NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL BIOLOGY AND GENETICS

INVESTIGATION OF PROMOTOR METHYLATION OF TRIM-3 GENE METHYLATION IN OBESITY

M.Sc. THESIS

Buğsem Hülya Öztenekecioğlu

Nicosia
June, 2022

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Supervisor
Prof. Dr. Rasime KALKAN

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Approval

We certify that we have read the thesis submitted by Buğsem Hülya Öztenekecioğlu titled **"Investigation of Promotor Methylation of *TRIM*-3 Gene Methylation in Obesity**" and that in our combined opinion it is fully adequate, in scope, and in quality, as a thesis for the degree of Master of Science.

Examining Committee	Name-Surname	Signature
Head of the Committee:	DOC. DR. PINAR TULAY	……
Committee Member:	YRD. DOC. DR. ÖZEL YÜRÜKER	……
Supervisor:	PROF. DR. RASİME KALKAN	……

Approved by the Head of the Department

16/06/2022

……..

DOC. DR. PINAR TULAY

Head of Department

Approved by the Institute of Graduate Studies

16/06/2022

…….

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

Declaration

I hereby declare that all material, documents, analysis, and findings included in this thesis have been acquired and presented by the academic regulations and ethical principles of the Near East University Institute of Graduate Studies. As required by these rules of conduct, I further declare that I have appropriately credited and referenced any material and data that are not unique to this work.

Buğsem Hülya Öztenekecioğlu

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Abstract

Investigation Of Promotor Methylation Of *TRIM-3* Gene Methylation In Obesity

Buğem Hülya Öztenekecioğlu

MA, Department of Medical Biology and Genetics

Supervisor: Prof. Dr. Rasime Kalkan

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It is extremely important to understand the causes of obesity which refers to an excessive percentage of body fat in the body. Environmental factors, epigenetic factors, and genetic factors play a key role in the pathogenesis of obesity. In this thesis, the *TRIM3* gene has been investigated to help understand the cause of obesity. The objective of this study was to establish a relationship between the methylation status of the *TRIM3* gene and obesity. Statistically significant differences were not observed between obese subjects and nonobese subjects. Based on literature this was the first study that shows the interactions between the *TRIM3* gene and obesity.

Keywords: Obesity, Genetics, Epigenetics, *TRIM 3* gene

Özet

Obezitede *TRIM-3* Gen Promotor Metilasyonunun Araştırılması

Buğsem Hülya Öztenekecioğlu

Yüksek Lisans, Tıbbi Biyoloji Ve Genetik Bilim Dalı

Danışman: Prof. Dr. Rasime Kalkan

Haziran 2022, 53 sayfa

Vücuttaki, aşırı yağ yüzdesini ifade eden obezitenin nedenlerini anlamak son derece önemlidir. Çevresel faktörler, epigenetik faktörler ve genetik faktörler obezite patogenezinde önemli bir rol oynamaktadır. Bu çalışmasında, obezitenin epigenetik değişikliklerini saptayabilmek amacı ile *TRIM3* gen metilasyonu araştırılmıştır. Bu çalışmanın amacı, obezite ile *TRIM3* gen metilasyonu arasındaki ilişkiyi belirlemektir. Çalımamızda *TRIM3* gen metilasyonu açısında obez ve kontrol grubu arasında istatistiksel olarak anlamlı bir farklılık gözlemlenmemiştir. Mevcut literatür bilgisi ile bu çalışma, *TRIM3* gen metilasyonu ve obezite arasındaki ilişkiyi araştıran ilk çalışmadır.

Anahtar Kelimeler: Obezite, Genetik, Epigenetik, *TRIM 3* gen

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List of Abbreviation

DNA: Deoxyribonucleic Acid

RNA: Ribonucleic Acid

CpGs: Cytosine Phosphate Guanine Dinucleotides

DNMTs: DNA Methyltransferases

BMI: Body Mass Index

IL-6: Interleukin 6

TNF-α: Tumor Necrosis Factor-α

MCP-1: Monocyte Chemoattractant Protein-1

T2DM: Type 2 Diabetes Mellitus

CRP: C-reactive protein

LEPR: Leptin Receptor

PCSK1: Proprotein convertase 1

MC4R: Melanocortin 4 Receptor

POMC: Pro-opiomelanocortin

ADRB3: Adrenoceptor Beta 3

BDNF: Brain-Derived Neurotrophic Factor

CNR1: Cannabinoid Receptor 1

PPARG: Peroxisome Proliferator-Activated Receptor

GWAS: Genome-Wide Affiliation Study

FTO: Fat Mass And **Obesity**-Associated

SNPs: Single Nucleotide Polymorphisms

CNV: Copy Number Variations

NEGR1: Neuronal Growth Regulator 1

***PPYR1*:** pancreatic polypeptide receptor 1

AGRP: Agouti-related protein

SH2B1: SH2B Adaptor Protein 1

PHIP: Pleckstrin Homology Domain Interacting Protein

MRAP2: Melanocortin-2 Receptor Accessory Protein 2

SIM1: Single-Minded Homolog 1

IRX3: Iroquois homeobox protein 3

***TMEM18*:** Transmembrane Protein 18

CADM1: Cell Adhesion Molecule 1

CADM2: Cell Adhesion Molecule 2

CpG: cytosine–phosphate–guanine

DNMT: DNA methyltransferase

DNMT1: DNA Methyltransferase 1

DNMT3A: DNA methyltransferase 3 alpha

DNMT3B: DNA (cytosine-5-)-methyltransferase 3 beta

SAM: S-adenosyl methionine

BMAL1: Brain and Muscle ARNTL-Like 1

CLOCK: Circadian Locomoter Output Cycles Protein Kaput

PER2**:** Period Circadian Regulator 2

PPARg: Peroxisome Proliferator-Activated Receptor Gamma

C/EBPa: Ccaat enhancer-binding proteins alpha

H3K4MTs: Histone 3, lysine 4 methyltransferases

MLL: Mixed-Lineage Leukemia

MLL3: Mixed-Lineage Leukemia 3

MLL4: Mixed-Lineage Leukemia 4

H4K20: Histone H4 Lysine 20

H3K27: Histone H3 lysine 27

Ezh2: Enhancer of zeste homolog 2

Wnt: Wingless-related integration site

PWS: Prader-Willi syndrome

BBS: Bardet-Biedl syndrome

BBS1: Bardet-Biedl syndrome 1

BBS10: Bardet-Biedl syndrome 10

LEP: Leptin

TRIM3: tripartite motif-containing 3.

RING: finger proteins

TRIM: tripartite motif proteins,

RBCC: 'RING-B-box-coiled-coil' proteins

RING: Ring finger proteins

mRNA: Messenger RNA

AT: abdominal fat

CSC: Cancer stem cells

shRNA: short hairpin RNA

TRIM13: Tripartite Motif Containing 13

TRIM41: Tripartite Motif Containing 41

TRIM52: Tripartite Motif Containing 52

LOH: loss of allelic heterozygosity

WHO: World Health Organization

CRP: C-Reactive Protein

CSC: Cancer Stem Cell

***UBASH3A*:** ubiquitin associated and SH3 domain containing A

ADIPOQ: *adiponectin Q*

PGC1α: peroxisome proliferator-activated receptor gamma coactivator 1α

IGF-2: Insulin-like growth factor II

RANKL: Receptor Activator of Nuclear Factor Kappa B Ligand

c-FOS: c-fos protein

GLUT4: Glucose transporter type 4

IRS-1: insulin receptor substrate

LY86: Lymphocyte antigen 86

PEG3: paternally expressed gene 3

***NNAT*:** Neuronatin

***PLAGL1*:** *pleomorphic adenoma gene 1*

MEG3: Maternally expressed gene 3

NPY: Neuropeptide Y

***TNF*:** Tumor Necrosis Factor

***TFAM*:** Mitochondrial transcription factor A

***TRIM26*:** Tripartite Motif Containing 26

***TRIM19*:** Tripartite Motif Containing19

***CMYA5*:** Cardiomyopathy associated 5

***TRIM76*:** Tripartite Motif Containing76

CHAPTER I

Introduction

Obesity is a complicated and multifactorial condition which has a severe effect on health. The condition known as obesity is caused by a disparity between the amount of energy that is consumed on a daily basis and the amount of energy that is expended, which ultimately leads to an excessive amount of weight gain. Obesity is a complex condition, which means that it is caused by a wide variety of causes, including genetic, environmental, cultural, and socioeconomic influences. Several genetic investigations have shown that obesity is widely heritable, with many genes having been linked to adiposity and weight gain. Additional factors that may contribute to obesity include a reduced amount of regular activities, sleeplessness, endocrine diseases, drugs, the availability of high-sugar and excessively carbohydrate-rich meals, and a slower rate of energy metabolism (Panuganti et al., 2022).

Epigenetic alterations have an influence on gene expression status without changing the nucleotide sequence. DNA methylation, histone modifications, RNA modifications, and noncoding RNAs are examples of epigenetic alterations. Epigenetic modifications are affected by both environmental and genetic factors. Several enzymes regulate epigenetic modifications, such as DNA methyltransferases, DNA demethylases, lysine methyltransferases, and demethylases. The reversible nature of epigenetic changes is critical, and this makes them a possible target during disease assessment (Aykac and Kalkan, 2022).

The tripartite motif protein family is one of the most abundant groups of potential RING finger E3 ubiquitin ligases. The acronym *TRIM3* stands for tripartite motif-containing 3. The *TRIM3* gene is found at 11p15.4. *TRIM3* is a protein-coding gene. The RING finger proteins produced by this gene are members of the tripartite motif proteins family, often known as 'RING-B-box-coiled-coil' proteins (NCBI, 2022).

TRIM proteins regulate pluripotency, cellular proliferation, cell signaling, transcription, and repair of DNA. TRIM protein variations may cause clinical and physiological disorders. There are several ways in which TRIM proteins' physiological activities might be compromised. We need to understand how amino acid changes in TRIM proteins affect their respective association pathways and target specificities. Researchers found that *TRIM3* expression was higher in schizophrenia

subjects' postmortem dorsolateral prefrontal brain specimens compared with healthy subjects (Watanabe et al., 2016).

A study of genome-wide methylation in peripheral blood leukocytes discovered that a CpG location in the *TRIM3* gene has lower methylation levels in obesity (Wang et al., 2010).

The aim of studying the *TRIM*-3 gene methylation is to:

(a) determine the methylation level of the *TRIM-3* gene

(b) investigate the effect of methylation pattern of *TRIM-3* gene on obesity

(c) identify the methylation differences of the *TRIM-3* gene in obese subjects and control subjects

Given the study's purpose of investigating the relationship between methylation of the *TRIM-3* gene and obesity, the research questions are:

(a) is there any relationship between the methylation status of the *TRIM-3* gene and obesity

(b) is there any relationship between the methylation status of the *TRIM-3* gene and the anthropometric and metabolic characteristics

This thesis summarizes the global trends in obesity with a special focus on the epigenetic alterations of the *TRIM3* gene in obesity. In literature, the correlation of the methylation pattern of the *TRIM*-3 gene with anthropometric and metabolic characteristics has not been shown yet. This study will be the first to show the relationship between the methylation status of the *TRIM*-3 gene with obesity.

CHAPTER II

General Information

2.1. Obesity

The World Health Organization (WHO) predicts that the problem has reached epidemic proportions, with 4 million deaths caused by obesity each year according to the global burden of disease report, published in the previous years. Increasing rates of overweight and obesity are observed in both children and adults. In the period from 1975 to 2016, the incidence of overweight and obesity among children and adolescents aged five to nineteen years has risen from the percentage of four to eighteen globally (WHO, 2021). More than one billion individuals throughout the world are affected by obesity today, with six hundred fifty million aged people, three hundred forty million teenagers, and thirty-nine million children falling into this group. This number continues to rise. The World Health Organization is highlighting the global obesity crisis in several aspects. The crisis involves tracking overall trends and prevalence, establishing a huge variety of requirements for the management and protection of obesity and overweight, as well as counseling and assisting nations with implementation (WHO, 2022). Obesity is a condition that affects almost all body systems. Since it has a negative impact on the cardiovascular systems and reproductive systems (Cafasso, 2022). Obesity is connected with a wide range of noncommunicable diseases, namely type 2 diabetes, cardiovascular disease due to the effect of cardiovascular systems, hypertension, strokes, and different types of cancer. Obese subjects also have a threefold higher risk of being hospitalized with COVID-19 (Kompaniyets et al, 2021).

Obesity is described as the accretion of excessive adipose tissue in the body, in another word, more than 25 kg/m2 of the body mass ratio is represented as overweight. Method for diagnosing obesity, known as Body Mass Index (BMI) is frequently utilized. BMI is estimated by multiplying the current body weight by seven hundred three, dividing by height in inches, and afterward dividing by once again height in inches. To sum up, dividing body weight in kilos by height in inches' square (Panuganti et al., 2021). The following BMI values may be estimated based on a person's height and weight: To be considered underweight, a person's BMI must be less than 18.5, but the BMI range of 18.5-24.9 is considered normal. A BMI between twenty-five and twenty-nine indicates overweight, whereas a BMI between 30 and 39.9 indicates obesity. A body mass index (BMI) of more than forty indicates

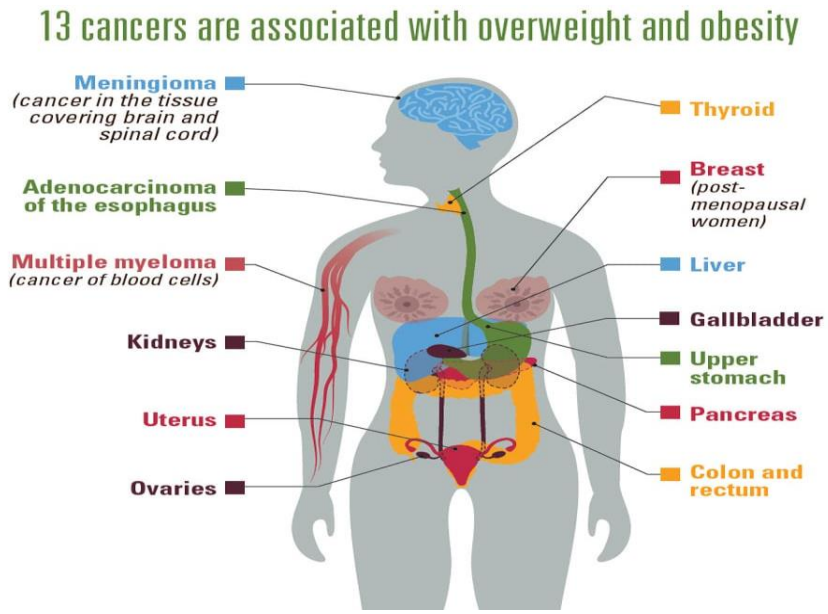
serious obesity (CDC, 2022). Persistently low levels of inflammation and an increase in inflammatory biomarkers are two of the characteristics of obesity (Karczewski et al 2019). In obese subjects, the process of chronic inflammation takes place more often, despite the fact that obesity is a component of metabolic instability. Chronic inflammation is frequently associated with metabolic syndrome because it is characterized by the release of inflammation adipocytes from adipose tissues, namely interleukin 6 (IL-6), resistin, monocyte chemoattractant protein-1 (MCP-1) tumor necrosis factor- (TNF-), and leptin. Therefore, inflammation has thought to be the source of the association between them (Ellulu et al., 2017). It is strongly connected with alterations in the physiological role of adipose tissue, which leads to dysregulation, resulting in elevated systemic levels of proinflammatory cytokines such as TNFα and IL-6. Secretion of pro-inflammatory cytokines will increase inflammation levels in the body, this action demonstrates that raising the risk of various chronic diseases (Kany et al., 2019, Karczewski et al., 2018). The overexpression of pro-inflammatory factors in obesity correlates with obesity and inflammation. Though the biological conditions, immune cells, and adipocytes are heterogeneous and react to extra nutrient stimulation through adipocyte hyperplasia and hypertrophy. Since necrosis and macrophage infiltration have a triggering effect on adipose tissue, hypoxia leads to the overproduction of pro-inflammatory mediators. This results in adipose tissue of regionalized inflammation which spreads throughout the body and leads to the development of obesity-related pathologies (Ellulu et al., 2017). Macrophages, which are components of adipose tissue, actively participate in their activities. Crosstalk with lymphocytes and adipocytes leads to immune regulation. Many various pro-inflammatory and anti-inflammatory factors are produced and released by adipose tissue namely leptin, adiponectin, and resistin, among with cytokines, chemokines, and TNF- α (Ellulu et al., 2017). Adipocytes are particularly crucial in this process as they sense energy storage in a cell-autonomous manner and produce hormones namely leptin to create a feedback loop that stimulates the sympathetic nervous system and lowers food intake (Karczewski et al., 2018). Adiponectin is a protein hormone generated from adipocytes that have gained prominence owing to its beneficial effects on inflammation, type 2 diabetes mellitus (T2DM), and insulin resistance; hence, it connects the adipose tissue directly with the foundations of metabolic disorders. Adiponectin enhances insulin sensitivity, reduces gluconeogenesis, and promotes oxidation in the liver. It is particularly

effective in controlling weight gain and increasing energy expenditure (Ellulu et al., 2017). Obesity is defined by excessive adiposity as a direct consequence of a sustained, positive energy balance. It is usually linked with a low-grade inflammatory state, with reduced leukocyte function and infiltration of nucleated blood cells at the location of insulin-sensitive organs (Zierath & Barrès, 2011). Obesity enhances insulin resistance. As a result, blood glucose levels will rise in the body, and insulin which is a hormone, causes to absorption of carbohydrates, resulting in low blood glucose levels (Wondmkun, 2020). A substantial association was found between obesity and high blood CRP (C-Reactive Protein) levels in both adults and children, which may be explained by the pathophysiological process linking the two conditions. The liver plays a crucial role in inflammation because it removes free fatty acids and circulating triacylglycerol, which causes adipose tissue to produce cytokines (IL-6), which in turn enhances hepatocyte expression and CRP production (Ellulu et al.,2017). In conclusion, obesity corresponds to an inflammatory state due to the increase in inflammatory cytokines, such as IL-6 and TNF-, and the decrease in the concentration of adiponectin, a potent anti-inflammatory hormone. According to Ellulu et al., TNF- is overexpressed in a condition of overweight, but IL-6 correlates closer to a state of obesity owing to the liver's role in synthesizing and secreting CRP, a marker of systemic inflammation (Ellulu et al.,2017). Reduced levels of adiponectin are associated with this condition, which is necessary for reducing metabolic abnormalities, improving insulin sensitivity, and adjusting energy expenditure (Vachharajani & Granger, 2009). There is significant evidence that higher levels of body fat are linked to an increased risk of diseases and a variety of malignancies such as ovarian cancer, endometrial cancer, and so on (Figure 1) (NCI, 2022). Genetic factors contribute up to seventy percent of obesity risk and can vary between individuals (Golden & Kessler, 2020). Several genes influence the regulation of appetite, food consumption, metabolism, body fat distribution, and body mass index (BMI) (Kalkan & Becer, 2019, Golden & Kessler, 2020).

Figure 1

Several cancers are associated with overweight and obesity (NCI, 2022).

Figure 1 (Continued)



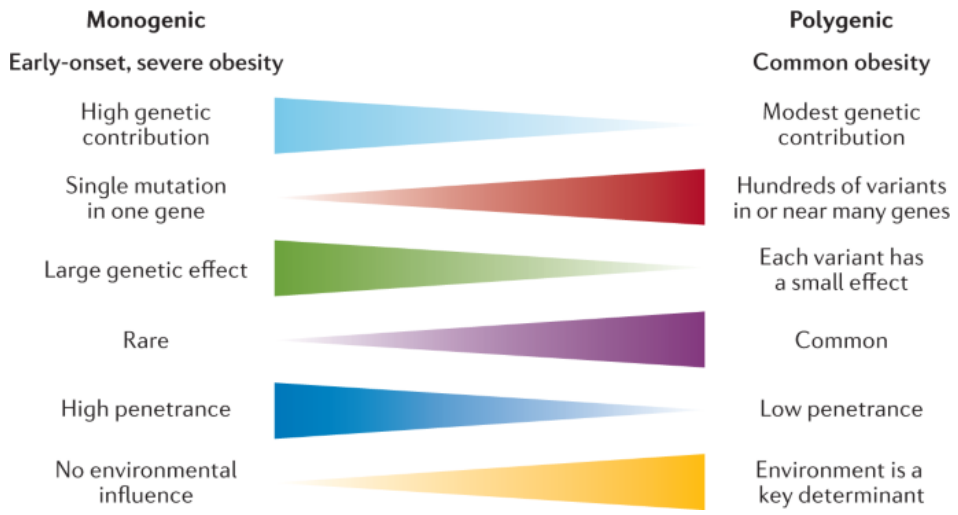
2.2. Genetic Factors

Genetic factors play a crucial role in obesity (García-Giménez, 2016). Obesity has usually been put into two broad groups: monogenic obesity, generally, has passed down through the Mendelian inheritance and has usually extreme, unusual, and starts early. Single-gene mutations or moderate or large chromosomal deletions are the underlying cause of monogenic obesity. The inheritance pattern of polygenic obesity is similar to that of other complex diseases. Studies indicate that an individual's polygenic obesity tendency may influence the development of mutations that contribute to monogenic obesity (Figure 2) (Loos & Yeo, 2021).

Figure 2

Significant aspects of the monogenic and polygenic forms of obesity (Loos & Yeo 2021).

Figure 2 (Continued)



Monogenic obesity is an uncommon form of early-onset obesity caused by spontaneous mutations in single genes, that are predominantly linked with the leptin or melanocortin axis, a key hypothalamus appetite regulation region that influences food-seeking and caloric intake (Huvenne et al., 2016). Syndromic monogenetic obesity is distinguished by extreme obesity that starts in early childhood with shows some phenotypic features such as cognitive impairments, different organ specific congenital malformations, consumption problems, typically hyperphagia, and dysmorphic features. Due to their prevalence in childhood, adolescents with excessive obesity and hyperphagia might be investigated for single-gene forms of obesity (Golden & Kessler, 2020). Leptin and its receptor genes were identified and these were considered candidate genes for human obesity, resulting in the identification of the first patients with congenital leptin deficiency. The majority of monogenic obesity mutations have been discovered in groups of individuals with severe and early-onset obesity (Dubern & Clement, 2012). In individuals with severe early-onset obesity, mutations in genes that encode the leptin receptor (LEPR) and numerous constituents of the melanocortin pathway, such as *PCSK1*, *MC4R*, and *POMC*, were determined (Yeo et al., 2021). Monogenic obesity often shows a recessive hereditary profile, and consanguinity within groups has enhanced the likelihood of identifying variants, given the larger deleterious variant's homozygosity. Researchers showed variations in genes containing *LEPR*, *MC4R*, and leptin responsible for thirty percent of severe obesity cases in Pakistani children of consanguineous parents and fifty percent of severe obesity cases

generally (Loos & Yeo, 2021). As the most common kind of obesity, polygenic obesity combines genetic predisposition with environmental factors such as caloric excess or sleep deprivation, making it the most complex form of obesity (Golden & Kessler, 2020). Common variations in candidate genes were investigated for their potential relationship through obesity incidence, BMI, or various body compositional features. Variants in candidate genes such as *ADRB3*, *BDNF*, *CNR1*, *MC4R*, *PCSK1*, and *PPARG* were shown to have a reproducible relationship with obesity outcomes (Loos and Yeo, 2021). The findings of the initial genome-wide association research for obesity traits found a cluster of common variations in the first intron of the *FTO* gene that was strongly related to BMI (Loos and Yeo, 2021). The *FTO* gene, which is found on chromosome 16, contains several variations that are thought to regulate and respond significantly to food intake versus energy consumption. The *MC4R* gene which is located on chromosome 18 assumes an administrative part in food consumption and energy balance. On the obese phenotype, it was proven that a common polymorphism of the *MC4R* gene had a synergistic influence with a variation of the *FTO* gene (Golden and Kessler, 2020). Subsequent genome-wide association studies (GWAS) and meta-analyses have identified over one hundred genes and single nucleotide polymorphisms related to body mass index (BMI) and obesity. These findings include genes that either increase or reduce the likelihood of becoming overweight (Golden and Kessler, 2020). GWAS research was able to identify hundreds of genes and genetic variations, most of which were SNPs, that are related to complicated human disorders (Huang, 2015, Voisin et al., 2015). Moreover, several research studies suggest that genomic copy number variants (CNV) may have especially strong effects on the severity and prevalence of obesity (Golden and Kessler, 2020). The genome-wide association research has also been used to evaluate the influence of copy number variants in obesity. These variations show a substantial link with body mass index. For instance, only a few CNVs have been identified, these CNVs include; deletion of the short arm of the chromosome 1, *NEGR1*, which is responsible for the encoding of cell adhesion molecules expressed in the brain; deletion of the short arm of the chromosome 16, which regulates insulin secretion and the 1p21.1 multi-allele CNV, which generates salivary -amylase, a crucial enzyme in the starch breakdown (Loos and Yeo, 2021). The risk of obesity may be attributed, in part, to genetic factors, which can account for up to seventy percent of the risk (Golden and Kessler, 2020). The control of hunger, food intake,

metabolism, the distribution of body fat, and body mass index are all influenced by several genes (Golden and Kessler, 2020, Kalkan and Becer, 2019). The single gene abnormalities can cause severe early-onset obesity namely *SIM, MRAP2*, *SH2B1, AGRP, PCSK1, MC4R, POMC, PHIP,* and *LEPR*. Single-gene abnormalities in *MC4R* are the most common cause of obesity and hyperphagia. It not only regulates food intake but also affects food preference, with those who have *MC4R* mutations preferring foods with higher fat content (Loos and Yeo, 2021). For instance, leptin deficiency is one of the inherited factors that might lead to obesity. Leptin is a hormone that circulates throughout the body and is mostly secreted by adipocytes. It helps to maintain energy balance by reducing feelings of hunger by activating leptin receptors in the brain (Lee et al., 2018). Leptin is a key hormone that is secreted by adipocytes and circulates in levels that are directly proportional to fat mass. Additionally, leptin is able to react to sudden shifts in energy status due to the fact that leptin levels go down during calorie restriction and then back up during refeeding (Loos and Yeo, 2021). The *LEPR* gene encodes the information necessary for the body to produce the leptin receptor, a particular protein. This protein serves a crucial function in regulating body weight (Rhythm, 2021). Several investigations found that leptin gene expression corresponded to leptin promoter DNA methylation patterns, implying that genetic variations influenced methylation percentages and, as a result, gene expression regulation (Lee et al. 2018). *FTO* gene variations influence satiety, increase food intake, and raise BMI. Variations in the *FTO* gene on chromosome 16 have been linked to both an increased risk of obesity and T2DM. *FTO*, which is predominantly expressed in the hypothalamus, is important for energy balance and food intake control (Nagrani et al., 2020). *FTO* has an essential function of controlling metabolism through modulating the expression of genes in metabolic active tissues. Genetic variations in the *FTO* gene, which suggest that *FTO* variants have a connection factor among the obesity-linked diseases, are functionally associated with an additional obesity-linked gene designated as *IRX3* (Nagrani et al., 2020). Furthermore, GWAS studies with functional investigations are located upstream of *CADM1* and *CADM2* genes, which generate cellular adhesion proteins that regulate synaptic formation towards the central nervous system. Upregulation of the *CADM1* and *CADM2* in the hypothalamus are linked to BMI-increasing alleles at each location. In a mice study, *Cadm1* or *Cadm2* deficiency resulted in decreased body weightt as well as higher insulin resistance, glucose tolerance, and energy

expended without affecting food intake (Loos and Yeo, 2021). To summarize, functional follow-up investigations for these genes are gradually extending our understanding of the pathophysiology that promotes weight gain.

2.3. Epigenetics

Epigenetics is a rapidly growing field in human and medical genetics, with significant implications on gene expression and phenotype in both healthy persons and a broad spectrum of diseases, such as obesity (Kalkan and Becer 2019), imprinting disorders (Butler, 2011) and cancer (Lu et al., 2020). The term "epigenetic" refers to heritable or acquired changes in gene expression that do not result from changes in the DNA sequence. Epigenetic changes are crucial since they can modify gene expression levels without causing any changes or variations in genomic DNA (Handy et al., 2011). The main four processes take place in epigenetics namely RNA modifications, DNA methylation, RNA interference, and histone modifications. They have linked processes that play an essential part in gene expression and transcription levels (Ling and Rönn, 2019). Generally, epigenetic modifications have direct attention to both mitotically and meiotically heritable alterations through the gene expression without affecting the DNA sequence (Felsenfeld, 2014).

2.4. DNA Methylation

DNA methylation is the process of attaching methyl groups to DNA. As soon as methyl groups are placed on a gene, it is switched off or silenced, which prevents the production of proteins. DNA methylation often takes place in sections of the genome with a maximum concentration of cytosine–phosphate–guanine dinucleotides known as CpG islands, which results in the suppressing of both coding and non-coding genes (Moore et al., 2013). The enzyme involved in the addition of methyl groups is called DNA methyltransferase (DNMT). In mammals, three well-known DNA methyltransferases, namely DNMT3A DNMT3B, and, DNMT1 perform the genomic methylation process. S-adenosyl methionine is a methyl group that can be transferred to DNA via DNMT1, DNMT3a, and DNMT3b. In the methylation process, DNMT1, DNMT3A, and DNMT3B have various functions. DNMT1 is required for the maintenance of full genome-wide methylation. During reproduction, DNMT1 restored a specific methylation pattern on the daughter strand

that matched the pattern of the parental DNA. Nonetheless, DNMT1 has been shown in vivo to exhibit *de novo* DNA methylation activity. The *de novo* methyltransferases DNMT3A and DNMT3B are involved in DNA methylation patterns from embryogenesis as well as the production of genomic imprints throughout germ cell development (Aydin and Kalkan, 2020). *De novo* methylation arises in embryogenesis and is sustained via the DNA methylation inheritance mechanism through DNA replication and cellular division (Hervouet et al., 2018). DNMT3A and DNMT3B production reduce with cell differentiation, despite being highly expressed in the earliest mammalian embryos. Those protein molecules have unique activities during embryogenesis, with variations in both temporal and spatial domains. DNMT3A predominantly methylates several genes and sequences in the late stages of embryonic development, particularly upon birth, however, DNMT3B affects a broader range of sequenced genomes in early embryos (Zhang and Xu, 2017). Hypermethylation and oncogenic activation are caused by the high expression of DNMTs, including DNMT1, DNMT3A, and DNMT3B, in a wide range of malignancies. In solid tumors, DNMT1 overexpression is associated with abnormal DNA methylation (Zhang and Xu, 2017). On the other hand, given the epigenetic control of gene expression in cellular activities, it is likely that alterations in DNA methylation led to the dysregulation of essential metabolic processes and raised the susceptibility to develop obesity and associated comorbidities (Samblas et al., 2019). Regarding the epigenetic regulation of gene expression in cell functions, alterations in DNA methylation are likely to have led to the dysregulation of essential metabolic pathways and enhanced vulnerability to obesity and associated consequences (Ouni and Schürmann, 2020). Multifactorial disorders are produced by the impacts and complicated interactions of numerous susceptibility genes, each of which has a minor influence, as well as environmental and epigenetic variables. Susceptibility genes are linked to a higher chance of developing the disorder. The detection of alterations in methylation patterns of certain genes has proven useful in predicting the onset of obesity and avoiding its effects. Multifactorial diseases, develop as a result of interactions between one or more genes and environmental variables. Polygenic diseases are those that are caused by the interactions of numerous genes but do not take into account the influence of environmental circumstances. The circadian clock system, which is primarily governed by epigenetic processes, regulates gene expression in practically all cells, including adipocytes. A review examined the

impact of weight and metabolic disorder highlights in clock quality methylation namely *CLOCK*, *BMAL1*, and *PER2* qualities (García-Giménez, 2016). Many of these genes' activities are believed to be controlled by external signals, hence epigenetic alterations in disease genes created by upstream signaling pathways are likely to be crucial in obesity. A cascade of adipogenic transcription factors interacts with each other and external cues during differentiation. It is crucial that the transcription factors PPARg and CCAAT enhancer-binding proteins alpha (C/EBPa) work together to promote the development of an adipocyte. Overall, they trigger hundreds of genes that are essential for adipocyte development (Youngson and Morris, 2013). Mixed-lineage leukemia (MLL) proteins MLL3 and MLL4, and their corresponding complex play an essential role in the adipogenesis process via the activation of the transcription factors PPARg and C/EBPa. According to recent research, the PPARg pathway is also associated with an increase in transcription of the histone H4 Lysine 20 (H4K20) monomethyltransferase, which in response promotes PPARg activity and activation of PPARg-targets to promote adipogenesis. Adipogenesis is inhibited by signaling pathways of the Wnt family and the b-catenin family, which is muted in pre-adipocytes according to the repressive histone mark H3K27 methylation. Activating the Wnt/b-catenin signaling, by deleting the histone methyltransferase, Ezh2, depresses Wnt genes, thus preventing the transcription of PPARg and C/EBPa (Youngson and Morris, 2013, Park et al., 2021). Weight gain may be caused by underlying medical conditions including Cushing's syndrome, which is an overproduction of steroids, and hypothyroidism, which is a deficiency of thyroid hormones (NHS, 2019). Bardet-Biedl syndrome is usually inherited in an autosomal recessive pattern. It is caused by biallelic loss-of-function pathogenic variations in at least twenty-six genes namely mutations in the *BBS1* and mutations in the *BBS10* gene (Forsyth and Gunay-Aygun, 2020). BBS can manifest with a variety of symptoms, including early-onset obesity and hyperphagia. Hyperphagia refers to an insatiable hunger, also described as an intense, difficult to control hunger (Rhythm, 2021). Although obesity is frequently a prominent symptom of these disorders, the underlying genetic alterations are frequently chromosomal abnormalities that include several genes, making it difficult to determine the particular processes directly connected to bodyweight control (Loos and Yeo, 2021). Gene function and metabolism may be altered by changes in epigenetic pathways throughout a person's lifetime due to long-term epigenetic changes.

Environmental and lifestyle variables, such as nutrition and physical exercise, are thought to impact epigenetic regulation. The association between lifestyle and disease risk may thus be explained by epigenetics. Various processes may be implicated in the control of epigenetic machinery when these lifestyle variables are utilized. Modifications in DNA methylation have been discovered to affect healthy aging and induce age-related health issues. According to accumulating data, changes in lifestyle, notably weight reduction, may affect the methylation of DNA and the expression of genes. DNA methylation, which preserves the memory of the metabolic state, is one of the processes that may influence the disease's appearance. The methylation of DNA causes changes in chromatin structure, which in turn lead to metabolic alterations associated with metabolic memory (Alegría-Torres et al., 2011). Environmental influences can produce the unique epigenetic profile associated with obesity on somatic cells, whereas multiple studies show familial environmental overexposure might enhance the epigenetic transgenerational inheritance linked with obesity by germline gene expression changes. Principally, studies had examined altered DNA methylation of potential gene expression among offspring according to a maternal or paternal variable (van Dijk et al., 2015). During pregnancy, certain nutrients namely methyl donors and vitamins, as well as a high or a low level of dietary protein diet, can be influenced by the epigenetic process implicated through the obesity etiology and metabolic syndromes in the child (Mahmoud, 2022). According to genes involved in insulin metabolism, inflammation, and DNA methylation, obesity features and DNA methylation patterns differed considerably (García-Giménez, 2016).

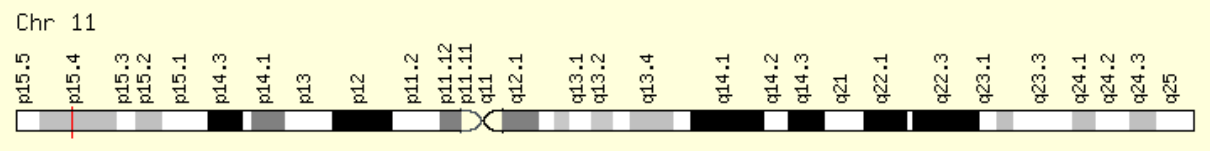
2.5. *TRIM3* Gene

TRIM proteins are a vast, varied, and ancient protein family which is active in a multitude of situations namely cellular differentiation, autophagy, apoptosis, DNA repair, and tumor suppression (Williams et al., 2019). There are around eighty TRIM proteins found in humans that are engaged in several physiological processes, namely apoptosis, cell cycle regulation, viral response, cell proliferation, oncogenesis, and antiviral defense. Their change consistently leads to a wide range of pathological diseases, along with cardiovascular, neurological, immunological, musculoskeletal, developmental, and cancer-related issues (Venuto and Merla, 2019). Furthermore, numerous members of the family play important roles in

neuronal development, with specialized involvement in neurite formation, axon guidance, and polarization. The *TRIM3* subfamily is one of the members of these families (Williams et al., 2019). *TRIM3* is an abbreviation for tripartite motif-containing 3. The *TRIM3* gene is located at 11p15.4. (Figure 3). *TRIM3* is a gene that codes for a protein. RING finger proteins expressed by *TRIM3* belong to the protein family called tripartite motif proteins (TRIM), commonly known as RING-B-box-coiled-coil proteins (RBCC). Three zinc-binding domains make up the TRIM motif, including a RING, a B-box type 1, and a B-box type 2, as well as a coiled-coil region. TRIM proteins take place in the cytoplasmic filaments (NCBI The National Center for Biotechnology Information, 2022).

Figure 3

Localization of the TRIM3 gene (NCBI The National Center for Biotechnology Information, 2022).



The tripartite motif protein family is among the most numerous several other families of E3 RING finger protein ubiquitin ligases which have been implicated in particular protein functions. Ubiquitination has one main characteristic that controls a variety of biological activities namely certain signal transmission, protein integrity regulation, cell death, transcription, cell cycle, and differentation, which is a posttranslational alteration. In addition to its function as a regulator for a wide variety of signaling pathways, the ubiquitin system's primary responsibility is the degradation of proteins that are its targets (Zhao et al., May 2021). Numerous enzymes, besides E1, E2, and E3, are required for the biochemical reaction of ubiquitination, and E3 ubiquitin ligases operate as receptors for identifying the specific proteins. The great preponderance of proteins possessing a tripartite motif is E3 ubiquitin ligases. TRIM proteins have a different effect on each disease, including obesity, cancer, bacterial or viral infections, autoimmune diseases,

developmental disorders, neuropsychiatric disorders, and congenital anomalies (Watanabe & Hatakeyama, 2016, Zhao et al., May 2021). It has been found that mutations and changes in intronic and intergenic domains are strongly associated with phenotypic changes by altering transcription, splicing, mRNA export, and translation of the TRIM proteins. Variants that alter amino acid sequences, or non-synonymous variants, have several opportunities to disrupt the physiological functions of TRIM proteins (Watanabe and Hatakeyama, 2017). TRIM family proteins are involved in numerous physiological functions, namely cell proliferation, DNA repair, pluripotency, signal transduction, and transcription. According to the physiological functions, the participation of TRIM proteins is associated with many other diseases namely genetic defects, neuropsychiatric disorders, developmental diseases, coronary heart diseases, and pathogenic diseases (Watanabe and Hatakeyama, 2016). The variety of malignancies linked with abnormal TRIM protein expression or activity mirrors the diversity of TRIM protein-mediated cellular proliferation and differentiation processes. The TRIM subgroup has been demonstrated to have the oncogenic capability and to be overexpressed in malignant cells, while the others suggest that the expression is related to the loss of tumor-suppressor function (Vunjak and Versteeg, 2019). A potential role in tumor suppression appears to be associated with *TRIM3*, which is a loss of allelic heterozygosity gene. As a result, the analysis of allelic loss of heterozygosity in tumor DNA enables the indication and localization of a minimally lost region that might correspond to a tumor suppressor gene locus involved in tumor formation. The chromosome segment 11p15 displays a common loss of allelic heterozygosity in several different types of adolescent tumors, including those related to the lungs, esophagus, breast, stomach, brain, ovary, and other types of body. (Boulay et al., 2009). On the other hand, TRIMs, such as *TRIM3*, exert a tumor-suppressing effect by inhibiting the proliferation and migration of cells as well as increasing apoptosis (Venuto and Merla, 2019). The expression of *TRIM3* has been linked to the etiology of obesity. An investigation of genome-wide methylation analyzing peripheral blood leukocytes indicated that a CpG site in the *TRIM3* gene exhibits reduced methylation levels in obesity (Watanabe and Hatakeyama, 2017). The CpG site on the promoter was shown to have decreased methylation level in obese participants. The methylation pattern of the following CpG site that exhibited substantial variability

methylation degrees among obese subjects and control subjects in the genome-wide methylation research is similarly located on this CpG island (Wang et al., 2010).

CHAPTER III

Methodology

3.1. Study Case

This study included a total of 59 obese and 62 non-obese subjects. The objective of the research was to examine the DNA methylation state of *TRIM-3* and its association with obesity. The form of informed consent was confirmed among all of the participants, and the study was confirmed by the Near East University's Scientific Research Ethics Committee (YDU/2021/96-1425).

At enrollment, cancer, diabetes mellitus, hypertension, dyslipidemias, liver cirrhosis, thyroid, cardiovascular, or any active inflammatory disease were evaluated in each patient. Professional athletes were excluded in this study. Medical history was questioned and written informed consent form obtained from all the subjects. The study protocol was approved by the Research Ethics Committee of the Near East University and performed in accordance with the Declaration of Helsinki (Project No: SAG-2018-1-013).

3.2. Materials

The list of equipment and kits has been shown in table 2 and table 3.

3.2.1. Equipment

Table 1**.**

The list of equipment

Equipment	Informations
Primers	The EpiTect HRM PCR Kit was used to be designed primers
Nuclease-free (DNase-free) consumables	All chemicals and consumables needed to set up PCR should be handled with extreme caution to avoid nuclease contamination.
Tubes or plates for optical PCR analysis	The PCR tubes or plates with thin walls that are recommended by the real-time cycler manufacturer.

3.2.1. Kits

Table 2.

The list of kits

EpiTect HRM PCR Kit	Includes 2x EpiTect High-Resolution Methylation PCR Master Mixture
HotStarTaq Plus DNA Polymerase	HotStarTaq Plus DNA Polymerase has been a customized variant of a Thermus aquaticus-cloned ninety-four kDa DNA polymerase.
EpiTect HRM PCR Buffer	A PCR buffer was developed for highly specific amplification of bisulfite-treated DNA, followed by high evaluation of melting intensity.
EvaGreen	A double-stranded DNA associating fluorescent dye which is suitable for HRM analysis and is quite accurate and inhibition-free PCR amplification.
dNTP mix	Includes ultrapure dCTP, dATP, dTTP, and dGTP.
RNase-free water	PCR-grade extremely purified

3.3. Methods

3.3.1. DNA Extraction

In molecular cell biology, isolating biological molecules namely RNA, protein, and DNA are important step for future processes and product advancement, as well as diagnostic tools. To successfully purify nucleic acids, certain steps must be followed: apart from a contaminant, breakage of cells or tissue, denaturation of nucleoprotein interactions, and deactivation of nucleases, including RNase for RNA extraction and DNase for DNA extraction. Contaminants such as DNA and RNA should be avoided in the production of protein, carbohydrate, and lipid products as well as other nucleic acids including those produced from target nucleic acids. The purity and quality of the obtained nucleic acid would significantly impact the outcomes of all subsequent clinical studies.

Blood samples obtained from patients with obesity and normal control. DNA was extracted from all subjects using Qiagen AllPrep DNA/RNA/Protein isolation

kit (Qiagen, Manchester, UK) according to the manufacturer's protocol and NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA USA) was used to measure its quantity. To isolate intact DNA the following steps are followed:

1. Blood samples were incubated into the erythrocyte lysis (EL) buffer at four degrees celcius for fifteen minutes. After the incubation process, samples were centrifuged at 1000 x g, four degrees celcius for fifteen minutes.
2. The pellet was added with Five milliliters of elution buffer and centrifuged at 1000 x g at four degrees for ten minutes to discarding the supernatant.
3. After the centrifugation procedure, the pellets (cells that had undergone lysis) were dried, and the supernatant was thrown away.
4. The cells were disrupted by the addition of 350-600 µl buffer RLT, then vortexed.
5. After the lysate was pipetted onto spinning columns that were located in a collecting tube with a diameter of two millimeters, the tube was centrifuged at the maximum speed of 14,000 revolutions per minute for three minutes.
6. 500 µl AW1 buffer (wash buffer) was put into the collection tube. The test tubes were centrifuged for one minute and fifteen seconds at a speed of 8000 x g (10 000 rpm). After the centrifugation period is done collection tubes should be checked and all of the filtrates should be in the collection tubes; if there is some in the spin column centrifugation step should be repeated. The process of discarding collection tubes and transferring spin columns to clean collection tubes was carried out.
7. Afterward, 500 µl of AW2 was put into each tube, and they were centrifuged at a speed of 20 000 x g or 14 000 rpm for a total of 2 minutes.
8. The DNA spin columns were transferred to 1.5 microlitre collection tubes. 100 µl EB buffer to each spin column and lids should be closed. After that, the samples underwent an incubation stage that lasted for two minutes and took place at room temperature. After the stage consisting of incubations had been completed, the samples were centrifuged at 8000 x g or 10 000 rpm for one minute in order to elute the DNA.

After the DNA extraction procedure was finished, a NanoDrop ND-1000 Spectrophotometer was used in order to determine the concentration of the DNA as well as its level of purity (Thermo Fisher Scientific).

3.3.2. Bisulfite Modification

In order to convert unmethylated cytosines into uracils without affecting the methylated cytosines, it is required to subject denatured genomic DNA to the sodium bisulfite treatment. The methylated cytosines are not converted, it remains the same. On the other hand, with the unmethylated sequence, all the cytosines are converted into uracil. Sodium bisulfite treatment was performed by using an Epitect Bisulfite Modification kit (Table 4) (Qiagen, Manchester, UK).

1. Before utilizing DNA in bisulfite processes, thaw it. To dissolve the appropriate number of aliquots of Bisulfite Mix, add 800 µl of RNase-free water to each aliquot of Bisulfite Mix. The Bisulfite Mix should be agitated until it is completely dissolved. This may need up to five minutes.
2. According to the Table 3, prepare 200 µl PCR tubes for the bisulfite reactions. Combine each constituent in the sequence specified. Solution of DNA and RNase-free water must be integrated to a volume of twenty µl.

Table 3.

Bisulfite reaction components

	Components				
Volume Per Reaction (µl)	DNA Solution (1 ng – 2 µg)	Rnase-Free Water	Dissolved Bisulfite Mix	DNA Protect Buffer	Total
	variable (maximum twenty µl)	variable	35	35	140

3. Following the closure of the PCR tubes, completely include the bisulfite processes. Place the tubes in a storage container at ambient temperature around fifteen or twenty-five degrees Celsius. After being added to the DNA–Bisulfite Mix, the DNA Protect Buffer's color must change from greenish to blues, indicating adequate mixing and the appropriate pH for the bisulfite transition phase.
4. Using a thermal cycler, convert the bisulfite DNA. Table 4 explains how to set up the thermal cycler. The entire cycle must require about 5 hours.

Table 4.

Bisulfite transition thermal cycler circumstances

Step	Denaturation	Incubation	Denaturation	Incubation	Denaturation	Incubation	Hold
Time	5 min	25min	5min	85min	5min	175min	Indefinite
Temperature	95°C	60°C	95°C	60°C	95°C	60°C	20°C

5. In the heat cycler, replace the PCR tubes that have the capacity for the bisulfite reactions. Begin the heat cycling incubation process. This might be critical to utilize properly closed PCR tubes.

Bisulfite-converted DNA cleanup

6. Immediately after the conversion of the bisulfite reactions, briefly centrifuge the PCR tubes that is containing the bisulfite process, followed by the transfer of the finished bisulfite reactions to pure 1.5 microliter micro centrifuge tubes.

7. Each sample should be added to 560 µl of newly produces Buffer BL having10 µl g/ml of carrier RNA. Vortex the mixtures to combine them, and after briefly centrifuge them.

8. Collection tubes and spin columns should be placed in a suitable rack.

9. Perform a one-minute centrifuge run at the maximum speed with the spin columns. After disposing of the flow-through, reintroduce the spin columns to their respective collecting tubes.

10. Combine five hundred µl of buffer BW to each spin column, and then centrifuge the mixture for one minute at the maximum speed. Take out the flow-through and put the spin columns back where they belong inside the collecting tubes.

11. Combine five hundred µl of buffer BD to each of the spin columns, and then leave them to incubate at ambient temperature between fifteen and twenty-five degrees Celsius for fifteen minutes.

12. Centrifuge the spin columns at the highest possible speed for one full minute. After the flow-through has been removed, the spin columns should be reinstalled into the collecting tubes.

13. Combine five hundred µl of Buffer BW to each spin column, then spin at full speed for one minute. Remove the spin columns from the collecting tubes and replace them with the flow-through.

14. Repeat one more Step 13.

15. Insert the spin columns into two ml collecting tubes and centrifuge at maximum speed for one minute to remove any residual liquid.

To prepare the spin columns, place them into purified 1.5 ml micro centrifuge tubes. Put the same amount of EB buffer in the middle of each membrane. One minute of centrifuging at around 12,000 rpm will provide pure DNA. As universal methylation and non-methylated control, the EpiTect PCR Control DNA Set was utilized. The NanoDrop Spectrometer was used to measure the concentration and purity of bisulfite-treated specimens.

3.3.3. Methylation Sensitive High-Resolution Melting Analysis

TRIM-3 promotor methylation was evaluated according to the methodology in the EpiTect® HRM^{TM} PCR Handbook (Table 5) (Table 6) (Rotor-Gene Q, Qiagen). The primer sequences were prepared in accordance with the EpiTect® HRMTM PCR Handbook (Qiagen, Manchester, United Kingdom). A universal methylated and unmethylated control DNA (EpiTect Control DNA Set) was utilized as a control to detect the methylation pattern of our samples.

Protocol: Analysis of TRIM-3 Methylation in Bisulfite Converted DNA

1. Several ingredients were dissolved including template DNA, as well as the control DNA samples, RNase free water, primer solutions, and EpiTect HRM PCR Master Mix.
2. The reaction mixture was made in table 5.

Table 5.

Reaction mixture

Component	Volume per 10 µl reaction	Final concentration
2X EpiTect Master Mix	5 µl	1x
10 µM (each) primer mix	0.75 µl	0.75 µM forward primer 0.75 µM reverse primer
RNase-free water	Variable	-
Template DNA	Variable	5-10 ng/reaction
Total volume per reaction	10 µl	

3. The reaction is well mixed, and suitable quantities are placed in PCR tubes. The patient's template DNA was combined into the PCR tubes and mixed.

4. Fill each PCR tube or well with identical quantities and volumes of template DNA and carefully mix.

5. Configure the real-time cycler according to the Table 6. By following the instructions in the table, the cycling routine on the Rotor-Gene Q was adjusted for HRM analysis.

Table 6.

Configure the real-time cycler

Initial PCR Activation Step	5 min	95 ^{0} C
3-step cycling		
Denaturation	10s	95 ^{0} C
Annealing	30s	62 ^{0} C
Elongation	10s	72 ^{0} C
Number of cycles 40-45		
Denaturation	30s	95 ^{0} C
Pre-hold	30s	50 ^{0} C
HRM Analysis for Rotor-Gene Q	2s	65-95 ^{0} C 0.1^{0} C increments

6. After inserting the tubes or plates of PCR into the real-time cycler, start the PCR cycle procedure while simultaneously conducting an HRM analysis.

7. Analyze the information

4. Statistical Analysis

The chi-square test and two-tailed Fisher's exact test were used for statistical analysis and their relationships with the characteristics of participants. The SPSS 15.0 software (SPSS, Chicago, IL, USA) was implemented for calculations, with a statistical significance of $P < 0.05$.

CHAPTER IV

4.1 Results

The mean age was 43.17 ± 9.62 years and BMI of 34.92 ± 5.83 kg/m2 in patients with obesity. The mean age of control group was 42.92 ± 13.14 years and their mean BMI was 23.78 ± 2.5 kg/m2. The anthropometric and metabolic characteristics of the patients has been presented in Table 7.

Table 7.

The Anthropometric and Metabolic Characteristics of Studied Patient Population.

Parameter	Non-obese subjects			Obese subjects		
	Methylated -31	Unmethylated -31	p	Methylated -24	Unmethylated -35	p
Age	42,16±13,68	41,35±13,09	0,81	43,58±11,21	44,34±9	0,77
BMI (kg/m²)	23,66±2,45	23,81±2,68	0,82	36,31±7,86	34,4±4,57	0,24
Waist circumference (cm)	83,52±8,17	84,94±8,69	0,51	117,9±15,65	112,5±13,14	0,15
Hip circumference (cm)	100,5±7,27	99,52±6,80	0,57	120,3±10,14	119,6±10,05	0,79
Fasting glucose (mg/dL)	88,16±7,74	89,19±5,64	0,55	101,5±18,64	104,3±22,85	0,61
Total cholesterol (mg/dL)	202±25	202,7±22,14	0,91	222,7±29,71	224,6±40,5	0,84
LDL-cholesterol (mg/dL)	127,6±25,83	130,7±25,73	0,63	137,4±25,77	138,5±34,31	0,89
HDL-cholesterol (mg/dL)	56,74±7,35	58,39±10,76	0,48	43,42±7,50	47,4±9,98	0,1

Table 7 (continued)

Triglycerides (mg/dL)	100,5±34,91	90,35±40,59	0,29	177,8±73	162,1±67,19	0,39
HOMA-IR	1,94±0,55	1,91±0,62	0,81	4,02±2,26	4,23±2,44	0,73
Leptin (ng/ml)	8,94±3,71	9,6±6,47	0,62	23,42±14,07	21,04±10,81	0,46
Adiponectin (µg/mL)	18,86±8,93	22,03±9,14	0,17	10,54±4,96	10,39±5,15	0,91
Resistin (ng/mL)	6,303±2,32	5,99±3,02	0,64	8,85±2,37	8,74±2,68	0,87

4.1.1 Methylation Pattern of the *TRIM-3* gene

TRIM-3 gene methylated in 24 out of 59 patients with obesity (40,68%) and 31 out of the 62 non-obese subjects (50%). There was no statistically significant difference between methylation status and obesity identified ($p > 0.05$) (Figure 5).

Methylation status of *TRIM-3* in obese and non-obese subjects has been shown in Table 8.

Table 8**.**

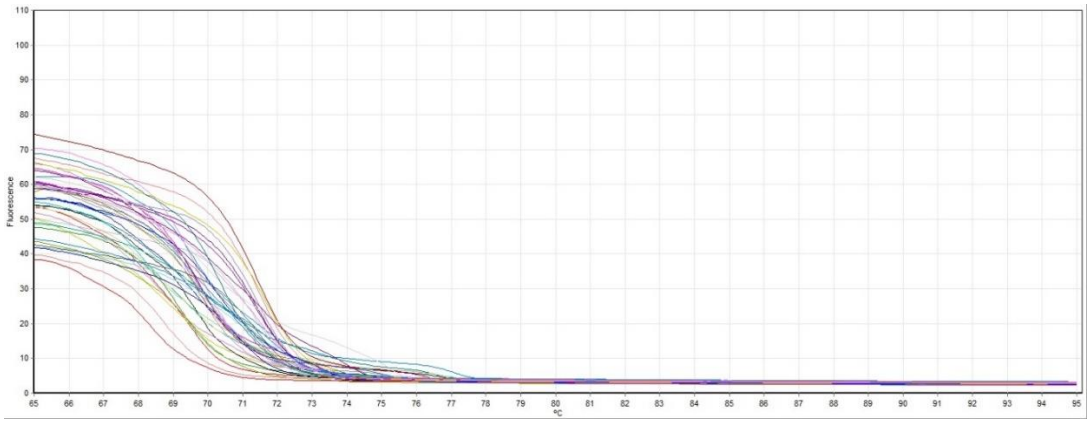
Methylation Status of The TRIM3 Gene in Obese and Non-Obese Subjects

TRIM3	OBESE STATUS				
		nonobese	obese	total	p value
unmethylated	Observed	31	35	66	p>0,36
	%within column	50.0%	0,5932	66.0%	
methylated	Observed	31	24	55	
	%within column	50.0%	0,4068	55.0%	
total	Observed	62	59	121	
	%within column	1	1	1,21	

The universal unmethylated control of the *TRIM3* gene was shown as purple, while universal methylated control of the *TRIM3* gene was shown as blue. Fluorescence (dF/DT) is shown along the Y-axis, while temperature (^{0} C) is plotted along the X-axis. In the *TRIM3* promoter region, the averaged melting curves of the dilution standards show the optimization process (Figure 5).

Figure 5

Increasing The Annealing Temperature Improves Assay Sensitivity When PCR Primers Include CpG Dinucleotides



Methylation and unmethylation levels for the *TRIM3* gene were estimated using a normalized HRM curve. Different colors are used to indicate methylation profiles of distinct patients. A high-resolution examination of the melting curve revealed that fluorescence increases with temperature (Figure 6)(Figure 7).

Figure 6

Figure Shows Starting Cycling Should Be Before The 30 ^{0} C Cycle

Fluorescence (dF/DT) is shown along the Y-axis, while CT (Cycle Threshold) values is plotted along the X-axis.

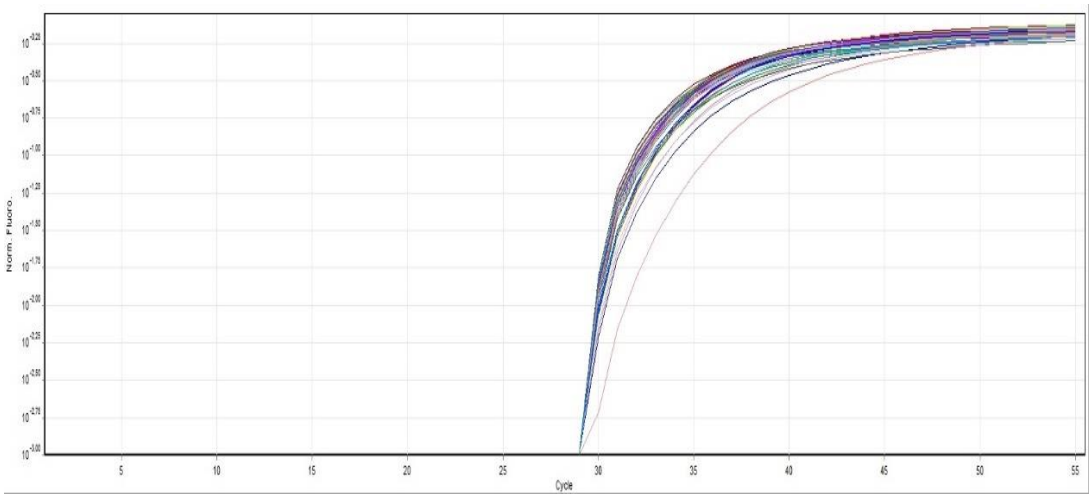
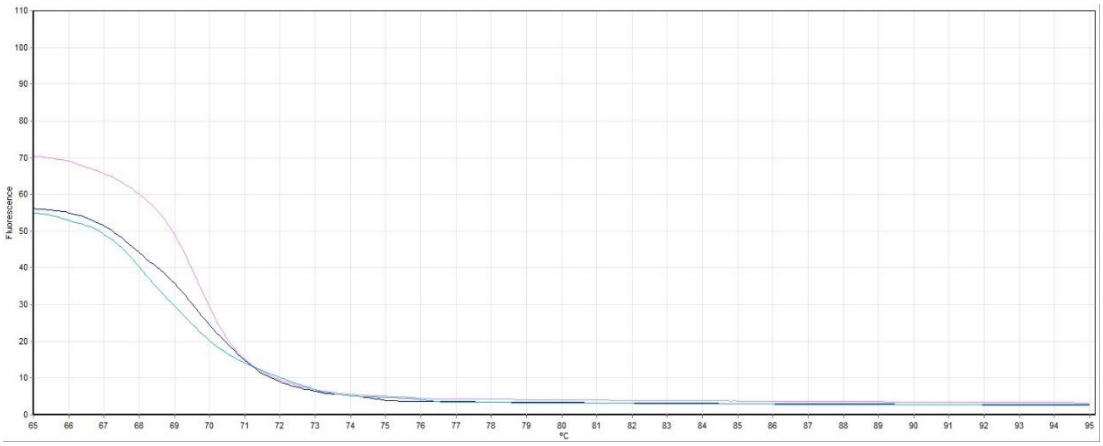


Figure 7

Unmethylated TRIM3 Patient

The unmethylated *TRIM3* control is shown in pink, whereas the methylated control is shown in blue (Figure 7). Fluorescence (dF/DT) is shown along the Y-axis, while temperature (^{0} C) is plotted along the X-axis.



4.1.2 Relationship between anthropometric and metabolic characteristics and of *TRIM3* methylation status

The relationship between *TRIM3* methylation status and waist circumference, hip circumference, BMI, age, circulating levels of serum glucose, triglycerides (TG), total cholesterol, HDL-C, and LDL-C, insulin concentration, HOMA-IR, leptin, adiponectin and resistin levels were investigated. However, no statistical association was found between anthropometric and metabolic characteristics and *TRIM3* methylation status (Table 8).

CHAPTER V

Discussion

The interaction between genetics and epigenetics plays a key role in the development of obesity. The interaction between obesity and epigenetic alterations has been demonstrated in several studies. DNA methylation is an epigenetic mechanism that is involved in the development of obesity and its metabolic complications (Kalkan & Becer, 2019, Samblas et al., 2019).

Until now, epigenetic alterations of *CLOCK, BMAL1, PER2, UBASH3A, TRIM3, LEP, ADIPOQ, PGC1α, IGF-2, IRS-1, LY86, MEST, PEG3, NNAT, PLAGL1, MEG3, NPY, IL6, TNF, TFAM, GLUT4, RANKL* and *c-FOS* had been reported related with obesity (Kalkan & Becer, 2019, Van Dijk et al., 2015, Carless et al., 2013, Xu et al 2013, Bouchard et al 2010).

TRIM3 methylation levels were found to be reduced in obese subjects after a genome-wide methylation analysis was conducted (Wang et al., 2010). Low et al. discovered spontaneous differentiation of glioma CSCs (Canser Stem Cells) after shRNA mediated downregulation of various TRIMs namely *TRIM3, TRIM13, TRIM41*, and *TRIM52*, indicating a role in CSC self-renewal (Jaworska et al., 2019).

TRIM3 located within the chromosome segment 11p15.5 upstream of the loss of allelic heterozygosity (LOH) hotspot, suggesting a role in tumor suppression (Boulay et al., 2009). Tumors of various types show LOH in 11p15 (Boulay et al., 2009). *TRIM3* inhibits tumorigenesis in liver cancer cells by decreasing proliferation, colony formation, migration, and invasion, as well as triggering cell cycle arrest, indicating that *TRIM3* may operate as a negative regulator for stem cell phenotypic acquisition (Jaworska et al., 2019).

A genome-wide approach was used to define the DNA methylation pattern in peripheral blood leukocytes of obese subjects. The genome-wide methylation study found substantial differences in CpG methylation between obese subjects and controls. The promoter CpG island exhibited decreased methylation in obese subjects in both the monitoring and replicating cohorts (Wang et al., 2010, Watanabe & Hatakeyama, 2017). One CpG site in the *UBASH3A* gene had increased methylation rates and one in the *TRIM3* gene had reduced methylation rates in obese subjects both in the genome-wide process ($P < 0.05$ and $P < 0.05$ for the *UBASH3A* and *TRIM3* genes respectively) and the verification process ($P = 0.008$ and $P = 0.001$ for the *UBASH3A* and *TRIM3* genes respectively) (Wang et al., 2010).

TRIM protein mutations are increasingly being linked to a wide range of disorders. Many diseases are considered to have TRIM protein abnormalities as a contributing factor. TRIM proteins have been linked to a wide range of disorders, including neurological diseases, neuropsychiatric disorders, developmental diseases, congenital anomalies, viral infections, and cardiac disorders (Liu et al., 2021). Postmortem dorsal prefrontal cortex samples from schizophrenia patients showed an increased level of *TRIM3* compared to those from healthy persons (Watanabe & Hatakeyama, 2017). Schizophrenia is a persistent, serious psychological condition that impairs an individual's manner of thinking, acting, expressing feelings, seeing realities, and interacting with one another (Bhandari, 2022). In schizophrenia subjects, sequences of exome and GWA have shown mutation of the *PML* gene and an intronic polymorphism of *TRIM26*. Additionally, Watanabe and Hatakeyama found that single nucleotide polymorphisms in another TRIM family member, *TRIM76* which was associated with schizophrenia (Watanabe & Hatakeyama, 2017).

Despite evidence linking obesity with the methylation of *TRIM3*, little is known about the exact processes involved. Based on emerging evidence that epigenetic regulation controls gene expression, we predicted that DNA methylation might play a role in obesity. The purpose of this research was to determine whether or not there is a connection between methylation of the *TRIM3* gene and obesity.

The mean age was 43.17 ± 9.62 years and BMI of 34.92 ± 5.83 kg/m2 in patients with obesity. The mean age of control group was 42.92 ± 13.14 years and their mean BMI was 23.78 ± 2.5 kg/m2. *TRIM-3* gene methylated in 24 out of 59 patients with obesity (40,68%) and 31 out of the 62 non-obese subjects (50%). There was no statistically significant difference between methylation status and obesity identified ($p > 0.05$). *TRIM-3* gene unmethylated in 35 out of 59 patients with obesity (59,32%) and 31 out of the 62 non-obese subjects (50%). There was no statistically significant difference between unmethylation status and obesity identified ($p > 0.05$).

In this thesis, we examined the relationship between the mean age of participants and their methylation and unmethylation status of the *TRIM3* gene. The mean age of the unmethylated *TRIM3* gene was 44,34±9 (mean ± Std. Deviation), and the mean age of the methylated *TRIM3* gene was 43,58±11,21 (mean ± Std. Deviation). According to the statistical analysis, no significant relationships were

found though the age of the obese subjects and their methylation status of the *TRIM3* gene (p = 0.77).

In this study, we identified changes in DNA methylation of the *TRIM3* methylation status between obese subjects and control subjects, and this research provides evidence that obesity is associated with methylation changes in the DNA.

The leptin levels of obese subjects are abnormally elevated. This phenomenon is referred to resistance of leptin (Obradovic et al., 2021). Through an aberrant generation of adipokines, visceral body fat might impact medical problems in obese subjects. Adiponectin represents a crucial function in energy homeostasis; the concentration of overall adiponectin and adiponectin with a high molecular weight drops in obesity and rises following weight reduction. The expansion of adipose tissue may influence the production and release of adiponectin (Nigro et al., 2014). In human investigations, those with extreme insulin sensitivity have greater levels of resistin than those with typical insulin action. Consequently, resistin may potentially play a significant key in insulin resistance (Su et al., 2019).

In our study, the mean level of leptin of the unmethylated *TRIM3* subjects was 21,04±10,81 (mean ± Std. Deviation), and the mean level of leptin of the methylated *TRIM3* subjects was 23,42±14,07 (mean ± Std. Deviation,) (p = 0,46). The mean the mean level of adiponectin of the unmethylated *TRIM3* subjects was 10,39±5,15 (mean ± Std. Deviation,), and the mean the mean level of adiponectin of the methylated *TRIM3* subjects was 10,54±4,96 (mean ± Std. Deviation,) (p =0,91). The mean level of resistin of the unmethylated *TRIM3* subjects was 8,74±2,68 (mean ± Std. Deviation,), and the mean level of resistin of the methylated *TRIM3* subjects was 8,74±2,68 (mean ± Std. Deviation) (p = 0,87). Therefore, *TRIM3* methylation status was not statistically associated with anthropometric or metabolic characteristics (Table 8).

CHAPTER VI

Conclusion and Recommendations

Genetic and epigenetic factors play a crucial role in obesity. Studies and alterations of epigenetic regulators highlight the importance of the interaction between genetic and environmental factors on the etiology of obesity. Researchers have identified several obesity susceptibility genes and their role during disease development. The development of obesity is significantly influenced by environmental and epigenetic variables. Interactions between genes and the environment contribute to the rising incidence of obesity. In this light, epigenetic modifications and their interaction with the environment hold promise as potential biomarkers of obesity. In accordance with these results, several researchers have investigated potential biomarkers of obesity in an effort to improve the quality of life of affected individuals, reduce their symptoms, help them adjust to daily life, and enhance and reinforce their attitudes toward human relationships. In this study, the relationship between the methylation pattern of the *TRIM3* and obesity could not be encountered. The number of epigenetic studies performed in this area is limited. Consequently, our work sheds light on epigenetics and gives vital information for future studies including biomarkers for obesity.

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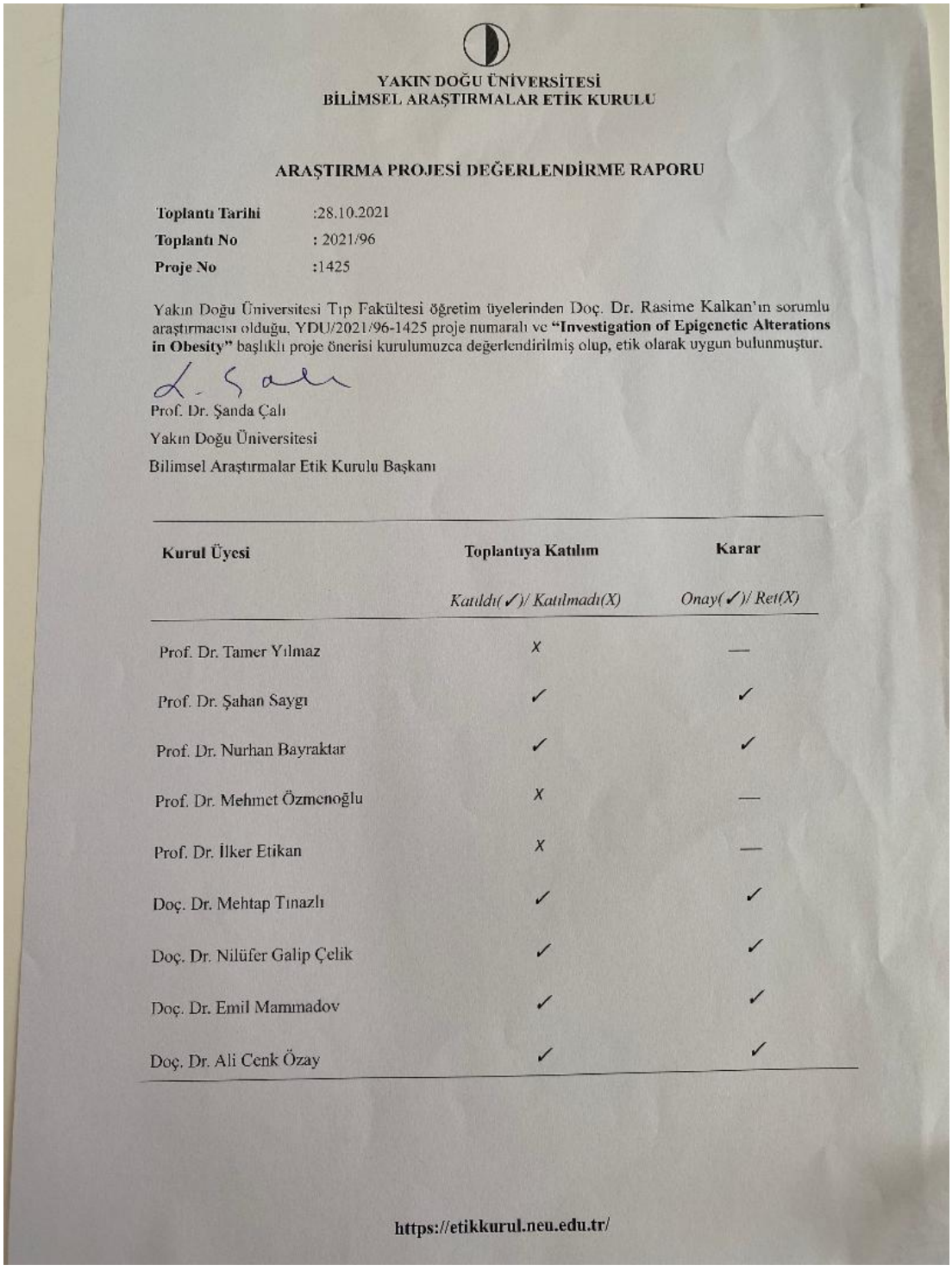
Appendices



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Yakın Doğu Üniversitesi Tıp Fakültesi öğretim üyelerinden Doç. Dr. Rasime Kalkan'ın sorumlu araştırmacısı olduğu, YDU/2021/96-1425 proje numaralı ve **"Investigation of Epigenetic Alterations in Obesity"** başlıklı proje önerisi kurulumuzca değerlendirilmiş olup, etik olarak uygun bulunmuştur.

Prof. Dr. Şanda Çalı
Yakın Doğu Üniversitesi
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Curriculum Vitae

Personal Information

Name Surname: Buğsem Hülya Öztenekecioğlu

Date and Place of Birth: 24.05.1998 / Nicosia

Education: Master's degree in Medical Biology and Genetics

Education

Degree	Field	University	Year
Bachelor	Molecular Biology and Genetics	Near East University	2021
Master	Medical Biology and Genetics	Near East University	2022