
Uzun M., et al [164]	Turkey	2015	382	9R/9R, 9R/10R vs 10R/10R	1.10
Agurs-Coollins T., et al [163]	USA	2011	1551	Any 9R vs 10R	0.04
Sikora M., et al [94]	Poland	2013	506	S/S vs L/L and L/S	0.03

In this study, the genotype frequencies of *COMT* (rs4680) were Val/Val 104(48.6 per cent), Val/Met 82(38.3 per cent), and Met/Met 28(13.1 per cent) in control group. On the other

side, the frequencies for obese group were Val/Val 163(69.7 per cent), Val/Met 44(18.8 per cent), and Met/Met 27(11.5 per cent). The allele frequencies of control group were Val 290(67.7 per cent) and Met 138(32.3 per cent). The frequencies were Val 370(79 per cent) and Met 98(21 per cent) in obese group. Consequently, the chi-square values explain that *COMT* (rs4680) polymorphism and obesity are related with each other in Turkish population (x^2=23.67, ***p*=0.001**). Val/Met genotype is significantly different in control and obese groups (***p*=0.04**). Wallace D.L., et al [199] has reported that there was no relationship between *COMT* polymorphism and BMI, on the other hand, Val/Val genotype affected unhealthy food preference and amount of food intake. Annerbrink K., et al [158] demonstrated that 51 years old male subjects had an association between Met/Met genotype and higher heart rate, blood pressure, waist circumference, waist-to-hip ratio. Galvao A., et al [157] reported that 3 or 4 years old male subjects who carried Val allele had an correlation with lipid-dense food intake in a larger amounts. On the other hand, Kring S., et al [200] interpreted that *COMT* polymorphism was not associated with obesity in male subjects. Need A.C., et al [201] has reported that there was no relationship between *COMT* polymorphism and BMI, obesity risk or weight.

Table 4.3. The distribution of *COMT* (rs4680) polymorphism in diverse populations.

Study	**Country**	**Year**	**n**	**Genotype**	**p-value**
Wallece D.L., et al [199]	USA	2015	61	Val/Val vs Val/Met and Met/Met	<0.005
Annerbrink K., et al [158]	Sweden	2008	240	Met/Met vs Val/Met and Val/Val	<0.005
Galvao A., et al [157]	Brazil	2012	354	Val allele vs Met allele	0.008
Kring S., et al [200]	Denmark	2009	1557	Val/Val vs Val/Met and Met/Met	0.43
Need A.C., et al [201]	UK	2006	1150	Val/Val vs Val/Met and Met/Met	0.75

In our study, the genotype distribution of *DAT1* (rs27072) were G/G 147(68.7 per cent), G/A 48(22.4 per cent), and A/A 19(8.9 per cent) in healthy subjects. Conversely, the frequencies were G/G 139(59.4 per cent), G/A 53(22.6 per cent), and A/A 42(17.9 per cent) in obese subjects. The allele frequencies for control group were G 342(79.9 per cent) and A 86(20.1 per cent). The allele frequencies were G 331(70.7 per cent) and A 137(29.3 per cent) in obese subjects. Eventually, the chi-square describes that *DAT1* polymorphism has an remarkable association with adult obesity (x^2=8.26, ***p*=0.016**). When obese and control groups are compared, A/A genotype is significantly higher in obese subjects (***p*=0.018**). Unfortunately, **no other studies are available about the relationship between rs27072 and obesity in the literature.**

In this study, the genotype frequencies of *VMAT2* (rs363399) were T/T 150(70.1 per cent), C/T 45(21 per cent), and C/C 19(8.9 per cent) in healthy subjects. On the other hand, for obese group, the genotype frequencies were T/T 179(76.5 per cent), C/T 36(15.4 per cent), and C/C 19(8.1 per cent). The allele frequencies of the control group were T 345(80.6 per cent) and C 83(19.4 per cent). The frequencies of alleles were T 394(84.1 per cent) and C 74(15.9 per cent) in obese individuals. As a consequence, the chi-square values define that there is no association between *VMAT2* polymorphism and obesity (x^2=2.66, p=0.26). Regrettably, there is not another population study about the association between the rs363399 polymorphism and obesity, and **our study is the first one in the literature.**

In this study, the genotype frequencies of *VMAT2* (rs4752045) in control group were C/C 97(45.3 per cent), C/G 94(43.9 per cent), and G/G 23(10.7 per cent). In a similar way, the frequencies of obese subjects were C/C 121(51.7 per cent), C/G 84(35.9 per cent), and G/G 29(12.4 per cent). The allele frequencies of healthy subjects were C 288(67.2 per cent) and G 140(32.8 per cent), in a similar manner, the frequencies were C 326(69.6 per cent) and G 142(30.4 per cent) in obese group. Accordingly, the chi-square values determine that *VMAT2* (rs4752045) polymorphism is not correlated with adult obesity in Turkish population (x^2=3.0, p=0.22). To add more, **our study is the first one** and unfortunately **there is no another population study in the literature.**

When control and obese groups are compared, eating behavior varies and the subjects who ate for reward were significantly higher in obese group. *DAT1* A/A genotype may be a risk factor for the pathogenesis of obesity in adults. *COMT* Val/Met genotype might be

protective against obesity. Our findings suggest that eating for reward may lead to the development of obesity via altered dopamine neurotransmission. It requires larger sample sizes and other population studies in order to understand the mechanism of obesity via DA pathway.

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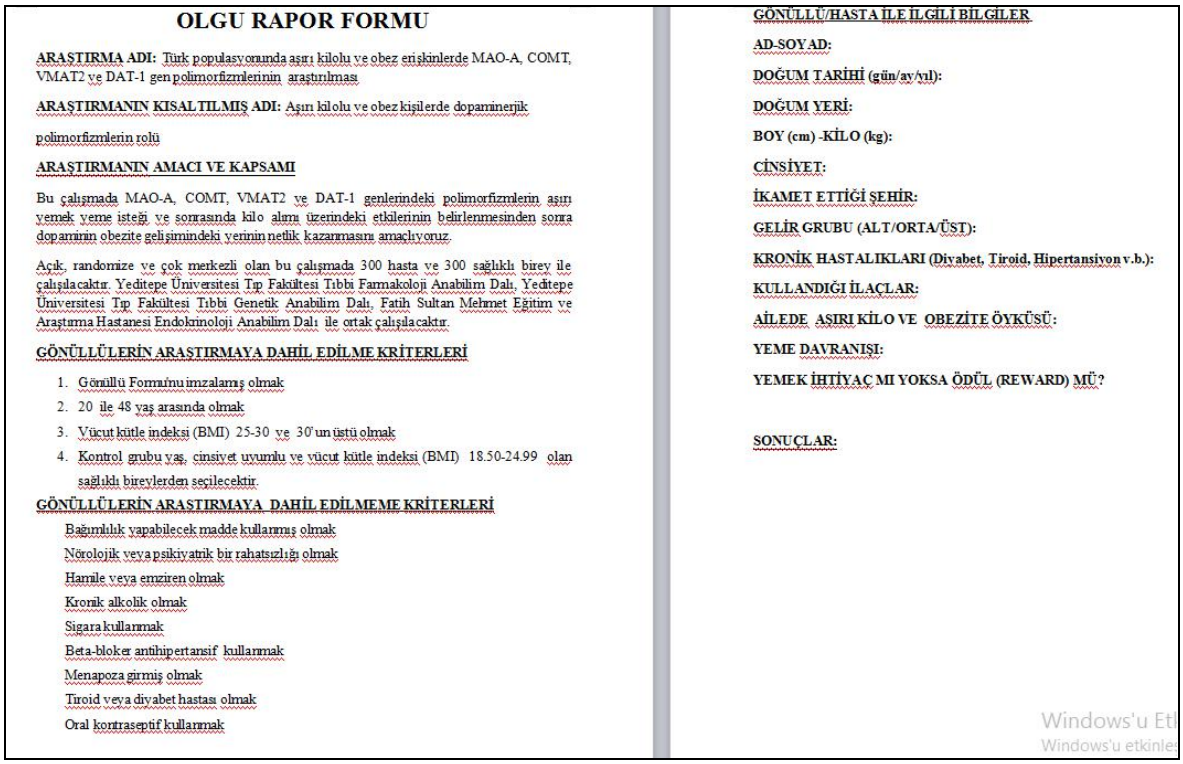
APPENDIX A: CASE REPORT FORM



OLGU RAPOR FORMU

ARAŞTIRMA ADI: Türk populasyonunda aşırı kilolu ve obez erişkinlerde MAO-A, COMT, VMAT2 ve DAT-1 gen polimorfizmlerinin araştırılması

ARAŞTIRMANIN KISALTILMIŞ ADI: Aşırı kilolu ve obez kişilerde dopaminerjik polimorfizmlerin rolü

ARAŞTIRMANIN AMACI VE KAPSAMI

Bu çalışmada MAO-A, COMT, VMAT2 ve DAT-1 genlerindeki polimorfizmlerin aşırı yemek yeme isteği ve sonrasında kilo alımı üzerindeki etkilerinin belirlenmesinden sonra dopaminin obezite gelişimindeki yerinin netlik kazanmasını amaçlıyoruz.

Açık, randomize ve çok merkezli olan bu çalışmada 300 hasta ve 300 sağlıklı birey ile çalışılacaktır. Yeditepe Üniversitesi Tıp Fakültesi Tıbbi Farmakoloji Anabilim Dalı, Yeditepe Üniversitesi Tıp Fakültesi Tıbbi Genetik Anabilim Dalı, Fatih Sultan Mehmet Eğitim ve Araştırma Hastanesi Endokrinoloji Anabilim Dalı ile ortak çalışılacaktır.

GÖNÜLLÜLERİN ARAŞTIRMAYA DAHİL EDİLME KRİTERLERİ

1. Gönüllü Formu'nu imzalamış olmak
2. 20 ile 48 yaş arasında olmak
3. Vücut kütle indeksi (BMI) 25-30 ve 30'un üstü olmak
4. Kontrol grubu yaş, cinsiyet uyumlu ve vücut kütle indeksi (BMI) 18.50-24.99 olan sağlıklı bireylerden seçilecektir.

GÖNÜLLÜLERİN ARAŞTIRMAYA DAHİL EDİLMEME KRİTERLERİ

Bağımlılık yapabilecek madde kullanmış olmak
Nörolojik veya psikiyatrik bir rahatsızlığı olmak
Hamile veya emziren olmak
Kronik alkolik olmak
Sigara kullanmak
Beta-bloker antihipertansif kullanmak
Menapoza girmiş olmak
Tiroid veya diyabet hastası olmak
Oral kontraseptif kullanmak

GÖNÜLLÜ/HASTA İLE İLGİLİ BİLGİLER

AD-SOYAD:

DOĞUM TARİHİ (gün/ay/yıl):

DOĞUM YERİ:

BOY (cm) -KİLO (kg):

CİNSİYET:

İKAMET ETTİĞİ ŞEHİR:

GELİR GRUBU (ALT/ORTA/ÜST):

KRONİK HASTALIKLARI (Diyabet, Tiroid, Hipertansiyon v.b.):

KULLANDIĞI İLAÇLAR:

AİLEDE AŞIRI KİLO VE OBEZİTE ÖYKÜSÜ:

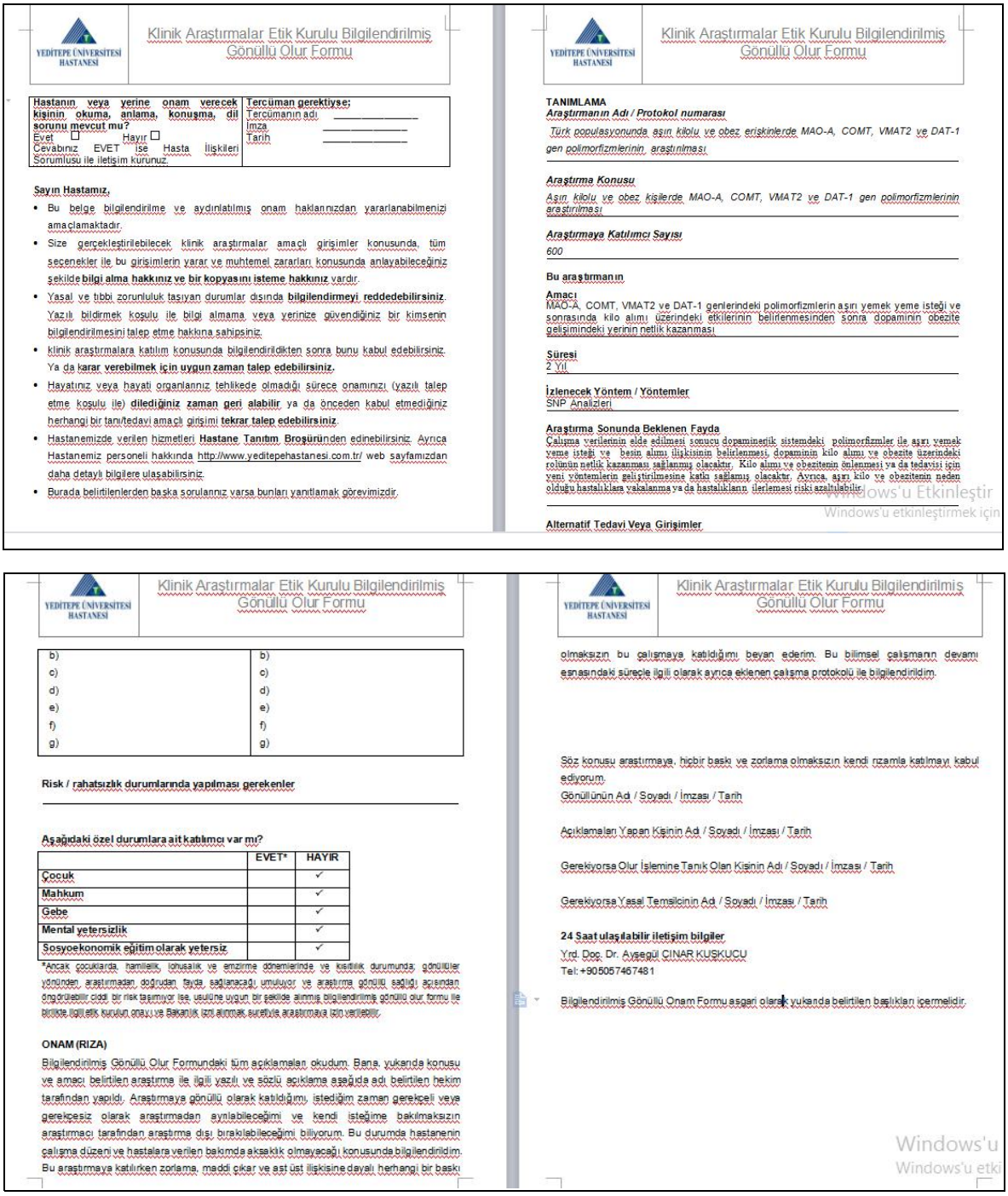
YEME DAVRANIŞI:

YEMEK İHTİYAÇ MI YOKSA ÖDÜL (REWARD) MÜ?

SONUÇLAR:

Figure A.1. Case Report Form.

APPENDIX B: CONSENT FORM



Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

YEDİTEPE ÜNİVERSİTESİ HASTANESİ

Hastanın veya yerine onam verecek kişinin okuma, anlama, konuşma, dil sorunu mevcut mu? Evet ☐ Hayır ☐ Cevabınız EVET ise Hasta İlişkileri Sorumlusu ile iletişim kurunuz.	Tercüman gerektiyse; Tercümanın adı _____ İmza _____ Tarih _____

Sayın Hastamız,

- Bu belge bilgilendirilme ve aydınlatılmış onam haklarınızdan yararlanabilmenizi amaçlamaktadır.
- Size gerçekleştirilebilecek klinik araştırmalar amaçlı girişimler konusunda, tüm seçenekler ile bu girişimlerin yarar ve muhtemel zararları konusunda anlayabileceğiniz şekilde **bilgi alma hakkınız ve bir kopyasını isteme hakkınız** vardır.
- Yasal ve tıbbi zorunluluk taşıyan durumlar dışında **bilgilendirmeyi reddedebilirsiniz**. Yazılı bildirmek koşulu ile bilgi almama veya yerinize güvendiğiniz bir kimsenin bilgilendirilmesini talep etme hakkına sahipsiniz.
- klinik araştırmalara katılım konusunda bilgilendirildikten sonra bunu kabul edebilirsiniz. Ya da **karar verebilmek için uygun zaman talep edebilirsiniz.**
- Hayatınız veya hayati organlarınız tehlikede olmadığı sürece onamınızı (yazılı talep etme koşulu ile) **dilediğiniz zaman geri alabilir**, ya da önceden kabul etmediğiniz herhangi bir tanı/tedavi amaçlı girişimi **tekrar talep edebilirsiniz**.
- Hastanemizde verilen hizmetleri **Hastane Tanıtım Broşüründen** edinebilirsiniz. Ayrıca Hastanemiz personeli hakkında http://www.yeditepehastanesi.com.tr/ web sayfamızdan daha detaylı bilgilere ulasabilirsiniz.
- Burada belirtilenlerden başka sorulanınız varsa bunları yanıtlamak görevimizdir.

Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

YEDİTEPE ÜNİVERSİTESİ HASTANESİ

TANIMLAMA

Araştırmanın Adı / Protokol numarası

Türk populasyonunda aşırı kilolu ve obez erişkinlerde MAO-A, COMT, VMAT2 ve DAT-1 gen polimorfizmlerinin araştırılması

Araştırma Konusu

Aşırı kilolu ve obez kişilerde MAO-A, COMT, VMAT2 ve DAT-1 gen polimorfizmlerinin araştırılması

Araştırmaya Katılımcı Sayısı

600

Bu araştırmanın

Amacı

MAO-A, COMT, VMAT2 ve DAT-1 genlerindeki polimorfizmlerin aşırı yemek yeme isteği ve sonrasında kilo alımı üzerindeki etkilerinin belirlenmesinden sonra dopaminin obezite gelişimindeki yerinin netlik kazanması

Süresi

2 Yıl

İzlenecek Yöntem / Yöntemler

SNP Analizleri

Araştırma Sonunda Beklenen Fayda

Çalışma verilerinin elde edilmesi sonucu dopaminerjik sistemdeki polimorfizmler ile aşırı yemek yeme isteği ve besin alımı ilişkisinin belirlenmesi, dopaminin kilo alımı ve obezite üzerindeki rolünün netlik kazanması sağlanmış olacaktır. Kilo alımı ve obezitenin önlenmesi ya da tedavisi için yeni yöntemlerin geliştirilmesine katkı sağlamış olacaktır. Ayrıca, aşırı kilo ve obezitenin neden olduğu hastalıklara yakalanma ya da hastalıkların ilerlemesi riski azaltılabilir.

Alternatif Tedavi Veya Girişimler

Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

YEDİTEPE ÜNİVERSİTESİ HASTANESİ

b)	b)
c)	c)
d)	d)
e)	e)
f)	f)
g)	g)

Risk / rahatsızlık durumlarında yapılması gerekenler

Aşağıdaki özel durumlara ait katılımcı var mı?

	EVET*	HAYIR
Çocuk		✓
Mahkum		✓
Gebe		✓
Mental yetersizlik		✓
Sosyoekonomik eğitim olarak yetersiz		✓

*Ancak çocuklarda, hamilelik, lohusalık ve emzirme dönemlerinde ve kısıtlılık durumunda; gönüllüler yönünden, araştırmadan doğrudan fayda, sağlanacağı umuluyor ve araştırma gönüllü sağlığı açısından öngörülebilir ciddi bir risk taşımıyor ise, usulüne uygun bir şekilde alınmış bilgilendirilmiş gönüllü olur formu ile birlikte, ilgili etik kurulun onayı ve Bakanlık izni alınmak suretiyle araştırmaya izin verilebilir.

ONAM (RIZA)

Bilgilendirilmiş Gönüllü Olur Formundaki tüm açıklamaları okudum. Bana, yukarıda konusu ve amacı belirtilen araştırma ile ilgili yazılı ve sözlü açıklama aşağıda adı belirtilen hekim tarafından yapıldı. Araştırmaya gönüllü olarak katıldığımı, istediğim zaman gerekçeli veya gerekçesiz olarak araştırmadan ayrılabileceğimi ve kendi isteğime bakılmaksızın araştırmacı tarafından araştırma dışı bırakılabileceğini biliyorum. Bu durumda hastanenin çalışma düzeni ve hastalara verilen bakımda aksaklık olmayacağı konusunda bilgilendirildim. Bu araştırmaya katılırken zorlama, maddi çıkar ve ast üst ilişkisine dayalı herhangi bir baskı

Klinik Araştırmalar Etik Kurulu Bilgilendirilmiş Gönüllü Olur Formu

YEDİTEPE ÜNİVERSİTESİ HASTANESİ

olmaksızın bu çalışmaya katıldığımı beyan ederim. Bu bilimsel çalışmanın devamı esnasındaki süreçle ilgili olarak ayrıca eklenen çalışma protokolü ile bilgilendirildim.

Söz konusu araştırmaya, hiçbir baskı ve zorlama olmaksızın kendi rızamla katılmayı kabul ediyorum.

Gönüllünün Adı / Soyadı / İmzası / Tarih

Açıklamaları Yapan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Olur İşlemine Tanık Olan Kişinin Adı / Soyadı / İmzası / Tarih

Gerekiyorsa Yasal Temsilcinin Adı / Soyadı / İmzası / Tarih

24 Saat ulaşılabilir iletişim bilgiler

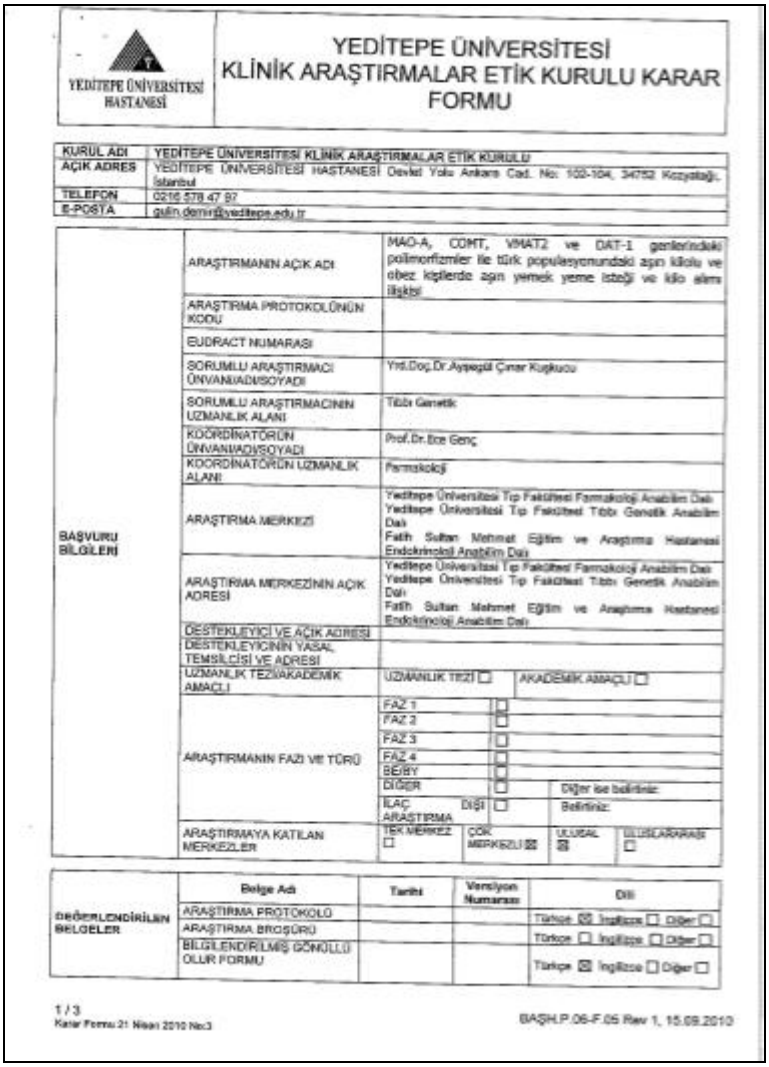
Yrd. Doç. Dr. Ayşegül CINAR KUŞKUCU

Tel: +905057467481

Bilgilendirilmiş Gönüllü Onam Formu asgari olarak yukarıda belirtilen başlıkları içermelidir.

Figure B.1. Consent Form.

APPENDIX C: ETHICS COMMITTEE APPROVAL FORM



YEDİTEPE ÜNİVERSİTESİ HASTANESİ

YEDİTEPE ÜNİVERSİTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU KARAR FORMU

KURUL ADI	YEDİTEPE ÜNİVERSİTESİ KLİNİK ARAŞTIRMALAR ETİK KURULU
AÇIK ADRES	YEDİTEPE ÜNİVERSİTESİ HASTANESİ Devlet Yolu Ankara Cad. No: 102-104, 34752 Kozyatağı, İstanbul
TELEFON	0216 578 47 97
E-POSTA	gulin.demir@yeditepe.edu.tr

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	MAO-A, COMT, VMAT2 ve DAT-1 genlerindeki polimorfizmler ile türk populasyonundaki aşırı kilolu ve obez kişilerde aşırı yemek yeme isteği ve kilo alımı ilişkisi
	ARAŞTIRMA PROTOKOLÜNÜN KODU	
	EUDRACT NUMARASI	
	SORUMLU ARAŞTIRMACI ÜNVANI/AD/SOYADI	Yrd.Doç.Dr.Ayşegül Çınar Kuşkucu
	SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	Tıbbi Genetik
	KOORDİNATÖRÜN ÜNVANI/AD/SOYADI	Prof.Dr.Ece Genç
	KOORDİNATÖRÜN UZMANLIK ALANI	Farmakoloji
	ARAŞTIRMA MERKEZİ	Yeditepe Üniversitesi Tıp Fakültesi Farmakoloji Anabilim Dalı Yeditepe Üniversitesi Tıp Fakültesi Tıbbi Genetik Anabilim Dalı Fatih Sultan Mehmet Eğitim ve Araştırma Hastanesi Endokrinoloji Anabilim Dalı
	ARAŞTIRMA MERKEZİNİN AÇIK ADRESİ	Yeditepe Üniversitesi Tıp Fakültesi Farmakoloji Anabilim Dalı Yeditepe Üniversitesi Tıp Fakültesi Tıbbi Genetik Anabilim Dalı Fatih Sultan Mehmet Eğitim ve Araştırma Hastanesi Endokrinoloji Anabilim Dalı
	DESTEKLEYİCİ VE AÇIK ADRESİ	
	DESTEKLEYİCİNİN YASAL TEMSİLCİSİ VE ADRESİ	
	UZMANLIK TEZİ/AKADEMİK AMAÇLI	UZMANLIK TEZİ ☐ AKADEMİK AMAÇLI ☐
	ARAŞTIRMANIN FAZI VE TÜRÜ	FAZ 1 ☐ FAZ 2 ☐ FAZ 3 ☐ FAZ 4 ☐ BE/BY ☐ DİĞER ☐ Diğer ise belirtiniz: İLAÇ ARAŞTIRMA DIŞI ☐ Belirtiniz:
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☐ ÇOK MERKEZLİ ☒ ULUSAL ☒ ULUSLARARASI ☐

	Belge Adı	Tarihi	Versiyon Numarası	Dili
DEĞERLENDİRİLEN BELGELER	ARAŞTIRMA PROTOKOLÜ			Türkçe ☒ İngilizce ☐ Diğer ☐
	ARAŞTIRMA BROŞÜRÜ			Türkçe ☐ İngilizce ☐ Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU			Türkçe ☒ İngilizce ☐ Diğer ☐

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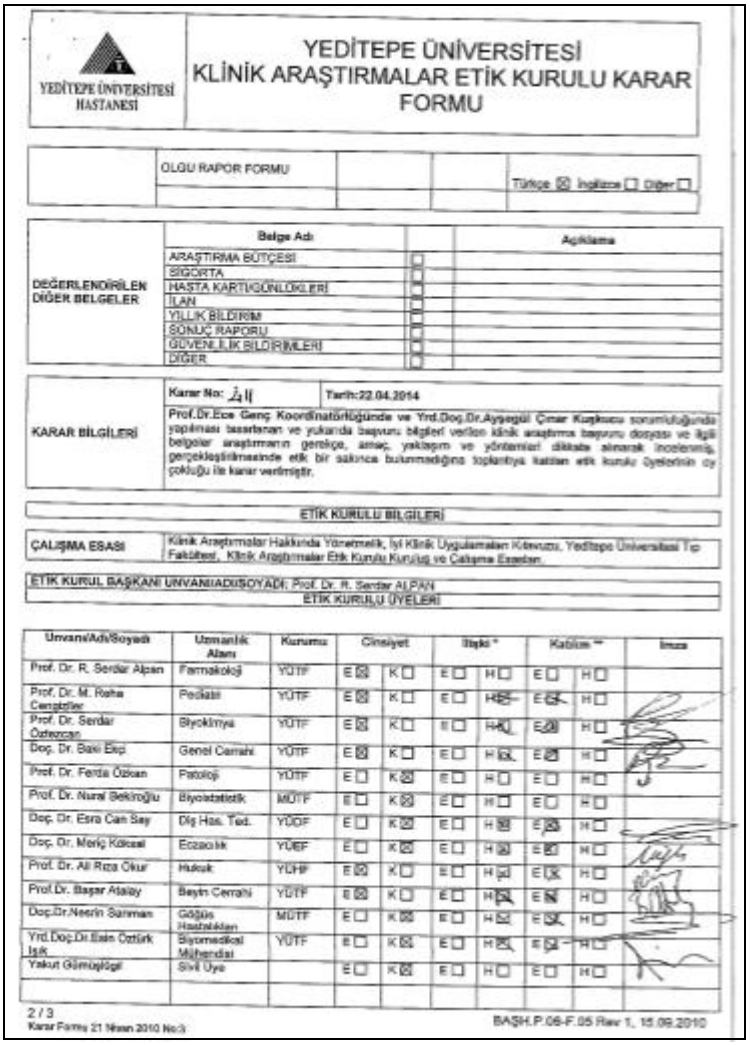
Karar Formu 21 Nisan 2010 No:3

BAŞH.P.06-F.05 Rev 1, 15.09.2010

Figure C.1. Ethics Committe Approval Form.



YEDİTEPE ÜNİVERSİTESİ HASTANESİ

YEDİTEPE ÜNİVERSİTESİ KLİNİK ARAŞTIRMALAR ETİK KURULU KARAR FORMU

	OLGU RAPOR FORMU			Türkçe ☒ İngilizce ☐ Diğer ☐

DEĞERLENDİRİLEN DİĞER BELGELER	Belge Adı		Açıklama
	ARAŞTIRMA BÜTÇESİ	☐	
	SİGORTA	☐	
	HASTA KARTI/GÜNLÜKLERİ	☐	
	İLAN	☐	
	YILLIK BİLDİRİM	☐	
	SONUÇ RAPORU	☐	
	GÜVENLİLİK BİLDİRİMLERİ	☐	
	DİĞER	☐	

KARAR BİLGİLERİ	Karar No: 11	Tarih:22.04.2014
	Prof.Dr.Ece Genç Koordinatörlüğünde ve Yrd.Doç.Dr.Ayşegül Çınar Kuşkucu sorumluluğunda yapılması tasarlanan ve yukarıda başvuru bilgileri verilen klinik araştırma başvuru dosyası ve ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş, gerçekleştirilmesinde etik bir sakınca bulunmadığına toplantıya katılan etik kurulu üyelerinin oy çokluğu ile karar verilmiştir.	

ETİK KURULU BİLGİLERİ

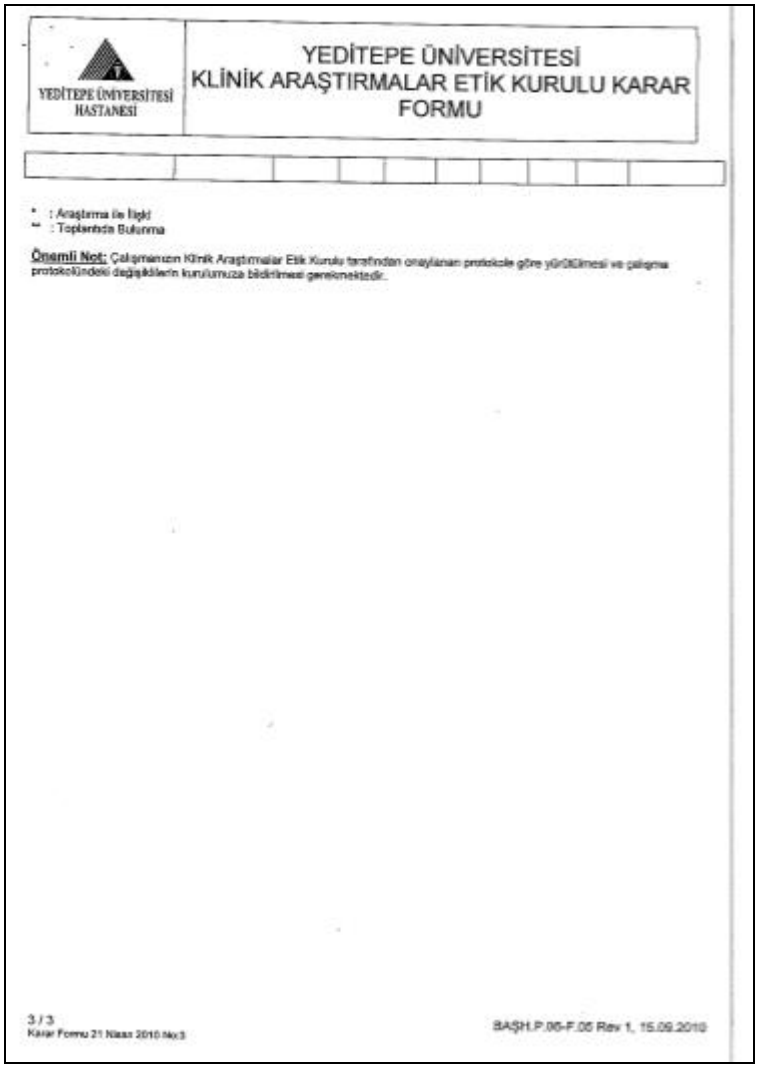
ÇALIŞMA ESASI	Klinik Araştırmalar Hakkında Yönetmelik, İyi Klinik Uygulamaları Kılavuzu, Yeditepe Üniversitesi Tıp Fakültesi, Klinik Araştırmalar Etik Kurulu Kuruluş ve Çalışma Esasları

ETİK KURUL BAŞKANI UNVANI/ADI/SOYADI: Prof. Dr. R. Serdar ALPAN

ETİK KURULU ÜYELERİ

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		İlişki *		Kabilim **		İmza
Prof. Dr. R. Serdar Alpan	Farmakoloji	YÜTF	E ☒	K ☐	E ☐	H ☐	E ☐	H ☐	
Prof. Dr. M. Reha Cengiz	Pediatri	YÜTF	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof. Dr. Serdar Özkorcan	Biyokimya	YÜTF	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Doç. Dr. Baki Ekçi	Genel Cerrahi	YÜTF	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof. Dr. Ferda Özkan	Patoloji	YÜTF	E ☐	K ☒	E ☐	H ☐	E ☐	H ☐	
Prof. Dr. Nural Bekiroğlu	Biyoistatistik	MÜTF	E ☐	K ☒	E ☐	H ☐	E ☐	H ☐	
Doç. Dr. Esra Can Say	Diş Has. Ted.	YÜDF	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Doç. Dr. Meriç Köksal	Eczacılık	YÜEF	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Prof. Dr. Ali Rıza Okur	Hukuk	YÜHF	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Prof.Dr. Başar Atalay	Beyin Cerrahi	YÜTF	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	
Doç.Dr.Nesrin Salman	Göğüs Hastalıkları	MÜTF	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Yrd.Doç.Dr.Esin Öztürk Işık	Biyomedikal Mühendisi	YÜTF	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	
Yakut Gümüşlügil	Sivil Üye		E ☐	K ☒	E ☐	H ☐	E ☐	H ☐	

Figure C.2. Ethics Committe Approval Form.



YEDİTEPE ÜNİVERSİTESİ HASTANESİ

YEDİTEPE ÜNİVERSİTESİ
KLİNİK ARAŞTIRMALAR ETİK KURULU KARAR FORMU

* : Araştırma ile İlgili
** : Toplantıda Bulunma

Önemli Not: Çalışmanızın Klinik Araştırmalar Etik Kurulu tarafından onaylanan protokole göre yürütülmesi ve çalışma protokolündeki değişikliklerin kurulumuza bildirilmesi gerekmektedir.

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Karar Formu 21 Nisan 2010 Rev3

BAŞH.P.06-F.06 Rev 1, 15.09.2010

Figure C.3. Ethics Committe Approval Form.